Quantifying *Culex quinquefasciatus* host feeding preference in semi-natural experimental enclosures

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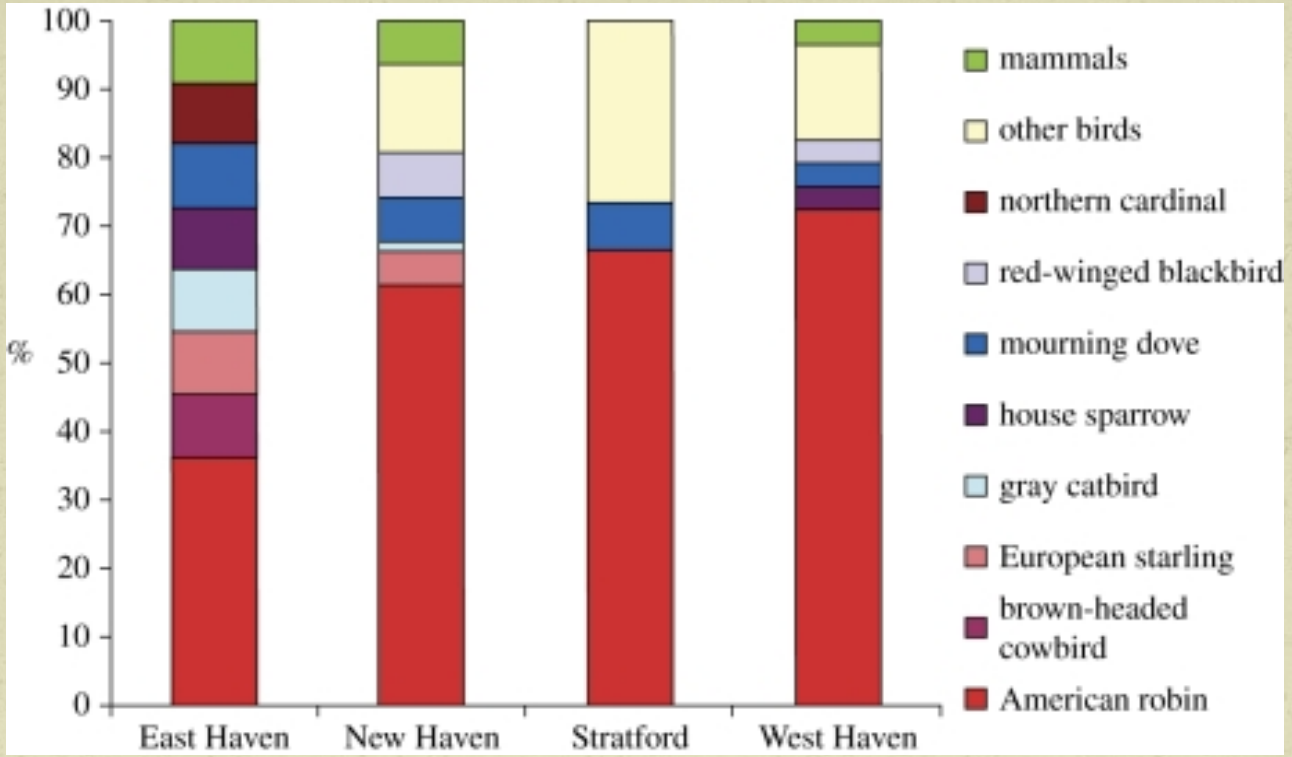
GEORGIA MOSQUITO CONTROL ASSOCIATION

Outline

- Background
  - Mosquito host feeding preferences – from the field
  - Recent proposals & experiments
  - Host defensive behaviors and mosquito feeding success
- Experimental Focus & Design
- Results
  - Blood feedig success
  - Blood source
  - Defensive behaviors
- Conclusion & Future Directions

Field studies of WNV transmission



"...measured strong feeding preferences for American robins (Turdus migratorius) by Cx. pipiens, quantified as the proportion of Cx. pipiens blood meals from robins in relation to their abundance (feeding index)."

