



# AMPHIBIANS

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## THANK YOU!

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## Disclaimers

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  - No warranty for accuracy
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## Objectives

- Taxonomy and research use
- Biology and behavior
- Housing, husbandry and care
- Infectious Diseases
- Anesthesia, analgesia and euthanasia

## Amphibian Taxonomy

- Approximately 4300 species
- Means "double life"
- Transition between aquatic and terrestrial vertebrates



## Amphibian Taxonomy

- Three orders
  - Gymnophiona (Apoda)
  - Caudata (Urodela)
  - Anura (Salientia)

## Amphibian Taxonomy

- Gymnophiona = "naked snake"
- Apoda = "without foot"
- Caecilians
  - Resemble earthworms
  - Burrowing
  - Tropical



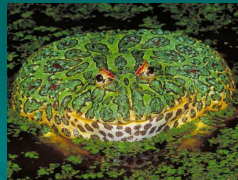
## Amphibian Taxonomy

- Caudata = "having tail"
- Urodela = "tail visible"
- Salamanders and newts
- Usually have well-developed limbs
- Aquatic, terrestrial, arboreal



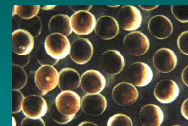
## Amphibian Taxonomy

- Anura = "without tail"
- Salientia = "jumping"
- Frogs and toads
- Powerful hindlimbs
- Most diverse of amphibians



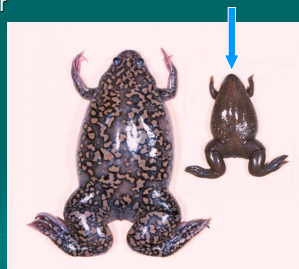
## Amphibian Research Use

- South African Clawed Frog or common plantanna (*Xenopus laevis*)
  - Developmental biology
  - Molecular biology
  - Toxicology (FETAX)
    - Frog embryo teratogenesis assay: *Xenopus*



## Amphibian Research Use

- Western Clawed Frog (*Xenopus tropicalis* or *Silurana tropicalis*)
  - Genetics
    - Short generation time <5mo
      - (*X. laevis* 1-2yrs)
    - Diploid 2N=20
      - (*X. laevis* 2N=36)



## Amphibian Research Use

- Leopard Frog (*Rana pipiens* or *Lithobates pipiens*)
  - Physiology
  - Endocrinology
  - Nociception
    - Acetic acid wiping response



## Amphibian Research Use

- Bull frog (*Rana catesbeiana* or *Lithobates catesbeianus*)
  - Physiology
  - Neurology
  - Infectious disease



## Amphibian Research Use

- Marine Toad (*Bufo marinus*)
  - Ophthalmology
  - Neurology



## Amphibian Research Use

- Axolotl (*Ambystoma mexicanum*)
  - Developmental biology
  - Limb/CNS/cardiac regeneration



## Amphibian Research Use

- Mudpuppy (*Necturus maculosus*)
  - Comparative anatomy
  - Physiology
  - Developmental biology



## Amphibian Research Use

- Tiger salamander (*Ambystoma tigrinum*)
  - Ophthalmology
  - Neurology



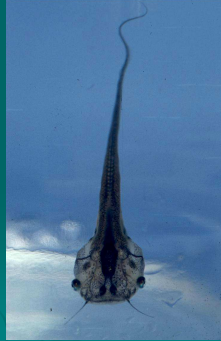
## Amphibian Biology

- Metamorphosis
  - Developmental process unique to amphibians
  - Requires iodine and thyroid hormone
  - Typically begin as fish-like aquatic larvae
    - Gills
    - Two chambered heart
  - Adult form usually very different
    - Lungs
    - Three chambered heart
    - Limbs

## Metamorphosis

### ➤ *Xenopus* tadpoles

- No limbs
- Well-developed tail
- Hover head down with undulating tail directing food to filter-feeding mouths



## Metamorphosis

### ➤ As *Xenopus* development progresses

- External gills resorbed
- Limbs develop
- Tail resorbed
- Limbs functional



## Metamorphosis

### ➤ When froglets emerge

- *Xenopus* totally aquatic so no ramp necessary
- Other species require ramp to leave water or will drown



## Metamorphosis

### ➤ Some species demonstrate pedomorphism

- Retention of larval characteristics
- Fully functional, reproductively active adult
- Axolotls are paedomorphic salamanders



## Integumentary System

- Skin typically moist, smooth, glandular
- Permeable for water absorption (vascular "drink patches" in some terrestrial species)
- Mucous glands
  - Present throughout skin
  - Protects against pathogens, trauma
  - Helps retain body fluids
  - Creates the "slimy" feel

