Two human cases of encephalitis (August 23)

Active surveillance for human cases initiated (Aug 30)

CDC.gov

Captive species and crows submitted to NVSL (Sep 10)

Dead crows reported (solid line)

Crows submitted (dashed line) and WNV-positive birds (bars)

Starting date of week bird was found

CDC.gov

PCR + for WNV like virus (Sep 23)

Incidence per 100,000
- 0.00
- 0.01 - 0.24
- 0.25 - 0.49
- 0.50 - 0.99
- >=1.00

1999

Incidence per 100,000
- 0.00
- 0.01 - 0.24
- 0.25 - 0.49
- 0.50 - 0.99
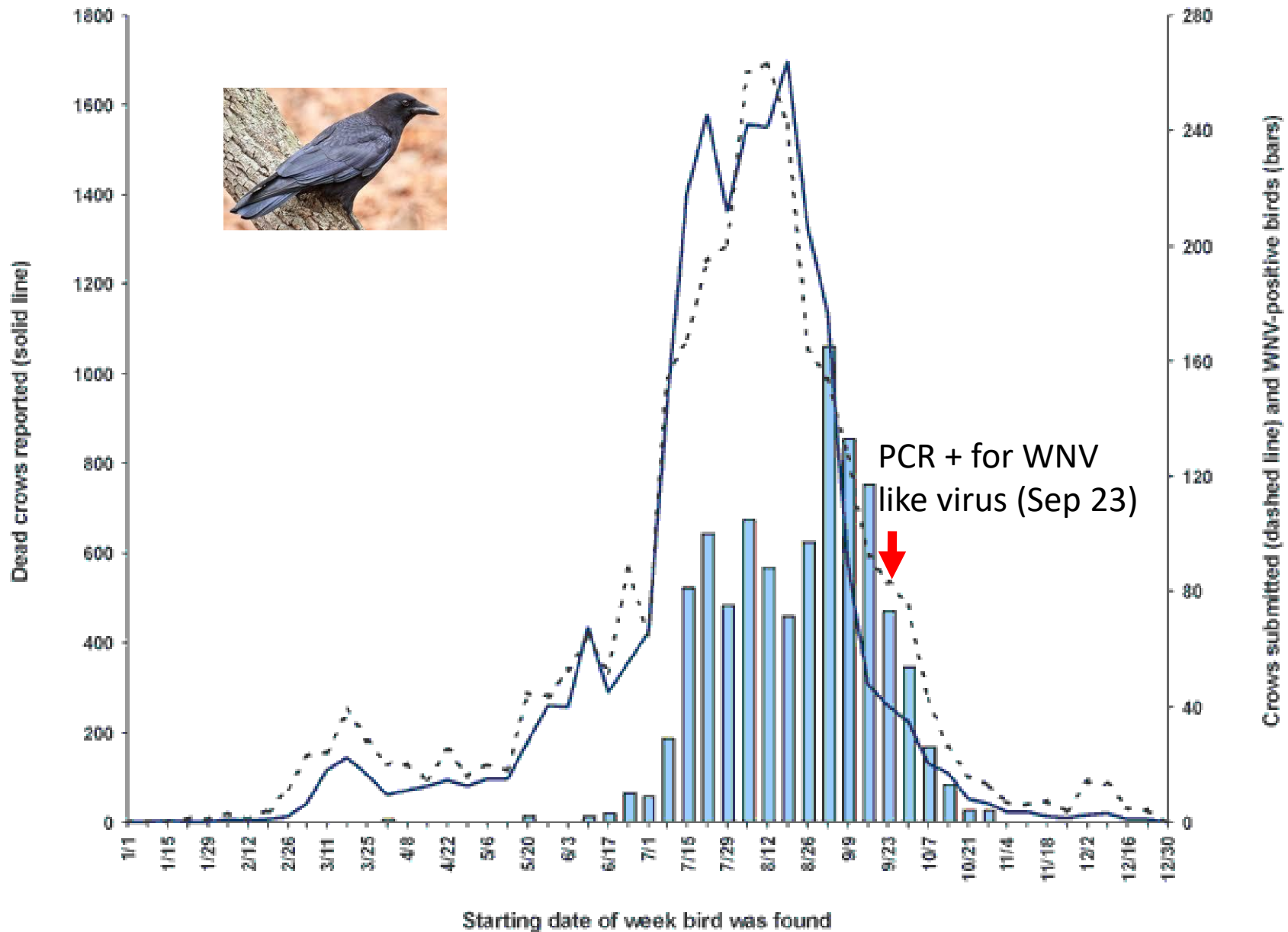- >=1.00

