Avian Tuberculosis & Captive Population Sustainability

WHITE-WINGED WOOD DUCK *(ASARCORNIS SCUTULATA)*

- KIMBERLY COOK, DVM

  DIRECTOR OF ANIMAL HEALTH AND CONSERVATION

  AKRON ZOOLOGICAL PARK

  AKRON, OHIO, USA

- FUTURE OF WATERFOWL CONSERVATION WORKSHOP 2016

  SYLVAN HEIGHTS WATERFOWL PARK

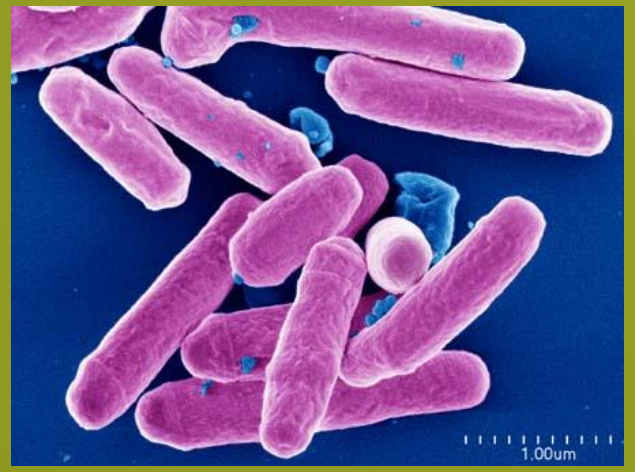
  SCOTLAND NECK, NORTH CAROLINA, USA

Photo credit: Nick Hill

Let's Begin With A Huge Thank You!

Mycobacterium avium basics

- Intracellular, gram positive, non-spore-forming, aerobic, rod-shaped bacteria with a lipid-rich waxy cell wall
  - This cell wall affords the organism resistance:
    - To the immune system: it can persist for long periods within the host's endosomal macrophage network
    - To detection and diagnosis
    - To disinfectants and treatment
    - To heat and cold.
      - M. avium survives above 55° C and freezing at –75° C
  - It also allows the organism to persist for long periods outside of the host in biofilms
    - Mycobacterial organisms are also able to remain viable in soil for up to 4 years [3]



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Mycobacterium Sources

- Opportunistic ubiquitous environmental mycobacterium
  - Found commonly world-wide in marshes, ponds, and rivers at the soil- water interface
  - Mycobacterial disease was reported in many avian species in zoological surveys as early as the late 1800's [2]
- They are routinely isolated from a variety of beddings, soil types, and commercial water sources
- Mycobacteria thrive in conditions of low pH, high zinc, high organic content, and increased fulvic and humic acid levels [3]

Susceptibility

- All bird species appear to be susceptible though to varying degrees
  - More common in captive than wild birds
  - Most common in large flocks of zoological and avicultural collections

Photo credit: Nick Hill

Prevalence

- Relatively uncommon in individual or small groups of captive held birds
- Marked prevalence variability occurs: retrospective avian necropsy reviews from three U.S. zoological institutions have reported 1.2%, 4% and 24.5% prevalence [1,2,5]
  - Institutional incidence may be significantly affected by husbandry, environmental factors, and the species involved
  - Mycobacteria can build up within an environment (fecal shedding), and in these cases waterfowl and water birds, which are normally quite resistant in the wild, may be infected in large numbers

aterfowl

- Emaciation (with good appetite)
  - We see a drop in body condition 3-6 months prior to death
- Dyspnea
- Granuloma formation
  - Cutaneous, subcutaneous and ocular
- Abdominal distention (ascites)
- Abnormal feathers or poor molt
- Diarrhea
- Lameness