## Integumentary System

- Granular glands
  - Produce variety of compounds, from toxins to pheromones to antimicrobial peptides (magainins)
  - Magainins have antibacterial, antifungal, and some antiprotozoal properties
  - Toxins can have neurotoxic, cardiotoxic, hallucinogenic, and hypotensive effects
  - Pheromones, opioids, and bioadhesives also secreted



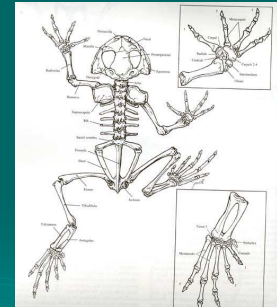
## Integumentary System

- In toads, granular glands are clustered behind eye and are called **parotoid** glands. Some species, such as the marine toad, can forcibly eject toxins from the parotoid gland into the eyes of the “predator.”



## Musculoskeletal System

- Frog ribs absent or greatly reduced
- Frog postsacral vertebrae fused into single urostyle
- Frog tibia and fibula fused into sturdier tibiofibula



## Musculoskeletal System

- Salamander skeleton predominantly cartilaginous
- Many salamanders regenerate tails (autotomy)

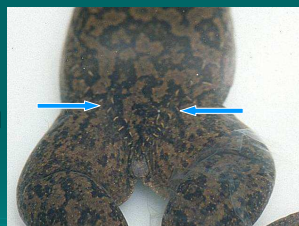


## Nervous/Sensory Systems

- Amphibians have 10 cranial nerves
- Vomeronasal organ (olfaction)
- Rods and two types of cones
- High frequency sound through tympanum, low frequency sound through forelimbs
- Lateral line system in all larval amphibians and aquatic adults

## Nervous/Sensory Systems

- Lateral line system
  - Linear arrangement of neuromasts
  - Detect changes in water currents and pressure



## Respiratory System

- Most larval amphibians breathe primarily through gills
- *Xenopus* tadpoles have functional gills and lungs and gulp air at water's surface
- Adult amphibians breathe through lungs, gills, both, or neither
- Skin must be kept moist to facilitate cutaneous respiration

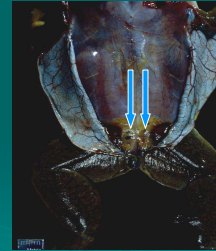


## Cardiovascular System

- Most adult amphibians have three chambered heart (two atria and a single ventricle)
- Larvae have a two chambered heart
- Lymphatic system drains directly into veins

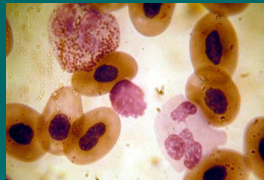
## Cardiovascular System

- Frogs have SC sinuses called dorsal lymph sacs
- Lymphatic hearts drain lymph sacs into venous system
- Lymph sacs IV injection site



## Cardiovascular System

- Plasma osmolarity 200-250 mOsm/Kg
- Large, nucleated RBCs
- Neutrophils like mammals
- Heterophil granules rod-shaped, small
- Eosinophil granules round or oval
- Thrombocytes nucleated



## Digestive System

- Pipid frogs (*Xenopus*) lack tongue
- Other frog tongues attached rostrally
- GI tract short in adults (carnivorous)
- Vomiting common as defense mechanism (some frogs evert stomach)
- Cloaca common emptying chamber for urinary, GI and repro tracts ("sewer")



## Excretory System

- Amphibians have mesonephric kidney (tadpoles pronephric)
- No loop of Henle so cannot concentrate urine
- Aquatic species excrete ammonia, terrestrial excrete urea, arboreal excrete uric acid
- Urinary bladder in many species
  - Serves as storage depot for water
  - Bladder contents released to dissuade predators

## Reproductive/Endocrine Systems

- Complex and varied courtship/reproductive strategies and life cycles



## Reproductive/Endocrine Systems

- Internal fertilization primarily in salamanders
- External fertilization primarily in frogs
- Amplexus in frogs
  - Nuptial pads on male (keratin)



## Reproductive/Endocrine Systems

- Egg caring/parental behavior present in many species



## Reproductive/Endocrine Systems

- Sexual dimorphism
  - *Xenopus* females larger than males



## Reproductive/Endocrine Systems

- Sexual dimorphism
  - Male bullfrog tympanum larger than eye
  - Also in pig and bronze frogs



## Amphibian Behavior

- Some amphibians will aggregate
- Many have home ranges, which may shift in response to environmental pressures
- Salamanders can be very territorial
- Some amphibians have "dear enemy" truce with adjacent conspecific



## Amphibian Behavior

- Acoustic and visual communication in frogs
- Chemical and visual communication in salamanders