2003

Incidence per 100,000
- 0.00
- 0.01 - 0.24
- 0.25 - 0.49
- 0.50 - 0.99
- >=1.00
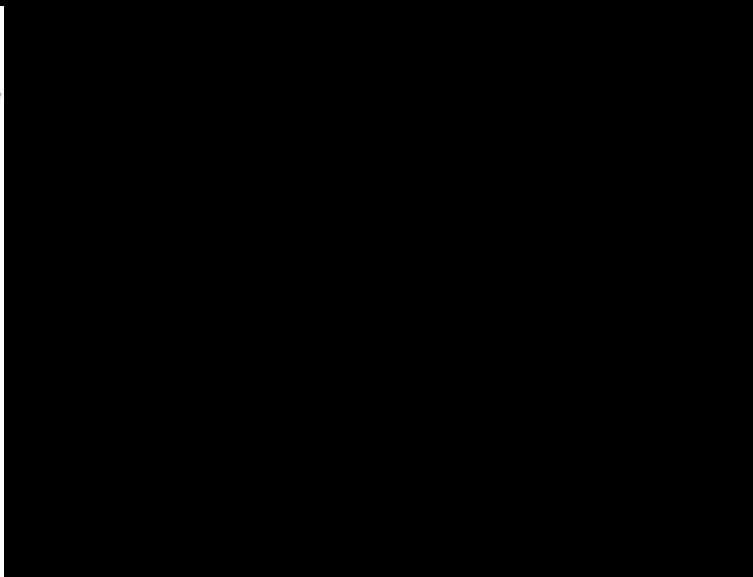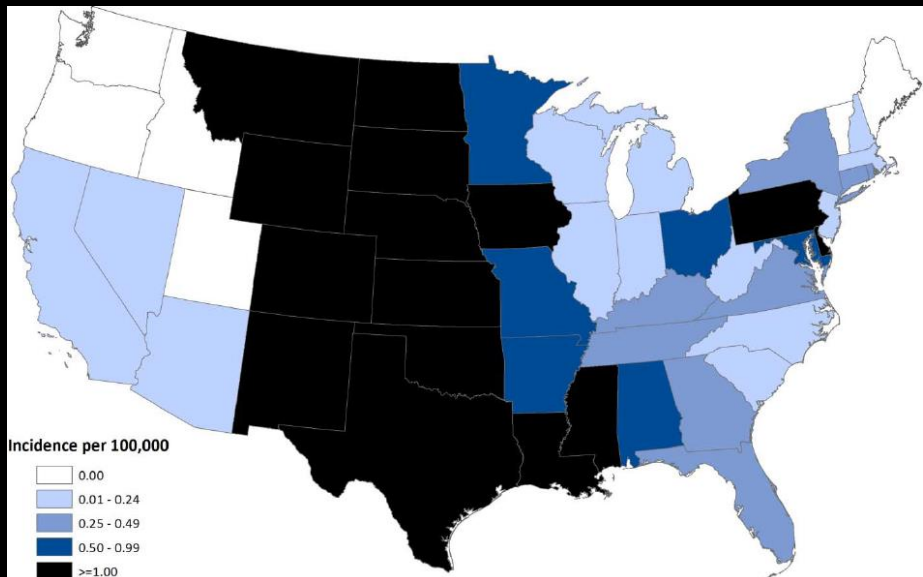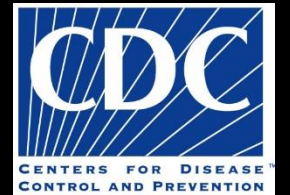
2001

...the following surveillance effort

- Centers for Disease Control: vector surveillance practices were insufficient to detect human and animal health threats from the mosquito-borne West Nile virus

- Lincoln Park Zoo, meeting of the minds

- Zoo was pivotal in WNV identification (McNamara, 2007)

A National Surveillance System for West Nile Virus in Zoological Institutions

The problem
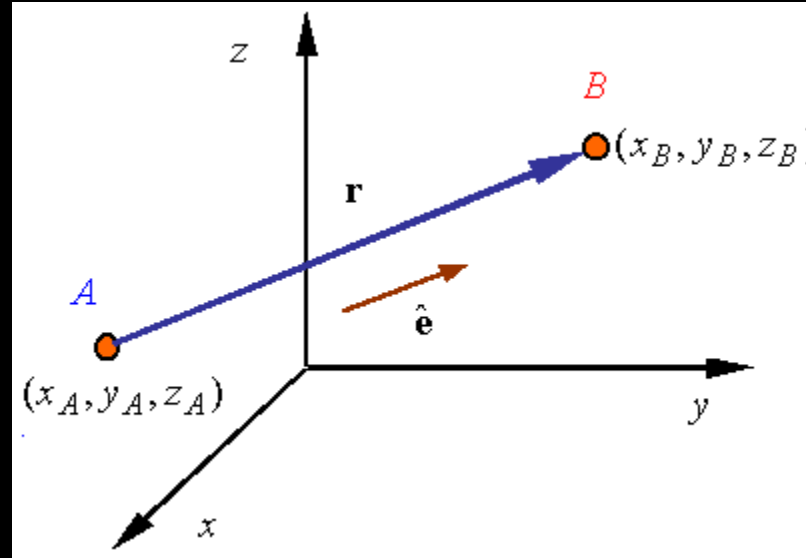
Zoos are **uniquely** suited to serve as biosurveillance platforms