Photos of *M. avium* Infection

Emaciation

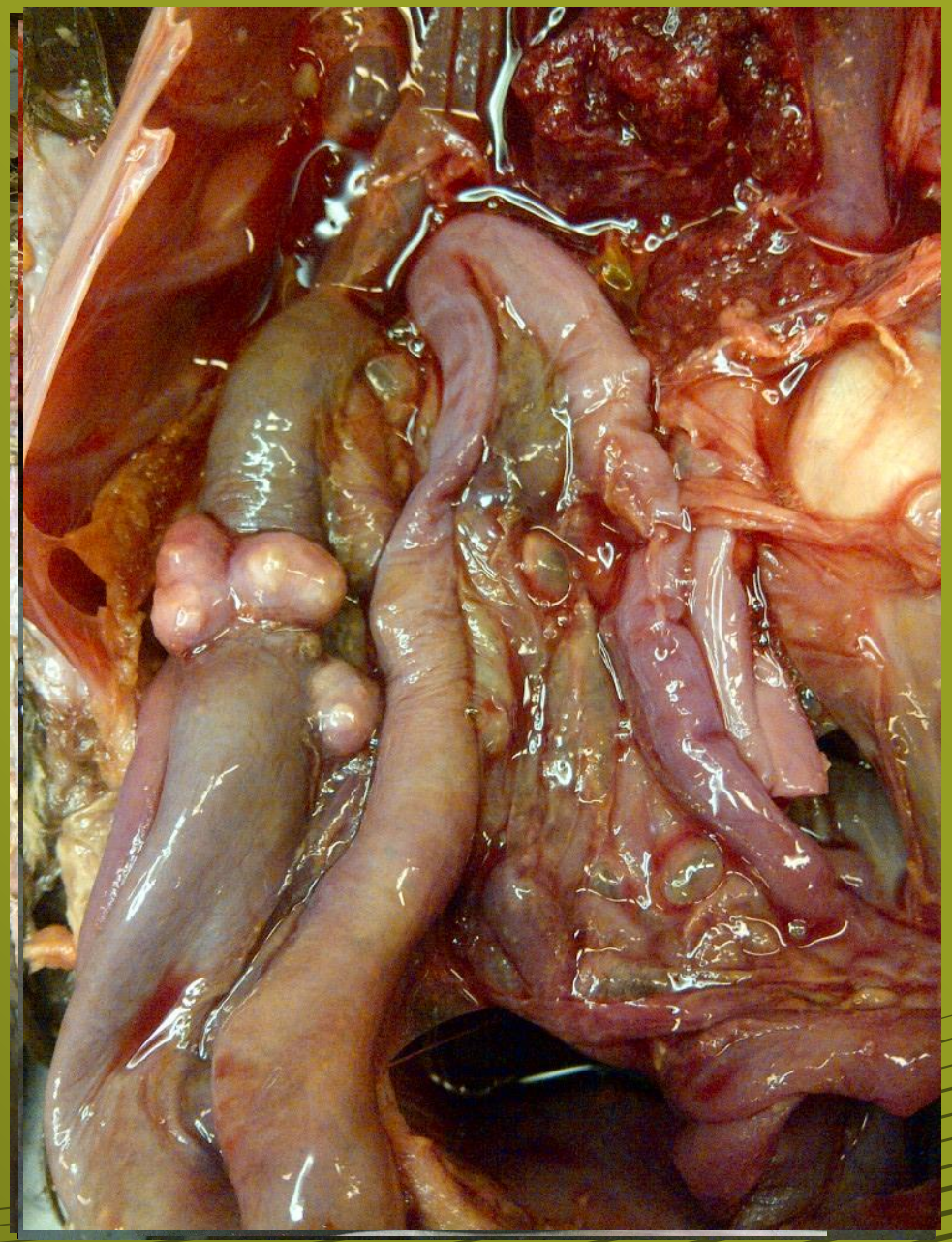
Widespread Infection

Liver

Spleen

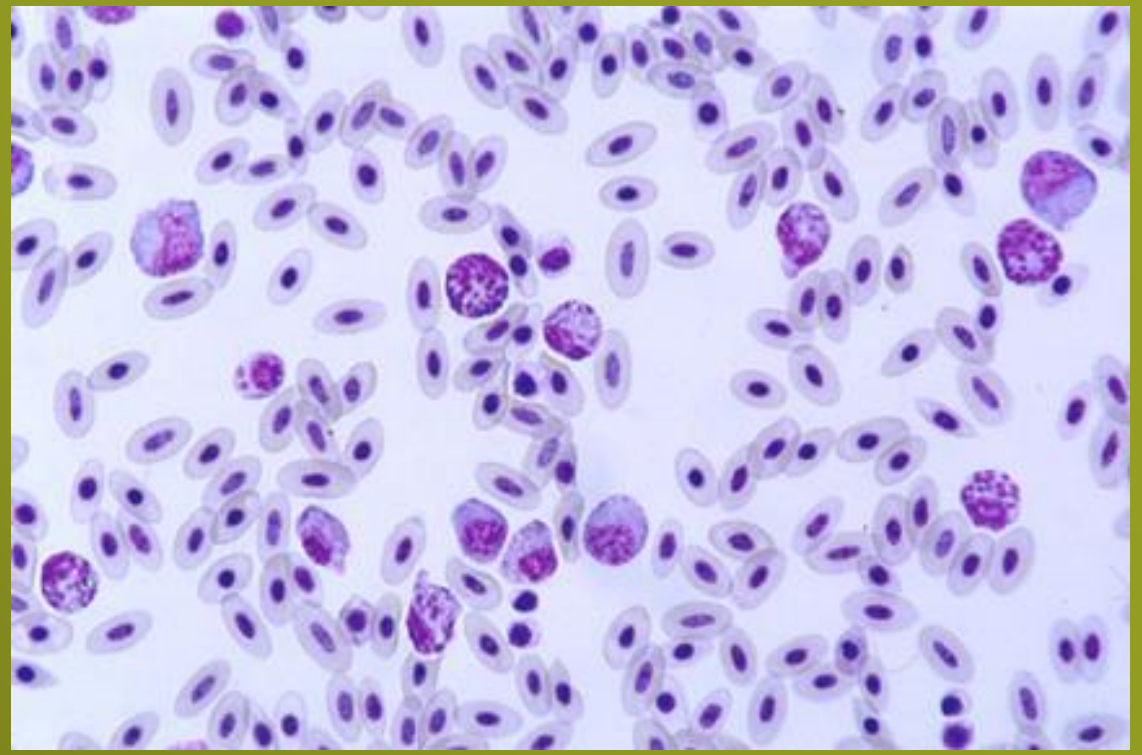
Respiratory

GI



Laboratory Findings/Diagnosis

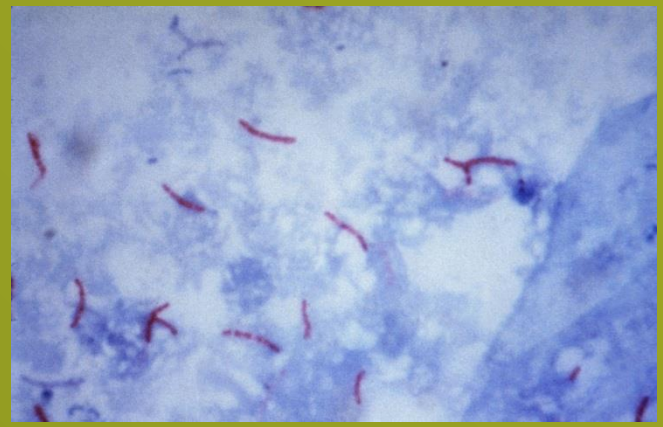
- Very difficult to diagnose in the early stages
  - High levels of organism shedding and environmental buildup occurs before infection is detected
- Increase in WBC 3-6 months prior to death
- Blood chemistry changes are variable based on the organ system involved



Laboratory Findings/Diagnosis

- Radiology
  - Non-specific and insensitive
- Endoscopy
  - More invasive, expensive (equipment, anesthesia, time) can collect samples for further diagnosis, more sensitive (if you know where to look)
- Culture
  - Difficult, time-consuming, and dependent on enough viable organisms being present in the sample
  - Low sensitivity and specificity because of environmental contamination issues and unpredictable organism shedding

https://en.wikipedia.org/wiki/Ziehl%E2%80%93Neelsen_stain



Laboratory Findings/Diagnosis

- Acid Fast Staining
  - Not an effective means of screening for disease
  - Acid-fast fecal screening in one study confirmed only 7% of culture-positive samples [2]
- Serologic Tests (ELISA; Western blot)
  - Have been used with varying success for flock diagnosis but the requirement for species-specific antigens makes widespread use of these tests difficult and costly for large mixed collections [2, 3]
- PCR
  - DNA probes and polymerase chain reaction (PCR) testing are the preferred methods of antemortem and postmortem diagnosis of infected tissues and samples [3]
  - Fecal screening hampered by the common issue of unreliable organism shedding, fecal inhibitors, and environmental contaminants

Treatment

- Many treatment protocols have been attempted but, because of our inability to diagnose disease until late in the course, they result in poor prognosis for long-term survival and…
  - Other negatives:
    - Risk of shedding and increased environmental contamination
    - Cost of treatment
    - Treatment is prolonged and it's impossible to know when to stop
    - Risk of developing resistance
    - Zoonotic risks
- In the end most simply do not choose to treat unless a bird is extremely valuable