## Xenopus Behavior

- Entirely aquatic in all life cycle stages
- Require no emergence surface
- Prefer still, warm waters in the wild and opaque containers (dark) in captivity
- Live in large groups with hierarchical structure in wild; territorial

## Xenopus Behavior

- Water deep enough to swim freely and suspend below surface
- Nocturnal
- Readily utilize retreats
  - PVC pipe
  - Ceramic tiles
  - Floating plastic "plants"



## Axolotl Behavior

- Do not require means to emerge from water (gills)
- Water deep enough to allow free movement while submerged
- Will utilize retreats (heavy)
- Overcrowding can result in bite wounds



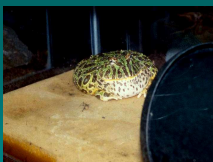
## Primary Enclosures

- Glass, plastic, plexiglas (modular units)
- Stainless steel, fiberglass
- Systems for aquatic species static, recirculating, or flow-through
- Lids prevent escape and retain humidity



## Primary Enclosures

- Moistened sphagnum moss substrate
  - Allows burrowing
  - Retains humidity
- Precut foam or sponge



## Primary Enclosures

- Bark, PVC pipe, or other retreats necessary because secretive
- Treefrogs and other perching species need branches or suitable structures





## Water Quality

- Chlorine, chloramines toxic
  - Activated charcoal will remove both
  - "Aging" works for chlorine
- Copper toxic
  - Avoid copper pipes



## Water Quality

- Larval amphibians exquisitely sensitive to toxins
- General hardness (high calcium and magnesium) most important factor in normal embryo development and survivability in *Xenopus*



## Temperature

- Varies with species
- Temperate salamanders and frogs prefer 60-70°F
- Tropical species prefer 70-80°F
- *X. laevis* prefer 68-72°F (20-22°C)
  - Become stressed if <14°C or >26°C
- *X. tropicalis* prefer 24-25°C

## Airflow

- Must be reduced to prevent desiccation
- In general, 80% relative humidity is adequate for most species

## Lighting

- Most species nocturnal or live under leaf litter or in streams in dark, cool environments
- Avoid direct exposure to bright light



## Sanitation

- Phenolics toxic
- Avoid soapy or chemical residues
- Many terrestrial salamander species mark territory with pheromones
  - Excessive cleaning can be stressful
  - Excreta buildup in humid environment facilitates pathogen growth
  - Must balance behavioral needs of species with health needs

## Nutrition

- Larval amphibians
  - Herbivorous or carnivorous
  - Tadpoles eat boiled dark lettuce, ground rabbit chow, flaked fish food, etc.
    - Spinach can cause oxalate toxicity
  - *Xenopus* eats strained green vegetable baby food or algae powder
  - Larval salamanders eat small whole or chopped vertebrates and invertebrates

## Nutrition

- Adult amphibians
  - Most carnivorous
  - Axolotls eat earthworms, fly larvae, commercial feed
  - Small salamanders eat fruit flies and their larvae



## Nutrition

- Leopard frogs require live prey
  - Crickets fed good diet and/or "gut loaded"
- *Xenopus* opportunistic
  - Commercial pellets because locate by olfaction
  - Trout chow
  - Organ meat is deficient in calcium and harbors pathogens



## Amphibian Handling

- Support the body as much as possible
- Restrain legs in frogs
- Large salamanders can bite
- Don't disrupt mucous layer
- Beware of tail autotomy



## Amphibian Handling

- *Xenopus* can be restrained for examination and transfer using decapicones



## Amphibian Zoonoses

- Atypical mycobacteriosis
  - *M. fortuitum*, *M. marinum*, *M. xenopi*
  - Usually self-limiting
  - Immune compromised individuals life-threatening disease
  - Recent severe cases in immune competent individuals



## Amphibian Zoonoses

- *Chlamydophila* (*Chlamydia*) *psittaci* and *C. pneumoniae*
  - Documented in *Xenopus laevis*
  - No reports found yet of frog to human transmission
- *Salmonella* Typhimurium outbreak 2009 associated with African dwarf frog



## Diagnostic Techniques

- Physical exam
- Blood samples
  - Abdominal vein
  - Lingual venous plexus
  - Femoral vein
  - Use lithium heparin
- Fecal exam
- Cultures
- Necropsy



## Therapeutic Techniques

- Oral medication
  - gavaging
- Injection sites
  - Dorsal lymph sacs
  - IM in quadriceps



## Infectious Diseases Red Leg

- Bacterial septicemia secondary to immune suppression and invasion by opportunists
- *Aeromonas* most frequently implicated, although other bacteria are commonly seen



## Red Leg

- Signs include petechiation and ulceration of abdominal and leg skin, lethargy, anorexia, ascites, ocular inflammation, and neurologic signs



## Red Leg

- Treat with enrofloxacin, aminoglycosides, chloramphenicol, tetracycline
- **Must** correct underlying stressor(s)

## Chrysobacterium (Flavobacterium)

- Found in soil, water, raw meat
- Stress predisposes
- Septicemia
- Resistant to many antibiotics and chlorine



From Green SL, et al., 1999, JAVMA 214(12): 1833-8.