Zoos are also uniquely **prone to have negative impacts** from blood-feeding vectors like mosquitoes

Zoological Parks

- More than 2800 licensed captive animal exhibitors in US

- > 200 are independently accredited
  - Aquarium and Zoo Association
    - Animal conservation programs
    - Public education programs
    - Involvement in Scientific research

The zoo environment

> 750 million annual visitors to zoos world wide

Staff & annual visitors

Native flora & fauna

Captive species
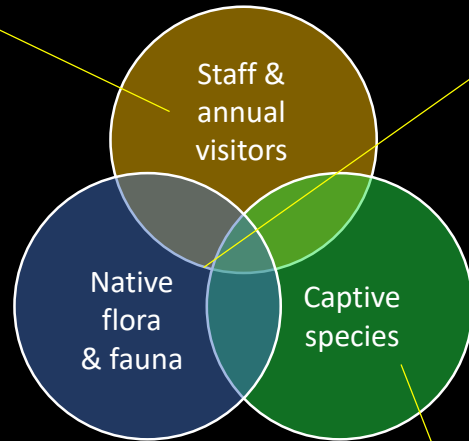
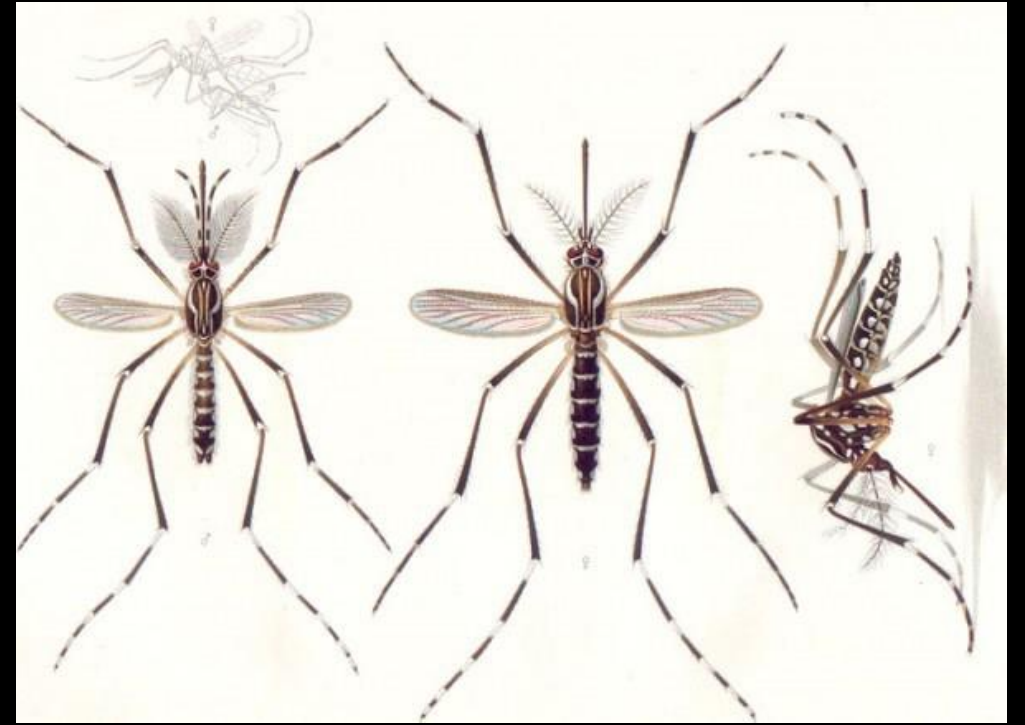**Insects of medico-veterinary importance (e.g., disease vectors):**
→ Ticks
→ Biting flies
→ Biting midges
→ **Mosquitoes**

- Often > 100 species
- Unique enclosures
- Maintained year

Mosquitoes

- Order Diptera (Di = two; Ptera = wings)
  - Family Culicidae
    - ~3500 species worldwide

- Pestiferous (annoyance)

- Most commonly reported insect vector, transmitting pathogens within zoos

Mosquitoes as vectors in zoos

- Viral pathogens
  - E.g., West Nile virus, other encephalitis causing viruses, Dengue virus, new emerging viruses (e.g., Chikungunya, Zika)

- Filarial pathogens
  - E.g., Canine heartworm

- Protozoal pathogens
  - E.g., *Plasmodium* spp. (human and avian malaria), hematoprotozoans

Entire colony of penguins die from avian malaria at Exmoor Zoo

Penguins have been part of the zoo⬀ since it opened in 1982

Vector surveillance in zoos?