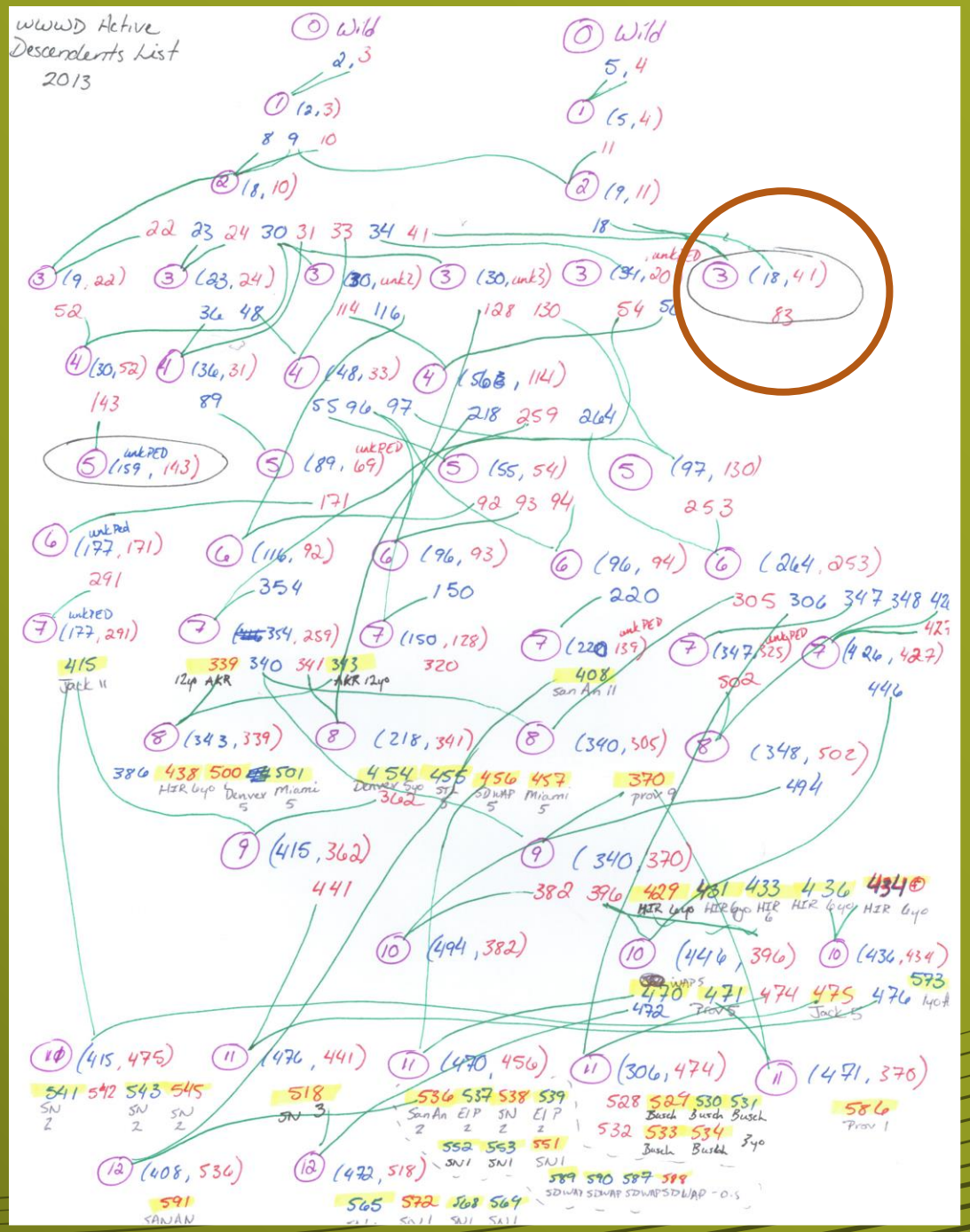
Photo credit: Nick Hill

N. America White-Winged Wood Duck Population Summaries

AS OF 9/15/2015

Where Did It All Begin?

- 2: wild capture, SE Asia, 1969
  - Transfer to Slimbridge 1970
  - Died 1982
- 3: wild capture, SE Asia, 1969
  - Transfer to Slimbridge 1970
  - Died 1974
- Inbred population
  - 12, maybe 13 generations now



Current Institutional Holdings

AZA INSTITUTIONS 21.20.0(41) at 11 Institutions

Institution	Male	Female	Unknown	Other	Total
AKRON	8	7	0	0	15
BUSCH TAM	2	3	0	0	5
DENVER	1	1	0	0	2
EL PASO	2	1	0	0	3
JACKSONVL	1	1	0	0	2
METROZOO	1	1	0	0	2
OMAHA	2	0	0	0	2
PROVIDNCE	1	1	0	0	2
SAN ANTON	1	3	0	0	4
SD-WAP	1	1	0	0	2
ST LOUIS	1	1	0	0	2
TOTALS	21	20	0	0	41

NON-AZA INSTITUTIONS 25.25.0(50) at 4 Institutions

Institution	Male	Female	Unknown	Other	Total
LIVINGRIP	2	0	0	0	2
PINOLA	3	2	0	0	5
SCOT NECK	19	22	0	0	41
STEINHARD	1	1	0	0	2
TOTALS	25	25	0	0	50

Total
46.45.0 (91)

15 Institutions

Annual Census – 20 years

Year	Male	Female	Unknown	Total
2015	46	45	0	91
2014	49	48	0	97
2013	44	40	0	84
2012	40	40	0	80
2011	40	39	0	79
2010	46	59	1	106
2009	49	45	2	96
2008	55	51	2	108
2007	46	42	1	89
2006	43	41	2	86
2005	38	38	3	79
2004	31	32	2	65
2003	31	32	2	65
2002	32	34	2	68
2001	44	55	2	101
2000	51	68	0	119
1999	84	113	0	197
1998	83	101	0	184
1997	86	103	0	189
1996	81	97	0	178
1995	89	94	0	183

Births since 2012

79 at 7 institutions

- Akron 1
- Denver 1
- Pinola 12
- Providence 1
- SanAnton 2
- ScotNeck 53
- SD-WAP 9