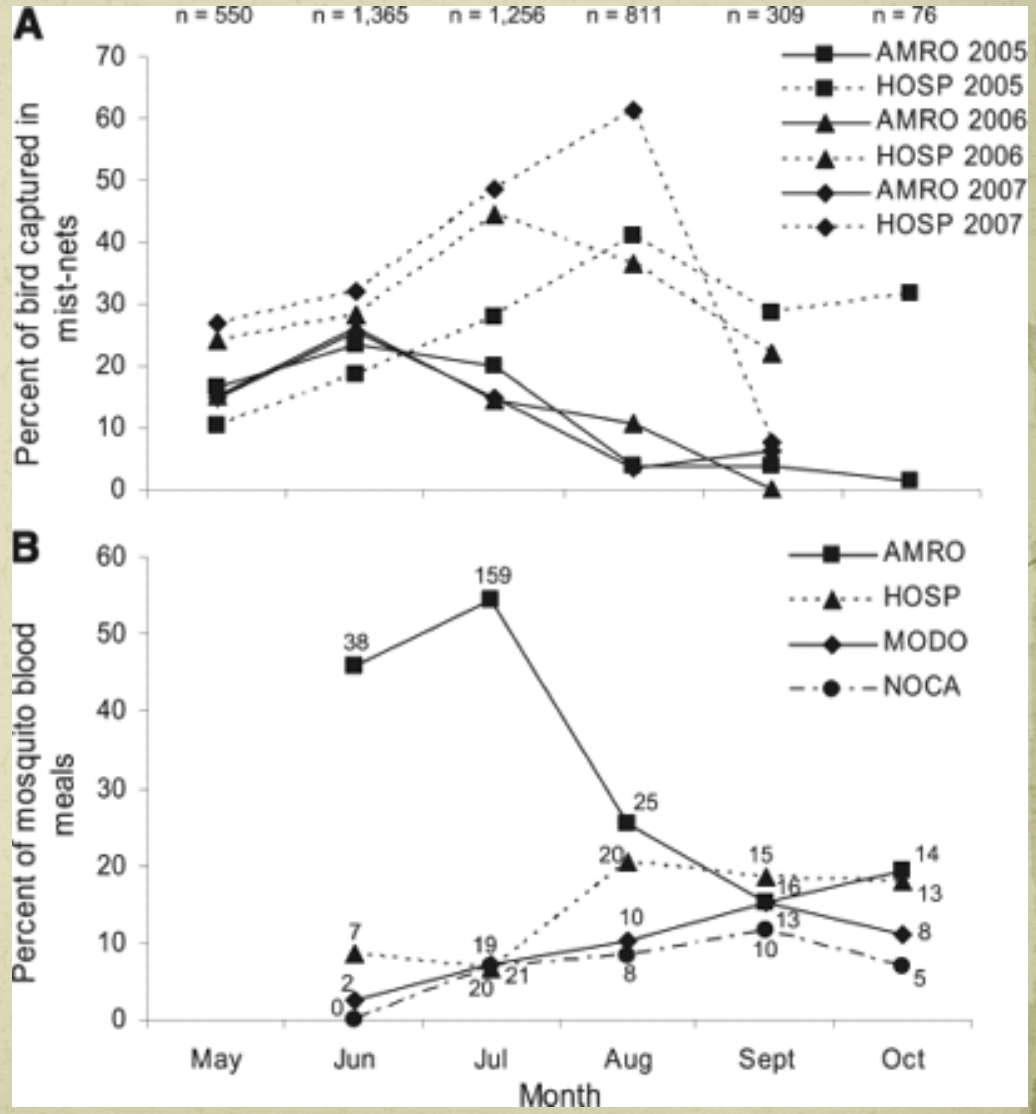
– Vector host-feeding preferences drive transmission of multi-host pathogens: West Nile virus as a model system, Jennifer E. Simpson[1,2], Paul J. Hurtado[3], Jan Medlock[4], Goudarz Molaei[5], Theodore G. Andreadis[5], Alison P. Galvani[1] **and** Maria A. Diuk-Wasser[1,*]

Field studies of WNV transmission

Late season shift to other avian species (not mammalian species)

Robins over utilized species, due to:

roosting behavior
nesting
defensive behaviors



Host Selection by *Culex pipiens*Mosquitoes and West Nile Virus Amplification Gabriel L. Hamer*, Uriel D. Kitron, Tony L. Goldberg, Jeffrey D. Brawn, Scott R. Loss, Marilyn O. Ruiz, Daniel B. Hayes and Edward D. Walker

Field studies of mosquito avian preferences

- Kilpatrick et al. 2006: American Robins (*Turdus migratorius*)
  - Over-utilized host for *Culex spp.* mosquitoes

Illustration by H. Douglas Pratt, National Geographic

- Gibbs et a. 2004: Northern Cardinals (*Cardinalis cardinalis)*
  - Highest seroprevelance among birds in GA
- Wu and Levine (unpublished) – ATL, GA
  - Cardinals make up the majority of blood fed mosquitoes collected from the field

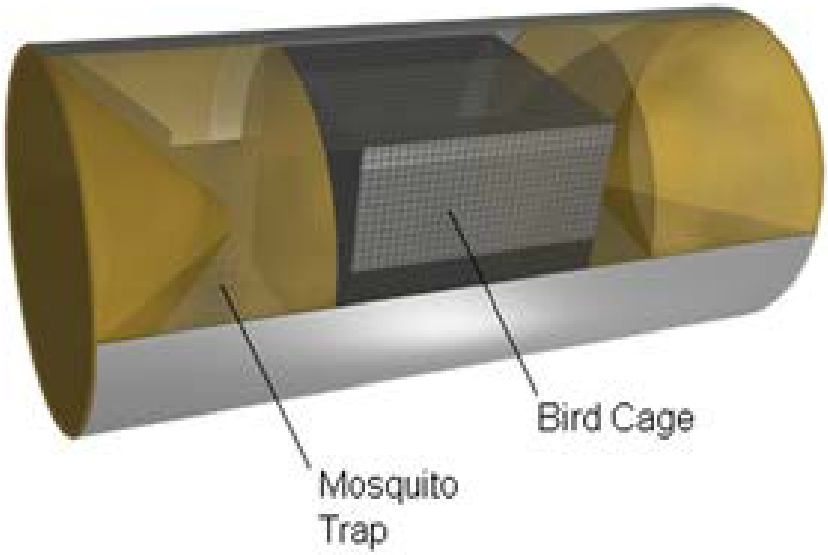
Simpson 2009: **Avian Host-Selection by Culex pipiens in Experimental Trials**

Tested *Cx. Pipiens pipiens* preference for American Robins with the use of "lard can" traps