- Limited time
- Limited resources
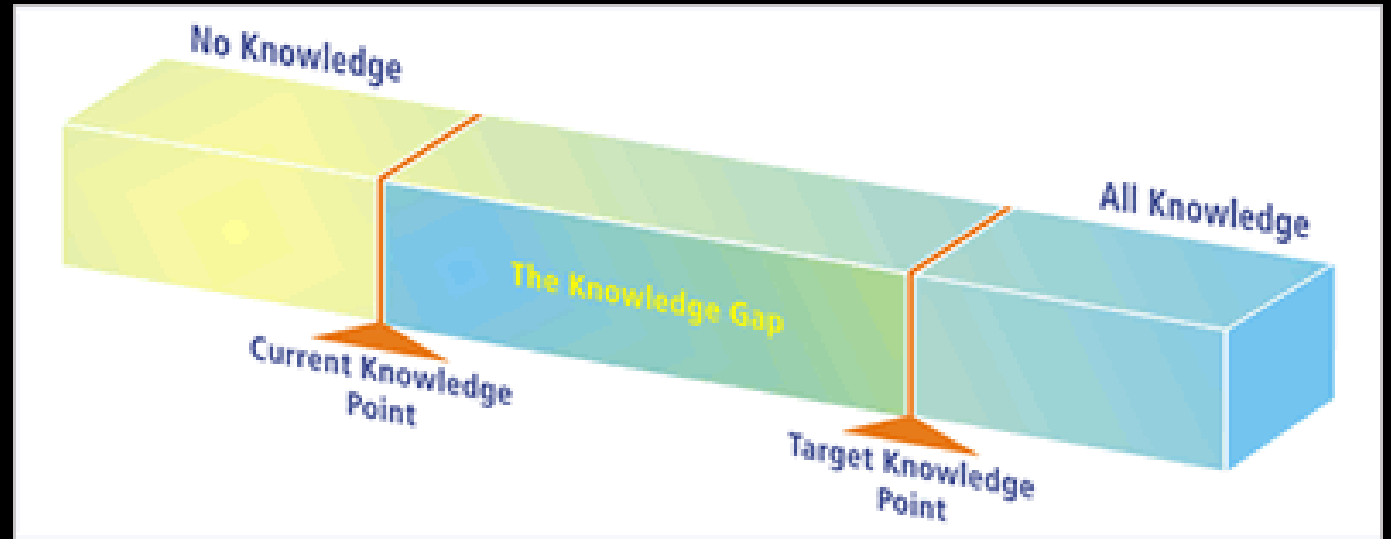
Study goals

1. **Characterize the abundance and diversity** of mosquito species within the Sunset Zoo.

2. **Assess potential risks** to the Sunset Zoo animal species and human visitor.

3. **Develop zoo specific surveillance goals and priorities.**

4. Determine the **minimum surveillance effort required** to make monitoring decisions.

Study location

- The Sunset Zoo (Manhattan, KS), AZA accredited
- More than 300 captive animals representing ~ 100 species