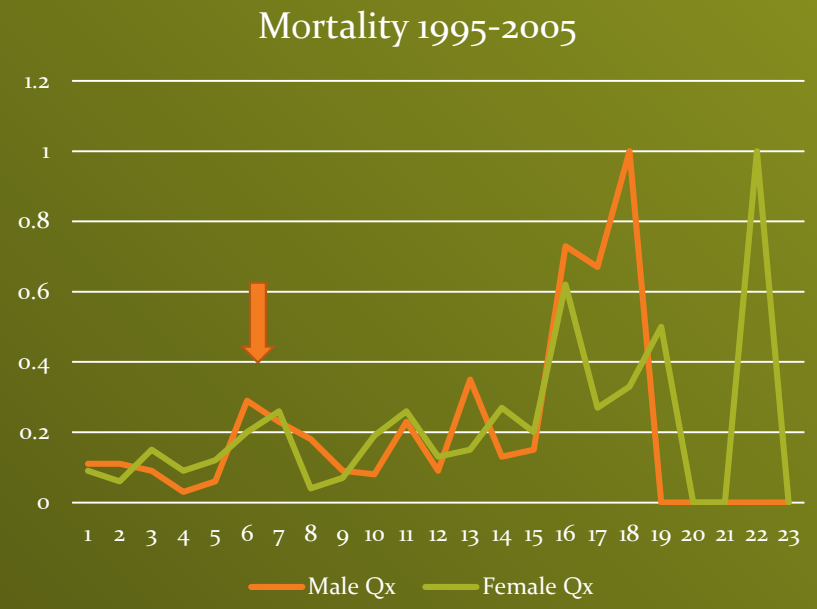
Deaths since 2012

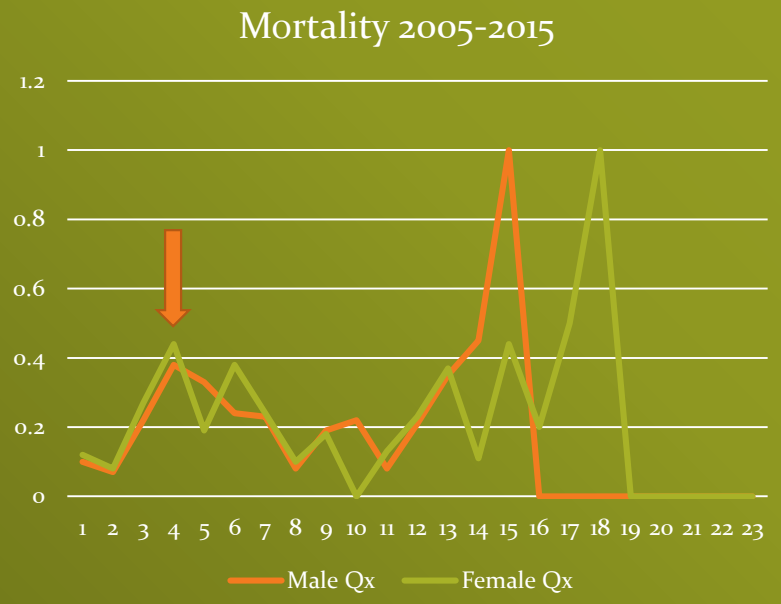
64 at 9 institutions

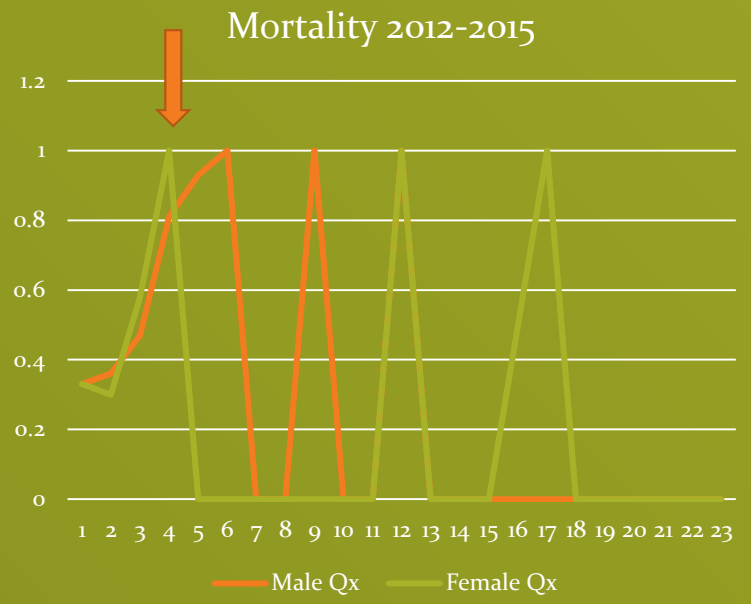
- Akron 1
- Audubon 1
- BuschTam 1
- Providnce 1
- SanAnton 1
- ScotNeck 53
- SD-WAP 1
- SiouxFal 3
- StLouis 2

Causes of Death
Mycobacteriosis x 25
Pending Necropsy x 17
Unknown x 13
West Nile encephalitis x 1