Mosquitoes more likely to be captured in an American Robin baited trap

A

B



Mosquitoes have greater success feeding on unrestrained vs. restrained hosts

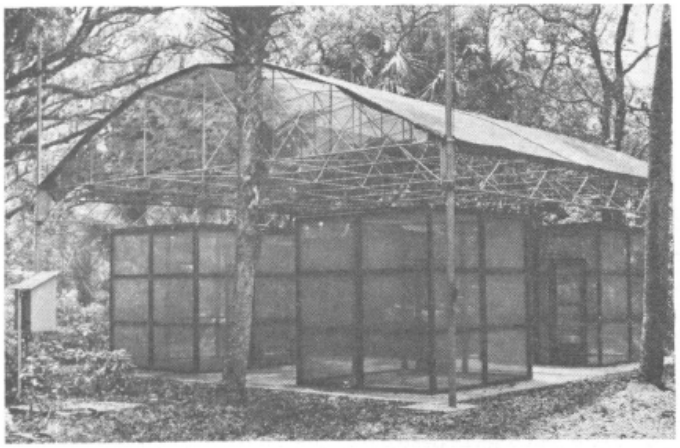


FIG. 1.—Experimental aviary for study of the influence of host behavior on feeding success of mosquitoes.

Table 3.—Feeding of mosquitoes on adult herons physically restrained and unrestrained.[a]

Host	Restrained			Unrestrained		
	Recovered (%)	Engorged (%)	Incomplete (%)	Recovered (%)	Engorged (%)	Incomplete (%)
Night heron	85	69	0	87	98	<1
Green heron	96	89	0	94	92	0
Little blue heron	91	76	0	75	8	6
White ibis	93	96	0	66	12	8
Cattle egret	93	66	1	71	15	13
Snowy egret	92	78	1	76	13	14

[a] Each bird was exposed for 4 hr (commencing ½ hr after sunset) with 300 *C. nigripalpus;* 1 night restrained and 1 night unrestrained.

Edman, J. D. and H. W. Kale (1971). "HOST BEHAVIOR - ITS INFLUENCE ON FEEDING SUCCESS OF MOSQUITOES." Annals of the Entomological Society of America **64**(2)

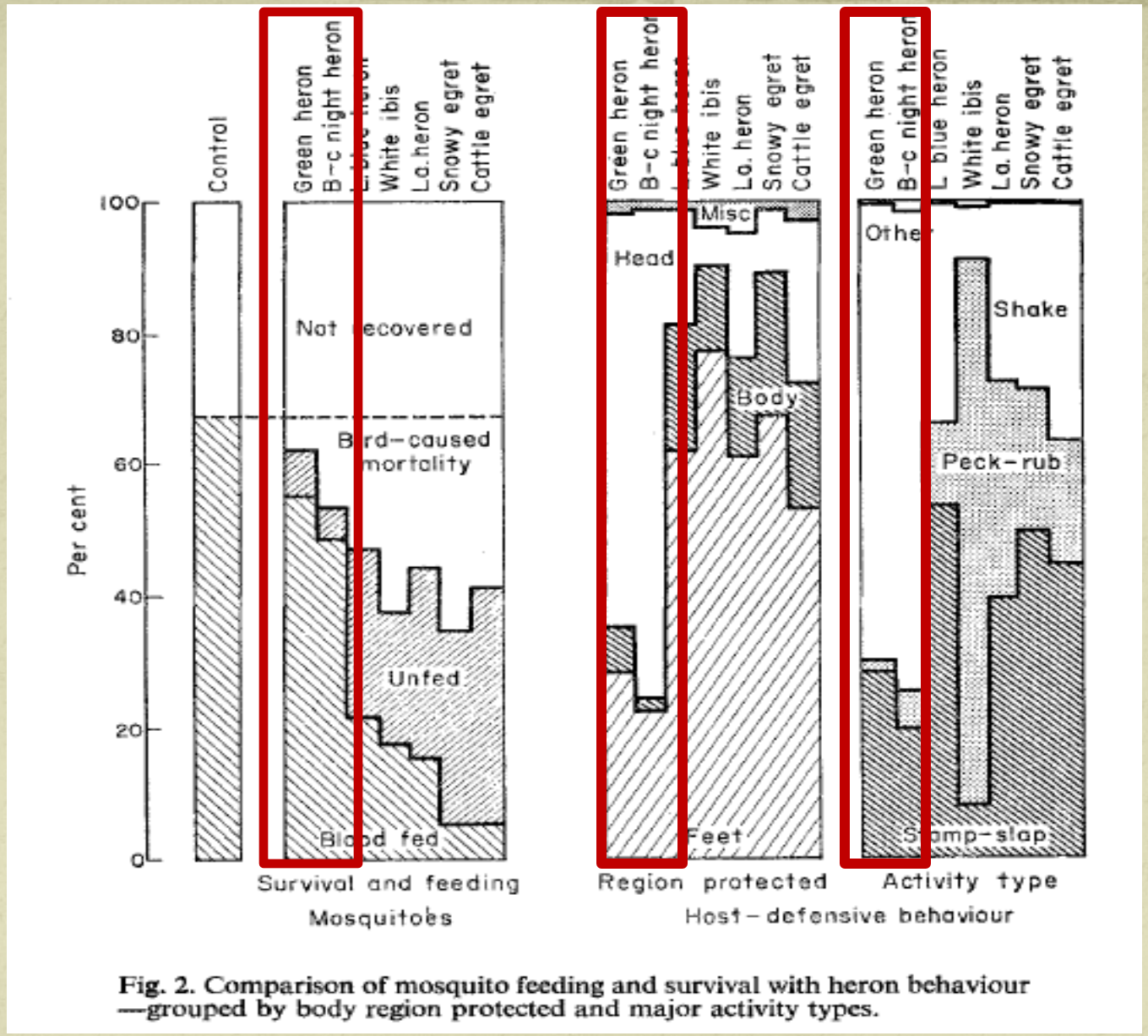


Fig. 2. Comparison of mosquito feeding and survival with heron behaviour —grouped by body region protected and major activity types.