Trapping locations and methods

1. Maintenance building
2. Chimpanzee
3. Maintenance buildings
4. Australian animal enclosures
5. Malaysian Tiger
6. Kansas native animal enclosures
7. Small ruminant and ungulate enclosures
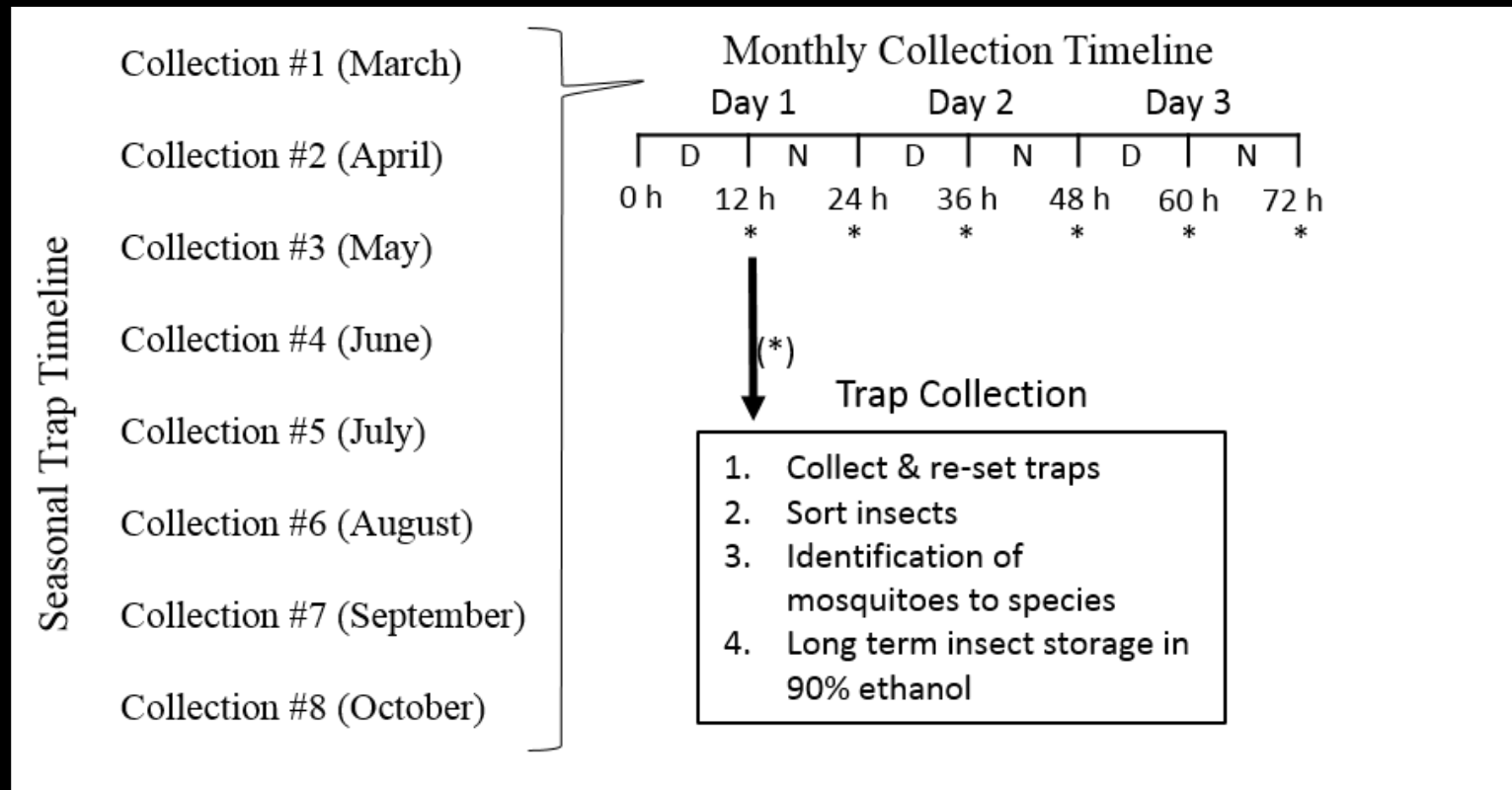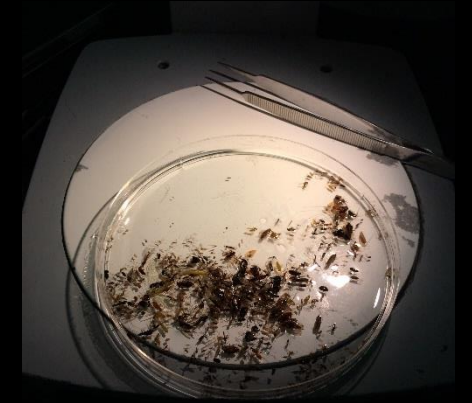8. Playground

Trapping locations and methods

Collection periods

- Goal: capture entire insect vector season
  - Collection initiation: > 15 °C for > 6 h / day
  - Collection termination: < 15 °C for > 6 h / day

Collection processing

Use of data

- Spatio-temporal abundance
  - When and where
  - Biting pressure (bites per unit time)

- Diversity indices to describe mosquito community
  - Diversity as an indicator of disease risk[(Keesing 2006)]
    - Species richness
    - Shannon Weaver index
    - Evenness

Vector-borne disease systems

Host

Pathogen

Vector (competent for pathogen)

Additional competent species

Additional competent species

Additional competent species

$$(H) = -\sum_{i=1}^{s}(p_i)\ln(p_i)$$

$$(E_H) = \frac{H}{\ln(S)}$$

What did we find?!