Demographics: Age at Death





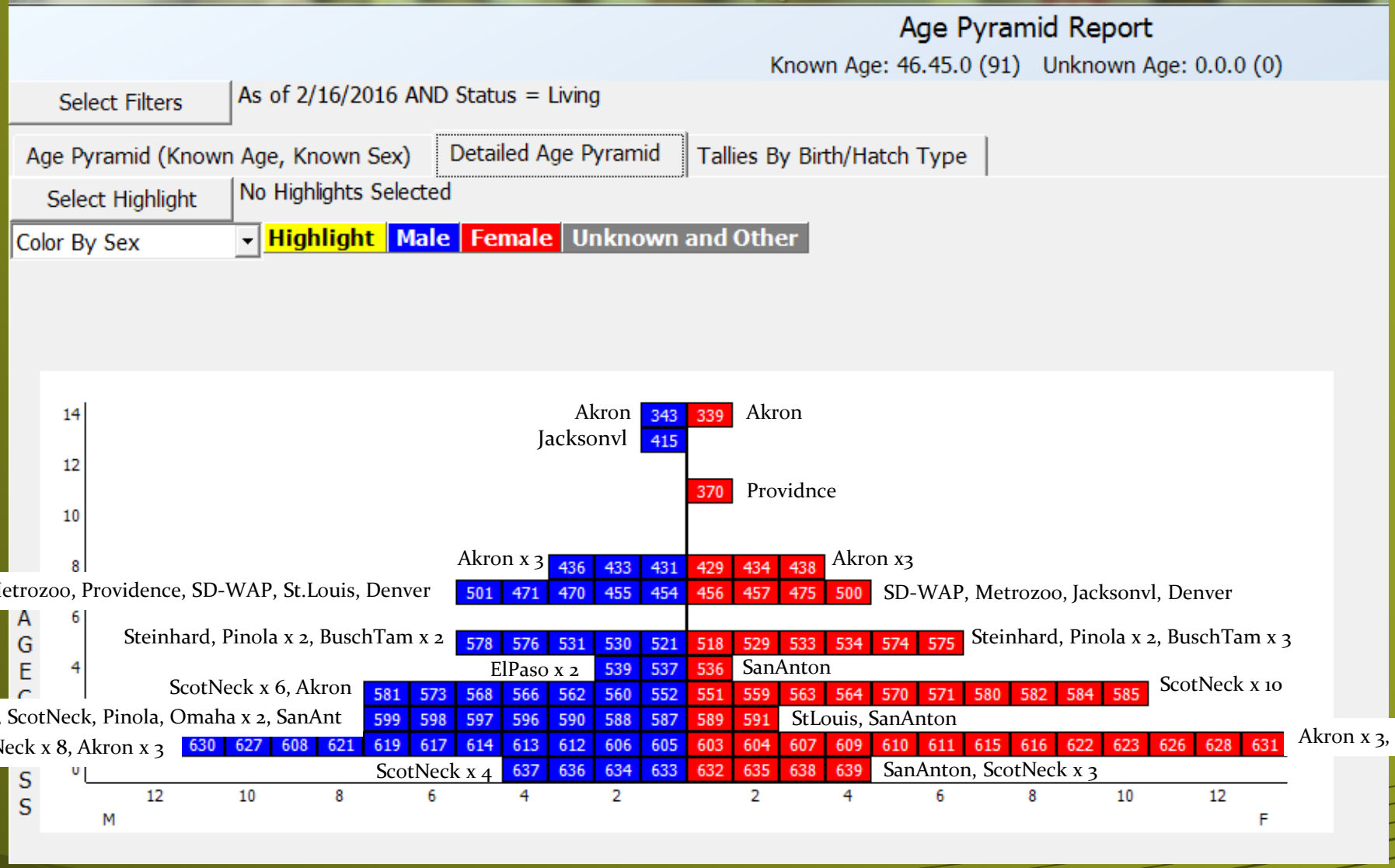


Age Pyramid

Overall
4y or older: 33
3y or younger: 51
Oldest birds: 15 y

AZA
4y or older: 27
3y or younger: 14
Oldest birds: 15 y

Non-AZA
4y or older: 6
3y or younger: 42
Oldest birds: 5 y



Experimental Population Observations

http://dailyalchemy.com/law-attraction-experiment-fail/

Akron Zoo/Hiram College Waterfowl Conservation Facility

“Biosecure” Population vs. “Natural”

- White-winged wood duck population at the Hiram College Field Station
  - Currently 15 birds
    - 15y x 2
      - This pair was at Akron Zoo in small sandy waterfowl exhibit, passerine aviary with a few other ducks, and indoors during the winter until last year
    - 9y x 6
      - These birds were brought to Hiram as eggs and have lived their entire lives in the facility
      - Originally there were x birds brought to Hiram
        - 2 died at the facility: 1 from probable West Nile virus encephalitis and 1 from egg coelomitis. Neither had evidence of mycobacterial infection on necropsy.
    - 4y x 1
      - This bird was born in the facility. The only successful hatch at this facility in 9 years of operating.
    - 2y x 6
      - These birds were born at Sylvan and transferred to the Hiram facility from the concrete ponds in the duckery. They were never outside.

“Biosecure” Population vs. “Natural”

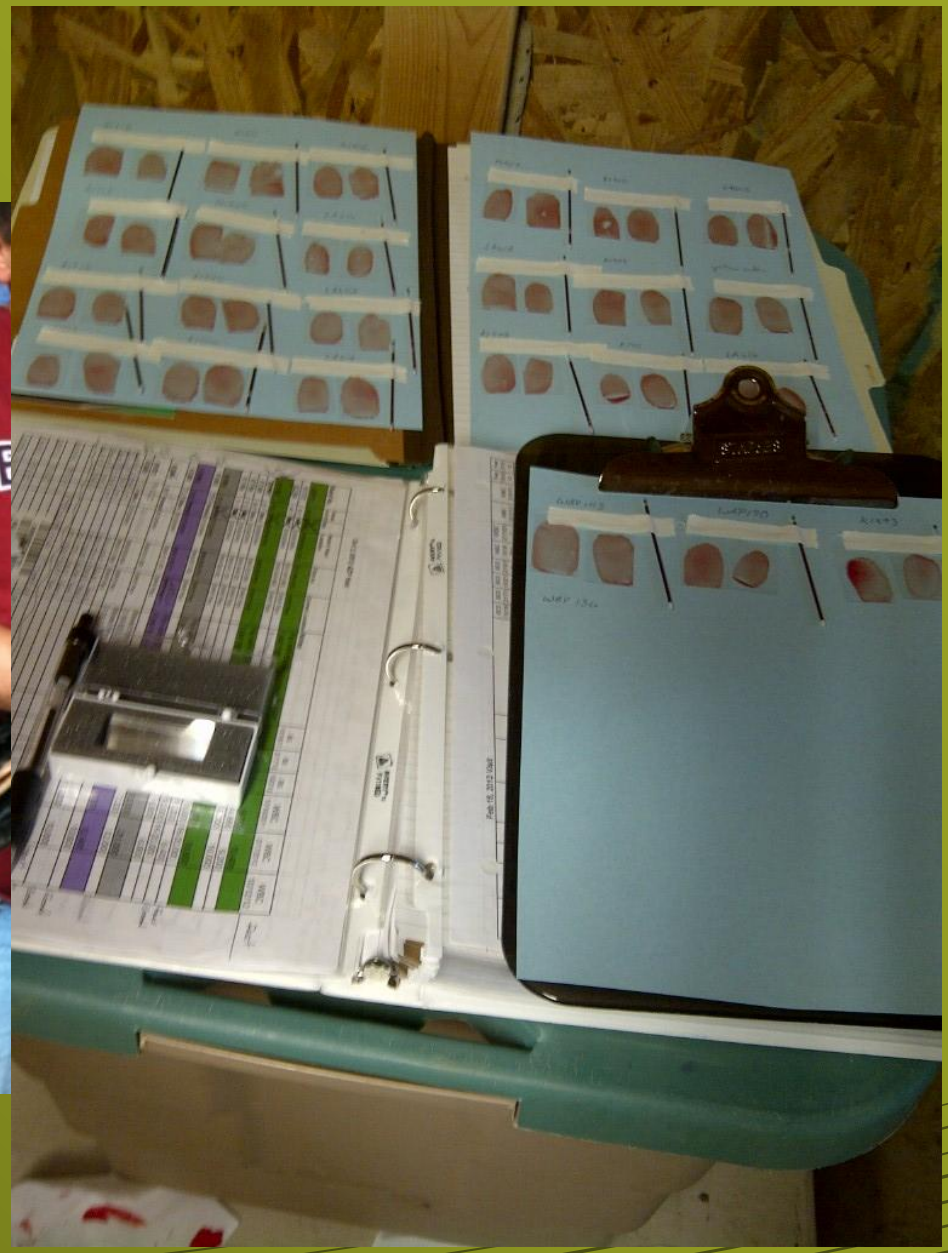
- White-winged wood ducks transferred from the Hiram College Field Station
  - 12 birds have transferred out of the Hiram facility
  - All were hatched in 2008
  - 1 (500) transferred to Denver 11/2010 and is still alive, has a 2 yo descendent
  - 1 (501) transferred to Miami 1/2011 and is still alive, has 1 descendent that died as a neonate