Webber, L. A. and J. D. Edman (1972). "ANTI-MOSQUITO BEHAVIOR OF CICONIIFORM BIRDS." Animal Behaviour **20**(MAY): 228-&

Recent host defense research

- Comparison tests with chickens and House Sparrows
  - Defensive behaviors had no influence on bloodfeeding success
  - Significant interaction between defensive behaviors and host species

- Field collections with European Starlings and American Robins
  - full blood meals from American Robins
  - partial blood meals from European Starlings
  - Mixed meals had > quantities from Robins
  - Concluded Starlings vigorously defenders – drove mosquitoes to Robin

Darbro, J. M. and L. C. Harrington (2007). "Avian defensive behavior and blood-feeding success of the West Nile vector mosquito, Culex pipiens." Behavioral Ecology **18**(4): 750-757

Hodgson, J.C., et al. (2001). "Interrupted Blood-feeding by *Culiseta melanura* (Diptera: Culicidae) on European Starlings." Journal of Medical Entomology 38(1): 59 - 66

Experimental Focus – can we use experimentally collected blood fed mosquitoes to quantify innate host preferences? How do host behaviors influence mosquito host feeding success?

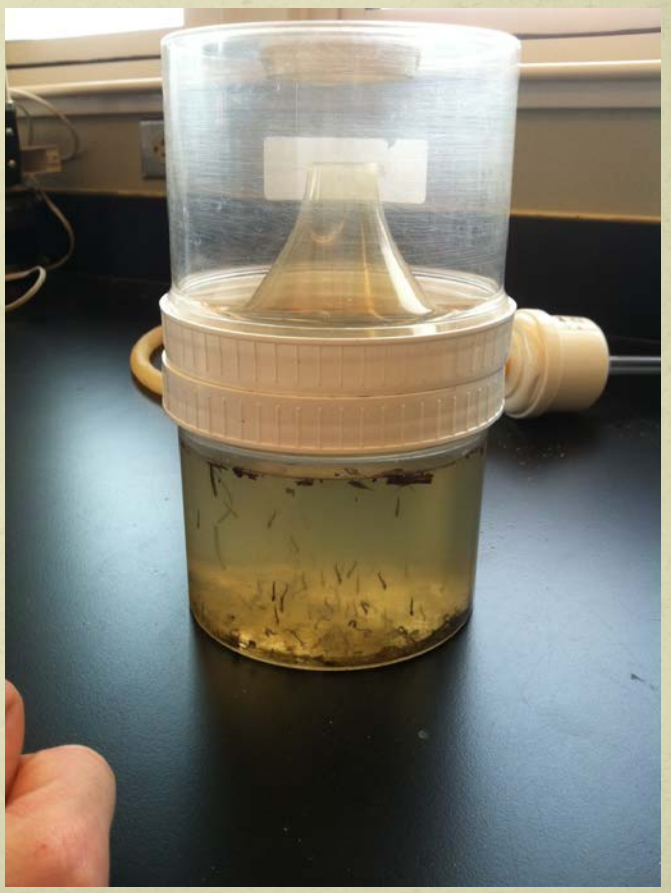
Credit: CDC/Jim Gathany(2003)

Illustration by Diane Pierce, National Geographic

Hypothesis: Observed field patterns are explained by a higher preference of Cx, quinquefasciatus to feed on Northern Cardinals in comparison to other available hosts

Experimental design

- Mosquitoes
  - *Cx quinquefasciatus* raised from field collected egg rafts



Experimental Design

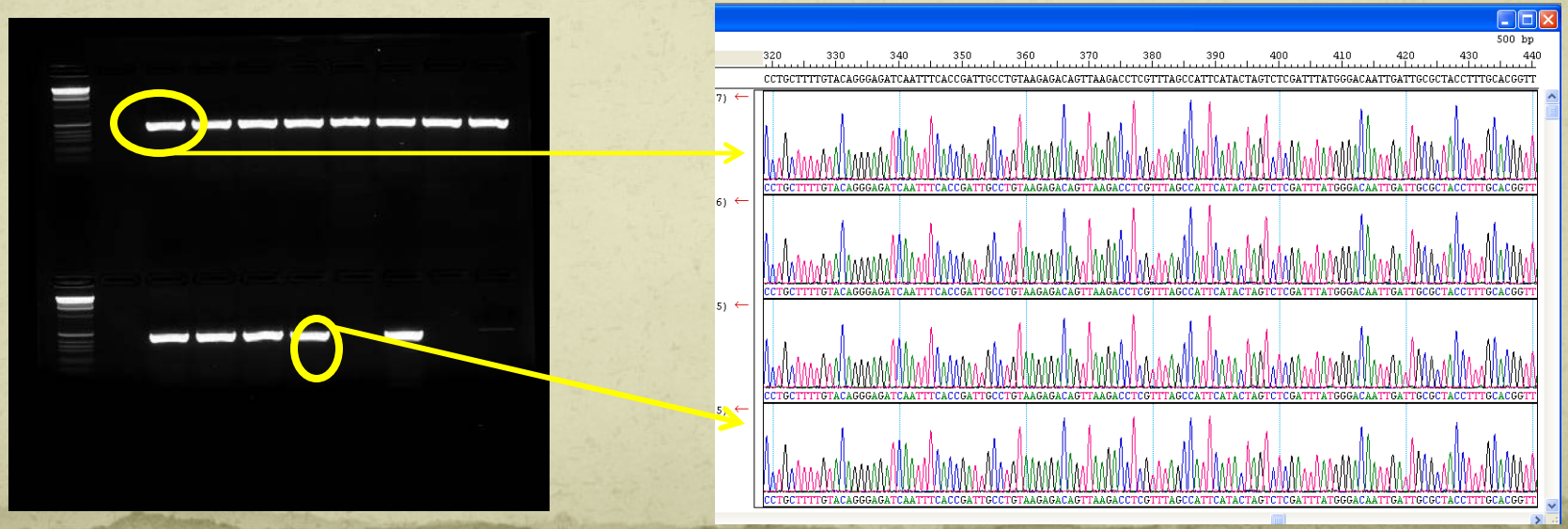
- Birds
  - Northern Cardinals, American Robins, Blue Jays, Brown Thrashers, and Gray Catbirds captured wild