- 22,652 mosquitoes collected

- Five genera representing twenty species

- 702 trapping periods
  - 356 day periods
    - 6,129 (27.06%)
  - 346 night periods
    - 16,479 (72.75%)

Species	Count Total	Proportion
Aedes albopictus	86.	0.56%
Aedes canadensis canadensis	3.	0.02%
Aedes hendersoni	6.	0.04%
Aedes nigromaculis	275.	1.78%
Aedes triseriatus	14.	0.09%
Aedes trivittatus	25.	0.16%
Aedes vexans	13791.	89.49%
Aedes zoosophus	1.	0.01%
Anopheles barberi	8.	0.05%
Anopheles punctipennis	120.	0.78%
Anopheles quadramaculatus	63.	0.41%
Anopheles walkeri	4.	0.03%
Culex erraticus	89.	0.58%
Culex quinquefasciatus	1.	0.01%
Culex restuans	272.	1.77%
Culex salinarius	2.	0.01%
Culex tarsalis	478.	3.10%
Culiseta inornata	164.	1.06%
Psorophora ciliata	7.	0.05%
Psorophora longipalpus	1.	0.01%
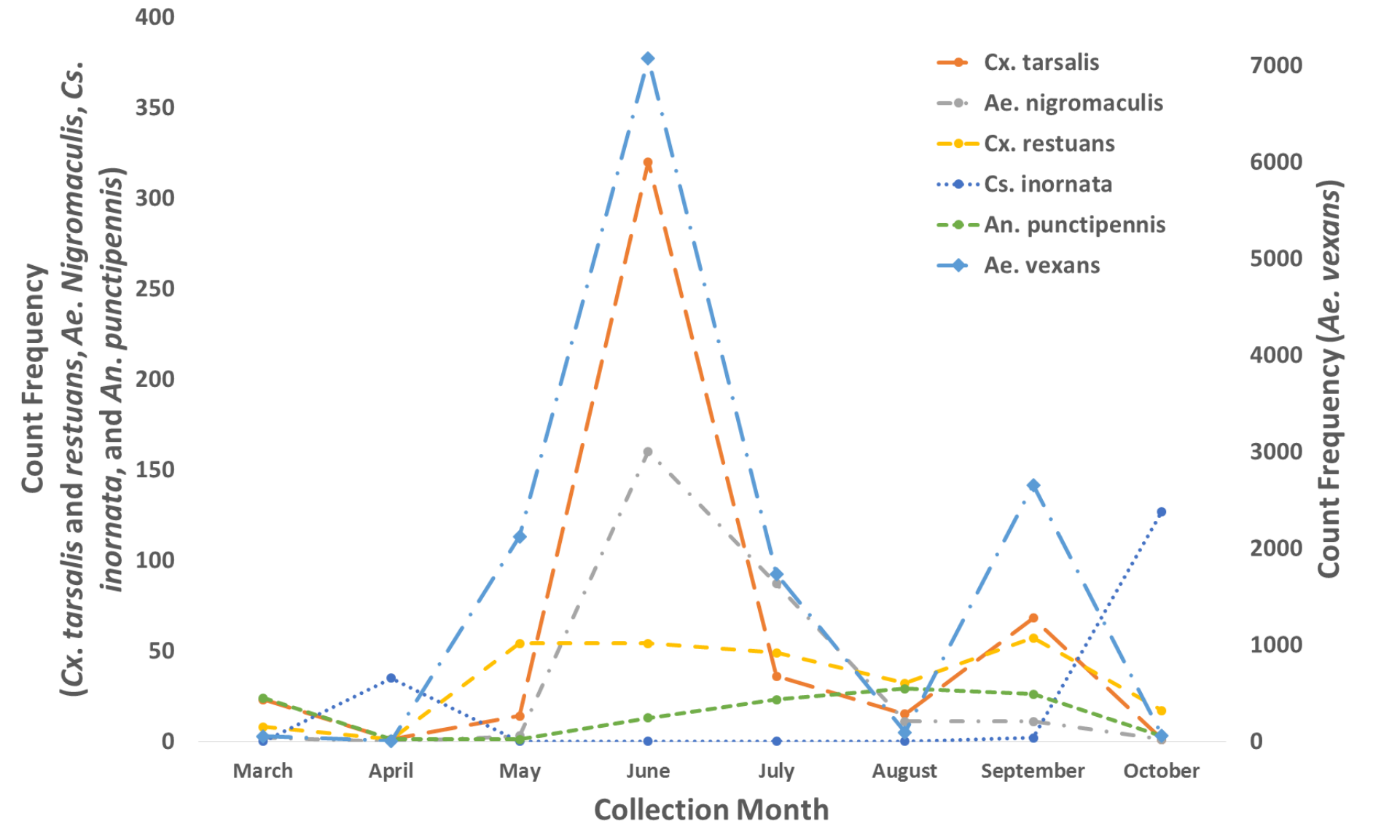
Diversity indices by location

Indices[a] and location	Location							
	1	2	3	4	5	6	7	8
Abundance	2033	2832	602	2297	2422	3162	1166	852
H	0.60	0.55	0.96	0.47	0.31	0.35	0.79	0.90
E_H	0.23	0.22	0.40	0.18	0.12	0.13	0.29	0.36
S	14	12	11	14	14	14	16	12
$S_{proportion}$	70%	60%	55%	70%	70%	70%	80%	60%

Mosquito abundance and biting pressures?