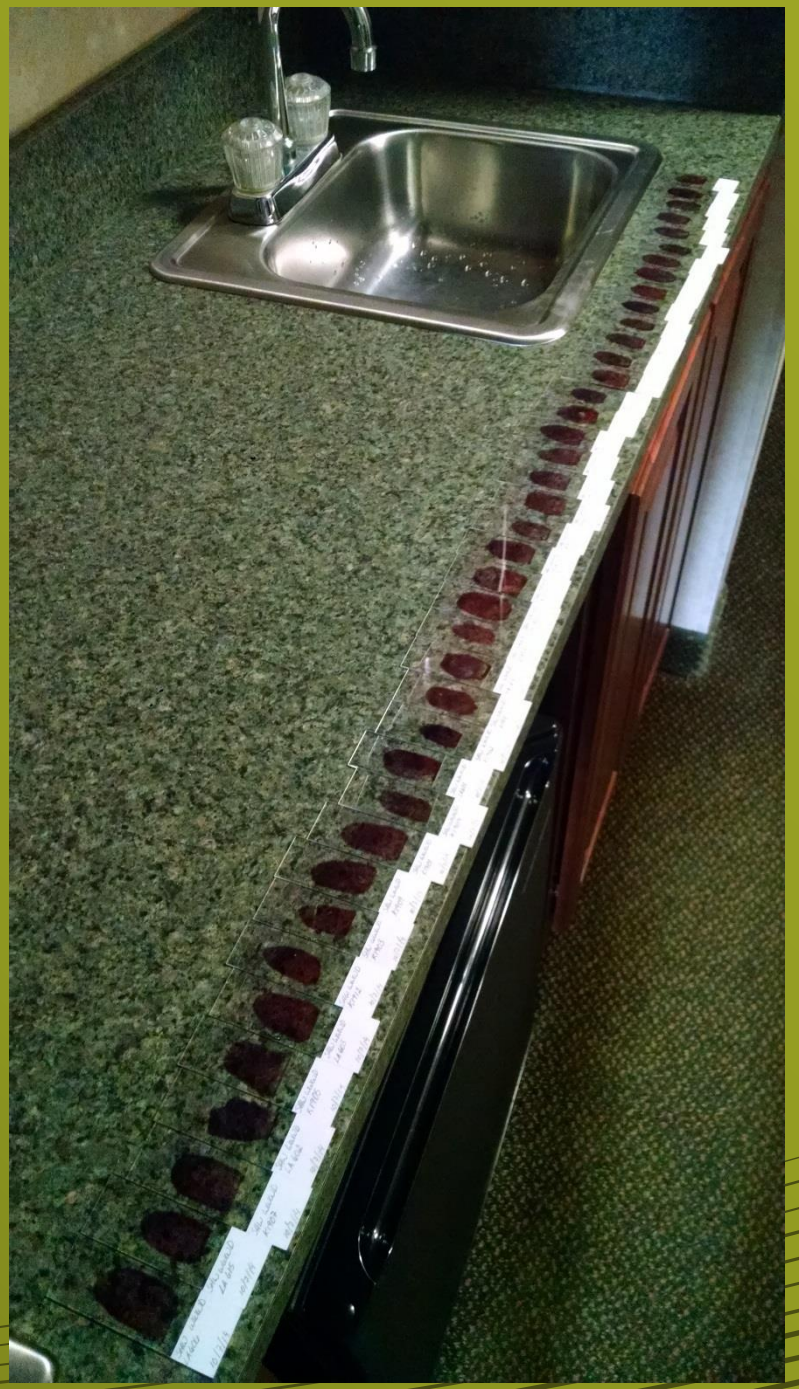
“Biosecure” Population vs. “Natural”

- 10 transferred to Sylvan 5/2010 (when they were 2 years old)
  - 447 – died ~1/2012 (18 mo later), no body for necropsy
  - 448 – euthanized 10/2011 (17 mo later), lesion in back of throat and respiratory distress, severe mycobacteriosis
  - 449 – died 8/2010 (3 mo later), no body for necropsy
  - 452 – Lost to follow up by 2/2011 (9 mo later)
  - 496 – died 9/2011 (16 mo later), mycobacteriosis in respiratory and liver
  - 497 – died ~1/2012 (18 mo yr later), had right eye infection on exam 10/2011, no body for necropsy
  - 498 – died ~6/2012 (24 mo later), mycobacteriosis in respiratory, spleen, and kidneys
  - 499 - died ~1/2012 (18 mo later), no body for necropsy, WBC 10/2011 ~100,000 with BCS 2/5
  - 451 – transferred to Central Park 11/2010 (6 mo later), died 9/2011 (10 mo later), mycobacteriosis
  - 453 – transferred to Central Park 11/2010 (6 mo later), died 7/2011 (8 mo later), mycobacteriosis

Research


POWER PACKER





Previous Research Findings

National Science Foundation: Highlight to Congress

"Undergraduate students undertake conservation efforts to repopulate the endangered White-winged Wood Duck (WWWD) in captivity"
Jody Modarelli, Hiram College, NSF-MRI grant # #1039259 Have Copy

The Relationship Between the IgG immunoglobulins in the Plasma of the Endangered White-Winged Wood Duck and its Susceptibility to Mycobacterium Avium

Audrey Andzelik, Claire McCarthy, Michael Majetich, Jooi Vyas, Abigail Hexamer, Tria Charnas, and Jody Modarelli. The relationship between the IgG immunoglobulins in the plasma of the endangered White-Winged-Wood Duck susceptible to avium TB, 58th Annual ASMS Conference on Mass Spectrometry and Allied Health, Salt Lake City, Utah, May 22nd – May 27th, **2010**. Poster abstract. ASMS

The relationship between the IgG immunoglobulins in the plasma of the endangered White-Winged-Wood Duck susceptible to avium TB" "
Audrey Andzelik; Claire McCarthy; Michael Majetich; Jooi Vyas; Abigail Hexamer; Tria Charnas, Jody Modarelli. Immunity. In preparation
Cannot find in internet search.