Experimental Design

- Experiments
  - Took place 2100 – 0700 hrs
  - 30 female mosquitoes
  - 2 avian hosts (all experiments contained 1 cardinal)
  - 1st hour of each exp recorded defensive behaviors

Experimental Design

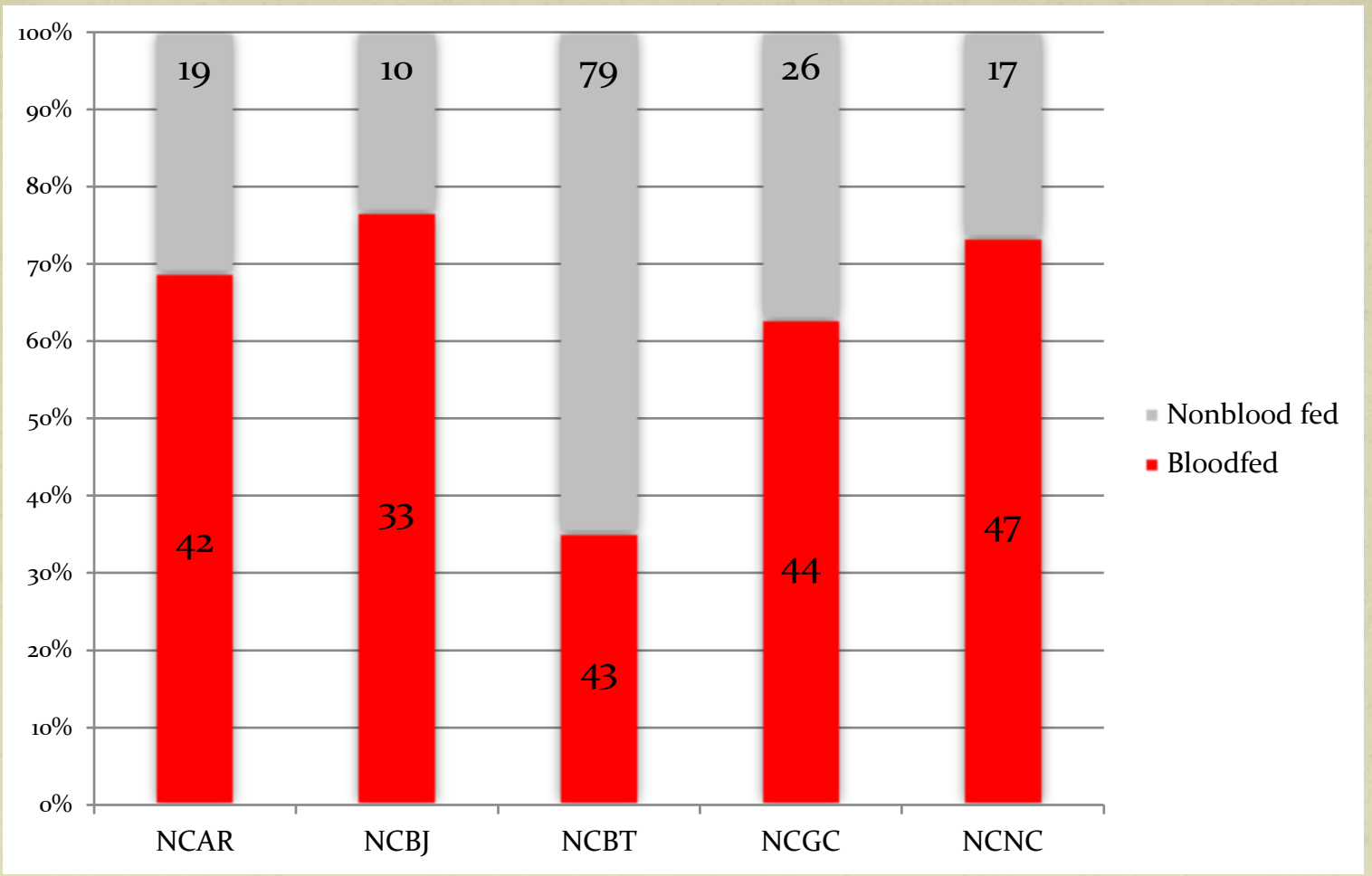
- Molecular analysis
  - DNA extracted from bloodfed abdomens
  - Amplified with direct PCR
  - Sequenced with ABI 3500 automated sequencer
  - Sequences compared to control sequences