- Highest density of mosquitoes (1,749 collected over 3 day period)
  - When – June
  - Where - <u>KS native species</u>
- Considerations:
  - Mosquito density in zoos[Derraik 2004]
  - Trap attractiveness range (30 m)[Service 1993]
  - Trap sensitivity to abundance[Cooperband 2006]

"True" mosquito abundance may be estimated to be 5-10x recorded values

8,745 – 17,490   = 12-24 mosquitoes/m^2

Making this more relatable → this room: ~ 54 m^2 → 650-1292

Impacts of captivity and biting insects

- Captive wild animals have elevated corticosteroid hormone levels

- Animals afflicted by biting insects and ecto-parasitism
  - Defensive behaviors:
    - Birds: stomping, wing shakes, and head movements
    - Other mammals: ear flicking, muscle twitching, leg stomping, and tail switching
    - All species:
      - Increased activity with decreased time at rest
      - Microhabitat selection (i.e., fleeing the insects)
      - Decreased food intake and weight of gain (cattle)
      - Decreased play behaviors
  - Energetic costs
  - Increased immune activity
  - Elevated corticosteroid hormones

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Corticosteroids and mosquitoes

- Mechanisms of host selection by mosquitoes:
  - Carbon dioxide
  - Body temperature
  - Odor (sweat)

- Animals with increased [CCS] are twice as likely to be bitten a mosquito (Gervasi 2017)

CO2   Odors   Heat   Host

Distance
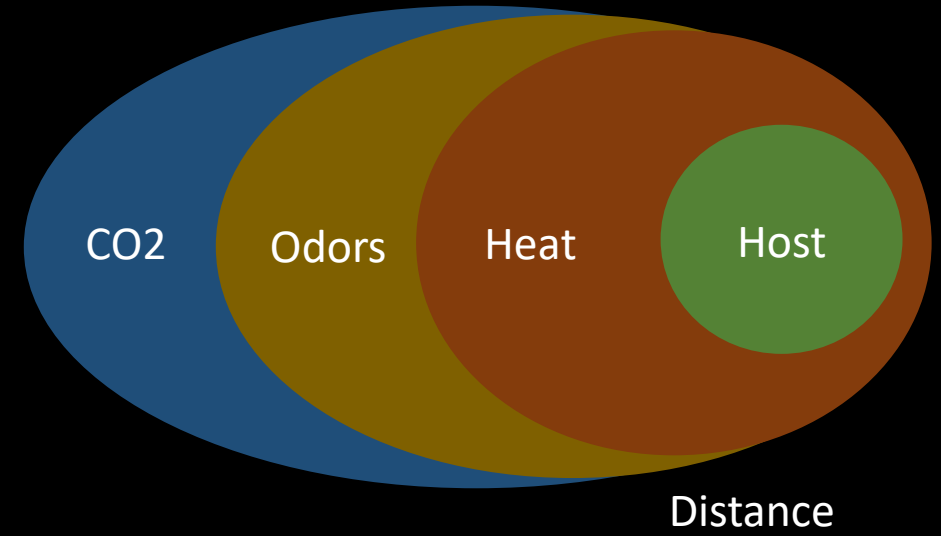
Diversity indices ...an indicator for disease risk?

- Species richness
  - 20 species identified in less than a 0.2 km^2 area
    - <u>Ungulate enclosures</u>
  - North-central KS [Ganser 2013]
    - 11 species identified over a 280 km^2 area

- Low relative evenness (highest: 0.4)

- Highest diversity (and evenness)
  - <u>Maintenance area</u> and <u>children's playground</u>

# Diversity indices ...an indicator for disease risk?

- The zoo environment
  - Numerous microhabitats within zoo [Adler 2011, Tuten 2011]
    - Year-round maintained indoor enclosures
    - Maintenance facilities (e.g., artificial breeding grounds)
    - Diverse animal enclosures
      - Forested
      - Water features

Priority species in the Sunset Zoo

| Priority species	Abundance (count)	Known pathogen competency[b] (reported in KS)
Aedes albopictus	86	SLEV, WEEV, WNV
Aedes triseriatus	14	WNV
Aedes vexans	13,791	*Haemoproteus* spp., *D. immitis*, EEEV, SLEV, WEEV, WNV, *Plasmodium* spp.
Anopheles punctipennis	120	*Haemoproteus* spp., *D. immitis*, WNV, *Plasmodium* spp.
Culex restuans	272	WEEV, WNV, *Plasmodium* spp.
Culex tarsalis	478	EEEV, SLEV, WEEV, WNV, *Plasmodium* spp.
Culiseta inornata	164	SLEV, WEEV, WNV