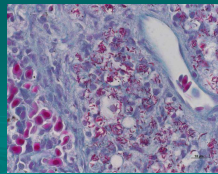
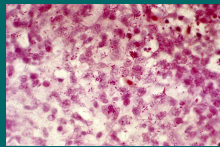
## Mycobacteriosis

- *Mycobacterium xenopi*, *M. marinum*, *M. fortuitum*, *M. chelonae*, *M. liflandii*, etc. implicated
- Signs include wasting, pneumonia, visceral and cutaneous granulomas, ulcers on skin, coelomic effusion



## Mycobacteriosis

- Acid fast organisms on histopath
- Euthanasia recommended (zoonosis)
- **Must** correct underlying problems

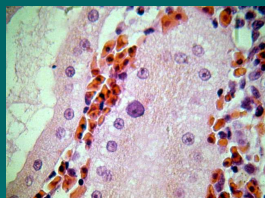


## Chlamydiosis

- Disease reported in *Xenopus* associated with *Chlamydia* (*Chlamydia*) *psittaci* and *C. pneumoniae*
- Frogs bloated, lethargic, patchy depigmentation and erythema of skin, coelomic effusion, gelatinous material in subcutaneous tissues, and hepatosplenomegaly

## Chlamydiosis

- Basophilic intracytoplasmic inclusion bodies
- Advisable to treat as potentially zoonotic



## Lucké Tumor Herpesvirus

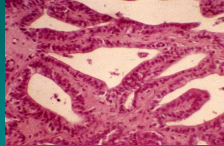
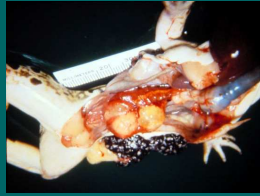
- Affects *Rana pipiens*
- Virus replicates in cool weather
- Virus shed during spawning
- Infected animals develop renal adenocarcinomas





## Lucké Tumor Herpesvirus

- Tumor growth occurs in warm months
- Signs include emaciation, lethargy, ascites
- No treatment



## Chytridiomycosis

- Caused by fungus, *Batrachochytrium dendrobatidis*
- Global threat to wild amphibians and reported in captive *Xenopus*
- Affects keratinized mouthparts in larvae and causes hyperplasia and hyperkeratosis in adults
- Mortality rates high

## Chytridiomycosis

- Signs included anorexia, lethargy, dark pigmentation, skin sloughing, no protective mucous layer
- Treat with itraconazole baths
- Elevating temperature may help



## Saprolegniasis

- Caused by *Saprolegnia* and related species in aquatic amphibians
- Hyphae appear as cottony tufts on skin



## Saprolegniasis

- Invasion by organism secondary to skin lesions, immune suppression
- Treat with saltwater or benzalkonium chloride baths
- Must correct underlying problem



## Pseudocapillariosis

- Caused by *Pseudocapillarioides xenopi*
- Common pathogenic nematode of *Xenopus*
- Transmitted by ingestion of sloughed skin from affected frog
- Parasite lives and burrows through epidermis
- Signs include desquamation and thick, pitted appearance to skin, progressing to ulceration and debilitation of the frog

## Pseudocapillariosis

- Diagnose by identifying nematodes and bipolar eggs on skin scrapings
- Treat with ivermectin, fenbendazole, levamisole

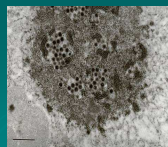
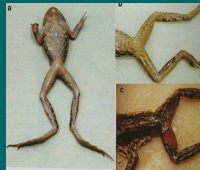


## Ranavirus

- Genus of family Iridoviridae
- Large, double stranded DNA virus
- Icosahedral; visible in paracrystalline arrays in cell cytoplasm
- Virus persistent in environment
- Direct and indirect transmission

## Ranavirus

- Limb and body swelling, erythema, hemorrhage, swollen livers, emaciation
- Death in days to weeks



## Amphibian Anesthesia/Analgesia

- Immersion anesthesia best
  - Buffered MS222
- Isoflurane
- Analgesics
  - Xylazine
  - Butorphanol
  - Morphine
  - Meloxicam



## Amphibian Surgery

- Aseptic technique



## Amphibian Euthanasia

- Buffered MS222 overdose followed by physical method (decapitation, double pithing)
- Euthanasia solution
- Hypothermia (freezing) is NOT acceptable