Experimental Design

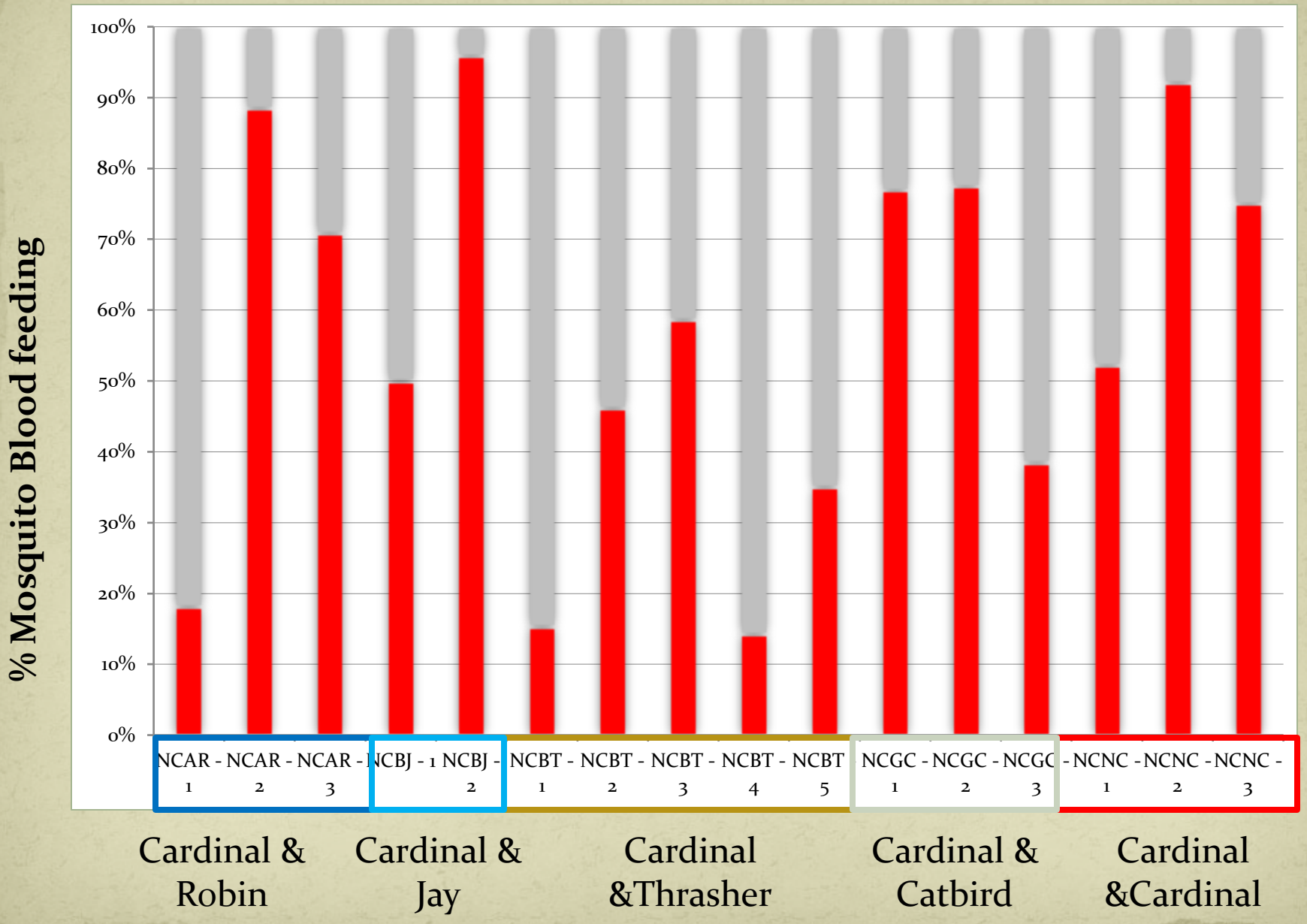
- Statistical Analysis
  - Odds of mosquito obtaining a bloodmeal
    - X^2 tests comparing experimental blood feeding results to Cardinal controls
  - Binomial test of proportions for bloodmeal sources
    - Is the proportion of bloodmeals from a Cardinal significantly different from random (50%)?
  - Defensive Ratios
    - Defensive behaviors of Cardinal in relation to experimental pair
    - Pearson Correlation Coefficient