Previous Research Findings

A Relationship Between IgY (DFc) and the Endangered White-Winged Wood Ducks' High Susceptibility to Mycobacterium Avium

Tessianna Misko, Phil D'Amico, Sarah Stevens, Claire McCarthy, Jooi Vyas, Michael Majetich, Audrey Andzelik, and Jody Modarelli. A Relationship Between IgY (DFc) and the Endangered White-Wood Winged Ducks High Susceptibility to Mycobacterium Avium, 59th Annual ASMS Conference on Mass Spectrometry and Allied Health, Denver, Colorado, June 4th – June 9th, **2011**. Poster abstract ASMS

Tessianna Misko, **Phil D'Amico**, **Sarah Stevens**, **Claire McCarthy**, **Jooi Vyas**, **Michael Majetich**, **Audrey Andzelik**, **Jennifer Pekar**, **Matthew DiMuzio** and Jody Modarelli. A Relationship Between IgY, IgY(DFc) and the Endangered White-Wood Winged Ducks High Susceptibility to Mycobacterium Avium. Submitted to Chem. Bio. **2012** Cannot find in internet search, have draft

Anita Iveljic- Summer 2012 (Collaborators- Akron Zoo and Sylvan Heights Waterfowl Park):
During the summer of 2011, research students used Matrix-Assisted Laser Desorption Time-of-Flight (MALDI-TOF) mass spectrometry and identified which duck antibody, the truncated IgY (ΔFc) or the untruncated IGY, increases in ducks under two years of age living in a soil-free environment and ducks over the age of two living in a soil-free or soil environment. Based on these findings, Anita isolated the cytokine IL6 from the plasma of ducks living in soil-water, soil, and TB plasma and quantitated the amount of IL6 in each sample via an ELISA assay. The cytokine IL6 is responsible for the inflammatory process and thought to increase the virulence of mycobacterium avium in patients exposed to the bacterium.

Anita Iveljic; Pyi Saw; Jody M. Modarelli. Interleukin 6: A Deterrent or an Indicator of Mycobacterium Infection in the Endangered White-Winged Wood Duck. 60th Annual ASMS Conference on Mass Spectrometry and Allied Health, Minneapolis, Minnesota, June 9th – June 13th, **2013**. Poster abstract ASMS, have draft

Previous Research Findings

The M. Avium Lipid Metabolites in the Endangered White-Winged Wood Duck

Lucas, Heather Balusabramanian, Divya, and Modarelli, Jody M. A conservation study to investigate Mycobacterium Avium lipid metabolites in the endangered White-Winged Wood Duck, 56th ASMS Conference Denver, CO, June 2nd – June 5th, **2008.** Poster abstract. ASMS

Claire. McCarthy; Audrey Anzelik; Michael Majetich; Jooi Vyas; Brittany Palmer; Margaret Pokryfki; Jody M. Modarelli. Identification of lipid metabolites in the plasma of the endangered White-Winged-Wood Duck infected with Mycobacterium Avium. 58th ASMS Conference on Mass Spectrometry and Allied Health, Salt Lake City, Utah, May 22nd – May 27th, **2010**. Poster Abstract ASMS

Sarah Stevens, Phil D'Amico, Tessianna Misko, Jennifer Pekar, and Jody Modarelli. The Search for Lipid Metabolites in the Endangered White Winged Wood Duck, 59th Annual ASMS Conference on Mass Spectrometry and Allied Health, Denver, Colorado, June 4th – June 9th, **2011**. Poster abstract ASMS

Identification of a family of glycosphingolipids in the plasma of the endangered White-Winged-Wood Duck infected with *Mycobacterium Avium*. Claire. McCarthy; Audrey Anzelik; Michael Majetich; Jooi Vyas; Brittany Palmer; Margaret Pokryfki; Jody M. Modarelli. J. of Lipid Research. In preparation. Cannot find in internet search

Previous Research Findings

Identification of a Unique Reverse Micellular Lipid in the Plasma of the Endangered White-Winged Wood Duck

Matt Dimuzio- Summer 2012 (Collaborators- Akron Zoo and Sylvan Heights Waterfowl Park): Hiram College undergraduate students identified during the summer of 2011 fatty acids from parasites found at the soil-water interface of ponds using mass spectrometry. Based on these results, summer 2012 research students grew Protozoa from water samples collected at the Hiram College FS, Akron Zoo, and Sylvan Heights Waterfowl Park and quantitated the amount of parasite in each sample. In addition, student Matt Dimuzio constructed a reed bed with different substrates (gravel, etc.,) as a means of eradicating or reducing the amounts of these organisms. Protozoa harbor MA nicely, and could be a mode of transmission. The study is still in progress.

Matt Dimuzio, Tessianna Misko, Jennifer Pekar, Sarah Stevens, Jennifer Razek and Jody Modarelli. Identification of a Unique Reverse Micellular Lipid in the Plasma of the Endangered White-Winged Wood Duck, 60th Annual ASMS Conference on Mass Spectrometry and Allied Health. Vancouver, British Columbia, Canada. May **2012**. Poster abstract. ASMS

Matt Dimuzio, Tessianna Misko, Jennifer Pekar, Sarah Stevens, Jennifer Razek and Jody Modarelli. Identification of a Unique Reverse Micellular Lipid in the Plasma of the Endangered White-Winged Wood Duck, Submitting to the Jour. Of Lipid Chem. **2012** Cannot find in internet search

Modarelli, Jody M., **Razek, Jennifer Lucas, Heather Tarase, Karly, Kinches, Brian, McCarthy, Claire, Marshall, John Gisemba, Eddie.** A Reverse Micellular Lipid in the Plasma of the Endangered White- Winged Wood Duck Infected with Avium Tuberculosis. Central Regional Meeting of the American Chemical Society, Cleveland, OH, **2009.** Poster abstract.