[a] St. Louis encephalitis virus (SLEV), Western equine encephalitis virus (WEEV), West Nile virus (WNV), and Eastern equine encephalitis virus (EEEV).
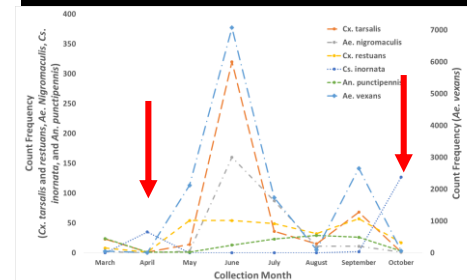
Recommendations for the Sunset Zoo and others

1. **Initial zoo wide survey recommended** if a zoological institution is interested in initiating surveillance
    - Describe mosquito community within zoo
    - Identify when and where highest risk is present
    - Identify peak diversity locations

2. Targeted monitoring → **directed towards zoo specific issues and priorities**
    - Priority species
    - High density, high diversity, and species rich locations
    - During peak abundance months

3. **Training programs**
    - Detection of biting insect specific defensive behaviors
    - Identification of key microhabitat breeding locations

4. **Collaborative relationships**

Role as regional biosurveillance platforms
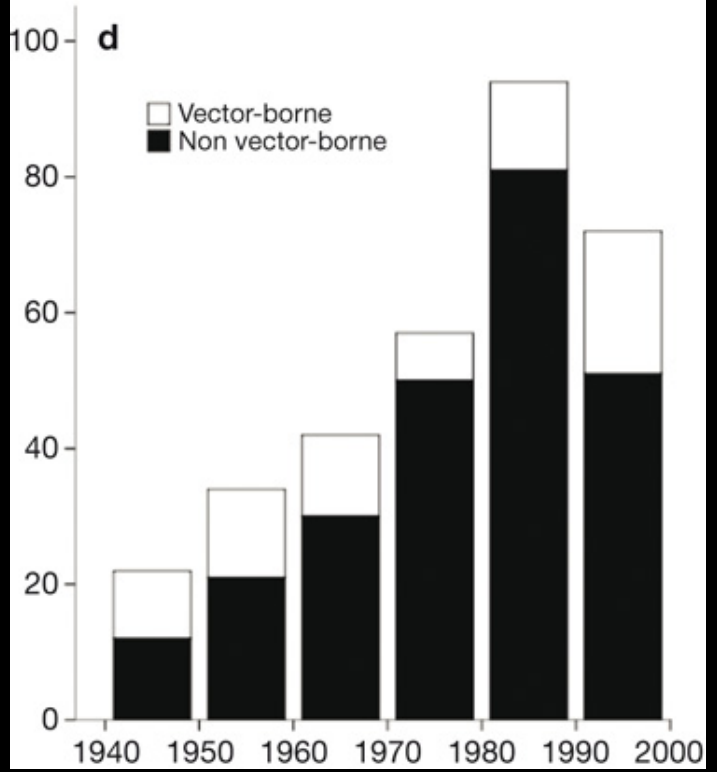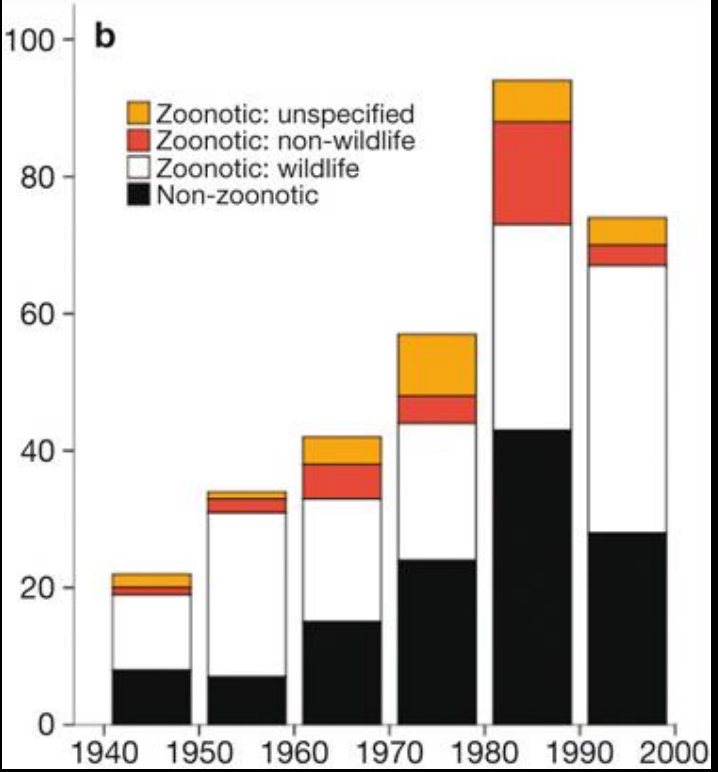
Acknowledgements

- Dr Lee Cohnstaedt
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Questions?