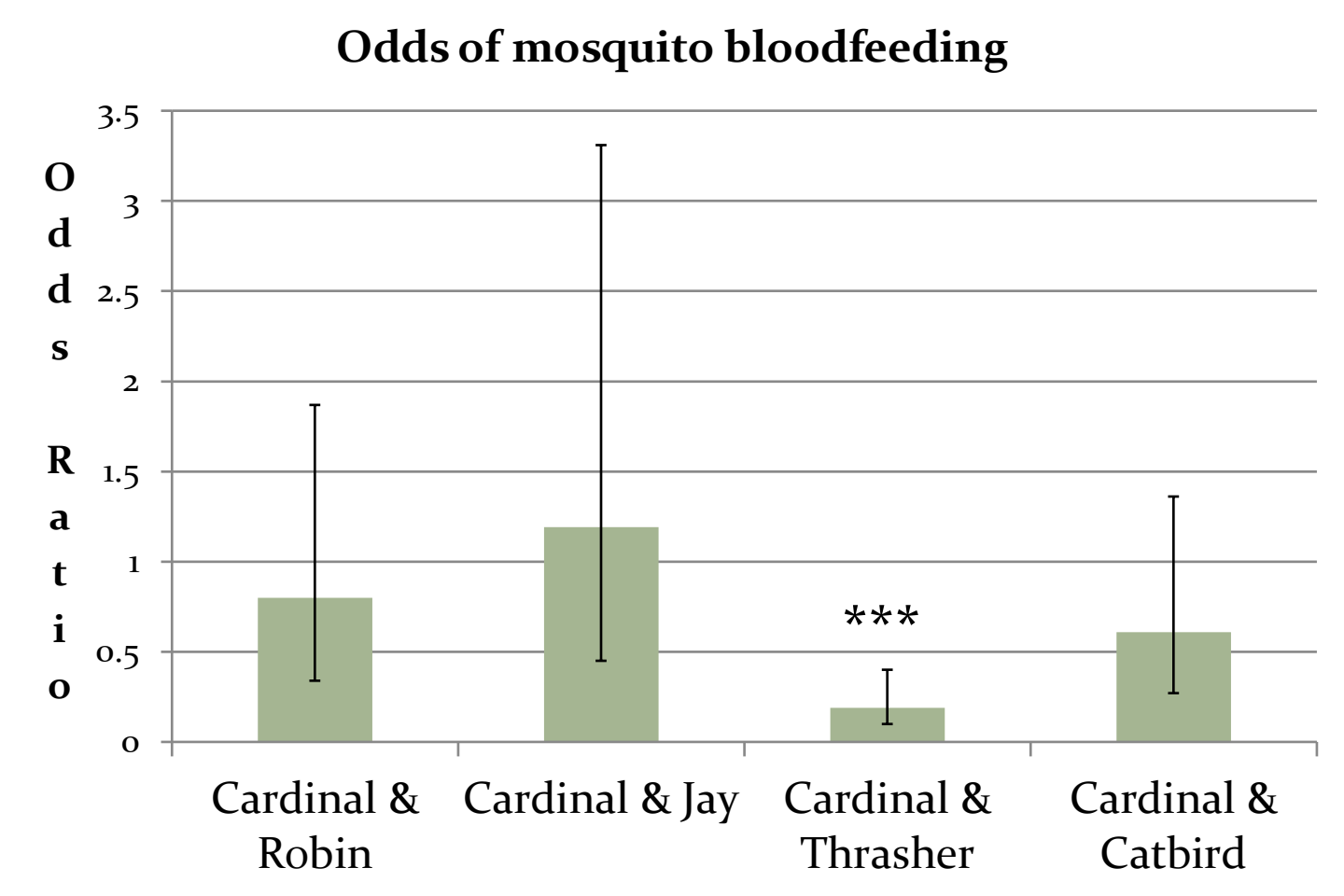
Overall mosquito bloodfeeding by species combination



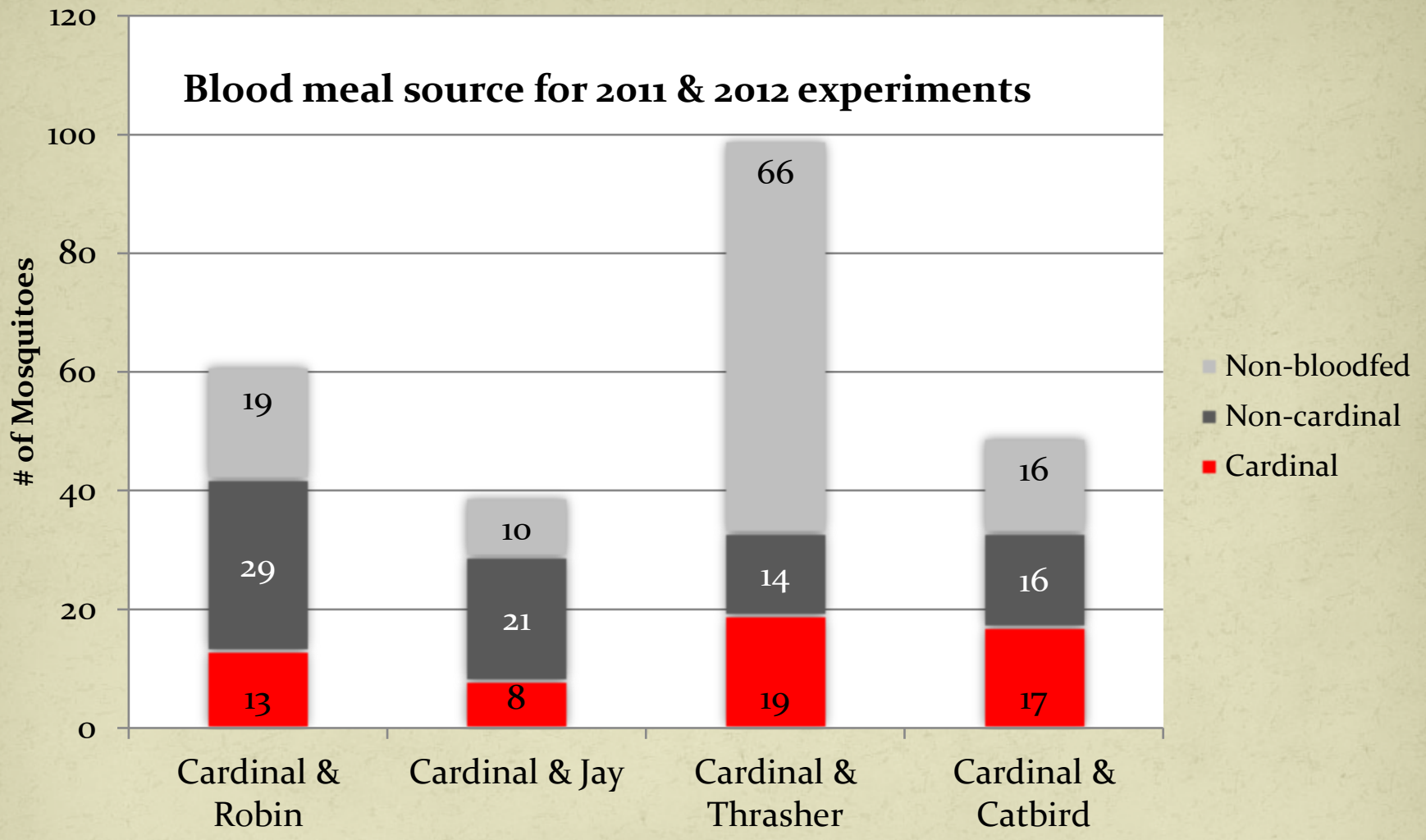
AVIAN SPECIES	American Robin	Blue Jay	Brown Thrasher	Gray Catbird	Northern Cardinal
Northern Cardinal	3 replicates	2 replicates	5 replicates	3 replicates	3 replicates