"A reverse micellular lipid in the plasma of the endangered White-Winged Wood Duck infected with Avium Tuberculosis" Claire. McCarthy; Audrey Anzelik; Michael Majetich; Razek, J.; Lucas, H.; Tarase, K.; Kinches, B.; McCarthy, C.; Marshall, J.; Gisemba, E. J. of Lipid Res. In preparation Cannot find in internet search

Previous Research Findings

Protozoa: A Potential Vector for Mycobacterium Infection in the Endangered White-Winged Wood Duck

Tessianna Misko, Jennifer Pekar, Matthew DiMuzio and Jody Modarelli. Protozoa: A Potential Vector for Mycobacterium Infection in the Endangered White-winged Wood Duck. Submitting to Jour. Of Bio Chem. **2012**
Cannot find in internet search

Reed Bed Filtration

Model Reed Bed System for the Filtration of water in order to reducethe influence of protozoa in regards to Mycobacterium Avium

Matt Dimuzio, Academic Advisor: Dr. Matt Hils, Department of Biology, Supervisor Dr. Jody Modarelli
Source of Funding: Paul and Maxine Frohring Foundation.

Abstract: Conservation efforts by zoos are being made to repopulate the endangered White-winged Wood Duck (WWWD) in captivity, a species highly susceptibility to Mycobacterium Avium (MA), the causative agent of Avium tuberculosis (TB). Changes in the environment can alter water and soil conditions by bringing in foreign debri and non-native organisms like protozoa. Protozoa have been shown to harbor MA and facilitated replication, which would put waterfall at risk that have ingested protozoa. The purpose of this study was to construct a model subsurface horizontal flow reed bed with different substrates to assess whether a reed bed system is effective in reducing or eliminating protozoa found in the water where ducks dabble. Water was collected at the Akron Zoo from the duck deep pool and filtered through the common reed, phragmites australis, in a susbtrate of either gravel or sand/gravel. Samples from unfiltered and filtered water was plated at 6 hours, 12 hours, and 24 hours. Colonies were grown in a CO_2 incubator at 40˚C and 5% CO_2 for approximately four weeks before being extracted and quantified. Preliminary results show that reed plus the sand/gravel substrate was the most effective in reducing the amount of protozoa in water. **(senior apex presentation)**

Current/Future Research Angles

- Reduce Environmental Load
  - Natural reed beds as filtration
  - Other forms of filtration/disinfection that are feasible for waterfowl facilities
    - UV
    - Chemical
  - Periodic exhibit disinfection
    - "Crop rotation"
      - Spring of 2013, SHW park pond was redone

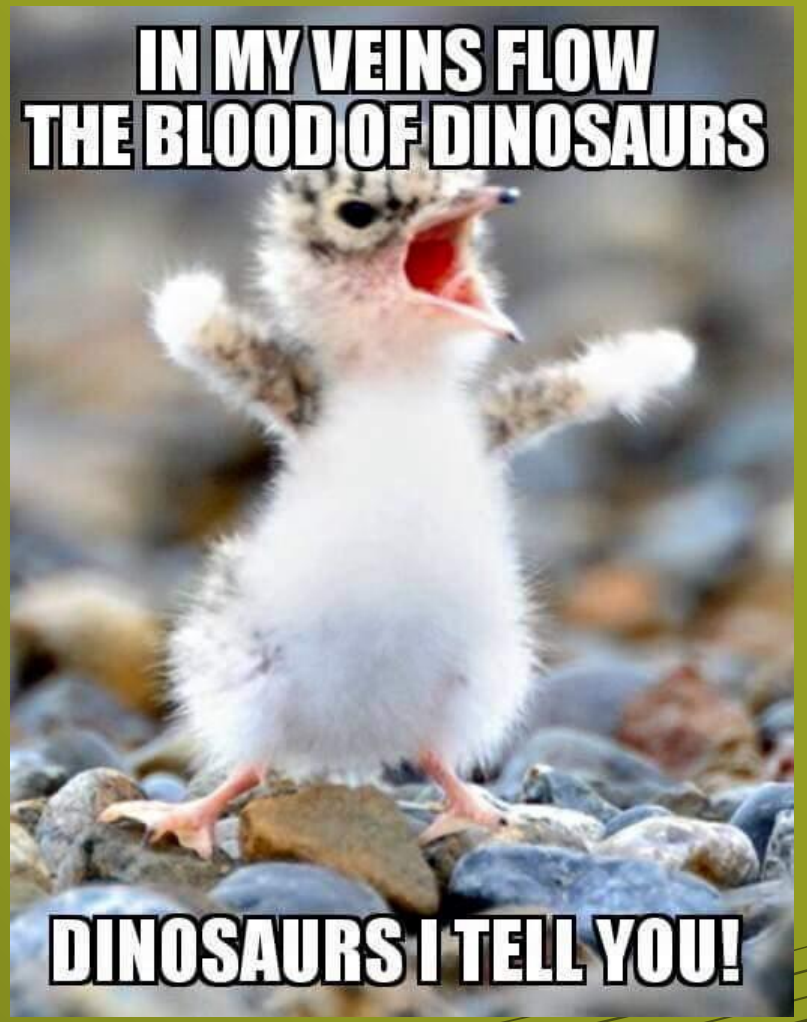
Current/Future Research Angles

- Reduce Susceptibility
  - Role of stress/cortisol
  - Head start to a certain age/immunological status
- Other Factors
  - Role of parasites or other secondary factors that increase virulence of mycobacterium within one environment vs another
- Genetic study that can be compared to European population
  - Role of genetic diversity and extreme susceptibility of certain species

The Future?

Population Management Strategies for Sustainability

- Pinioning?
  - Covered exhibits
- Indoor vs outdoor
  - Disinfectable exhibits
- Smaller, less populated exhibits
  - Spread the population: more institutions
- Moving eggs/birds from brooder tables prior to environmental exposure
  - Head starting in conservation facility
- Remove infected birds as soon as they are identified



That's All Folks!

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3. Miller, R. E., Fowler, M. E., & Fowler, M. E. (2012). *Fowler's zoo and wild animal medicine: Current therapy*. St. Louis, Mo: Elsevier/Saunders.

4. OIE (World Organisation for Animal Health): Manual of diagnostic tests and vaccines for terrestrial animals, 2010 (http://www.oie.int/eng/normes/mmanual/a_summry).

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