Mosquito blood feeding by replicate





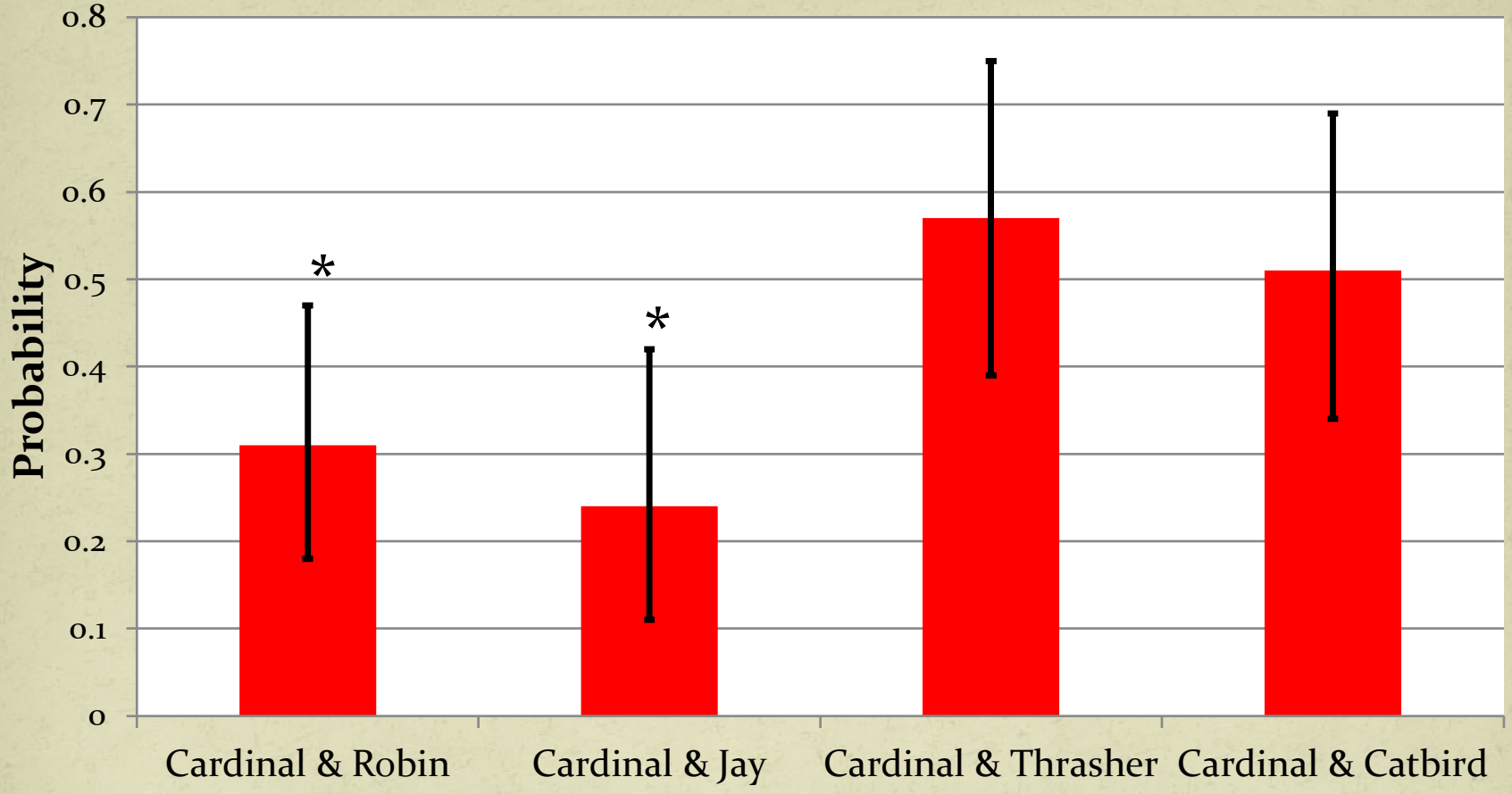
*** ***Odds of mosquito blood feeding was significantly less than 1 for Cardinal and Thrasher experiments***



AVIAN SPECIES	American Robin	Blue Jay	Brown Thrasher	Gray Catbird	Northern Cardinal
Northern Cardinal	3 replicates	2 replicates	5 replicates	3 replicates	3 replicates

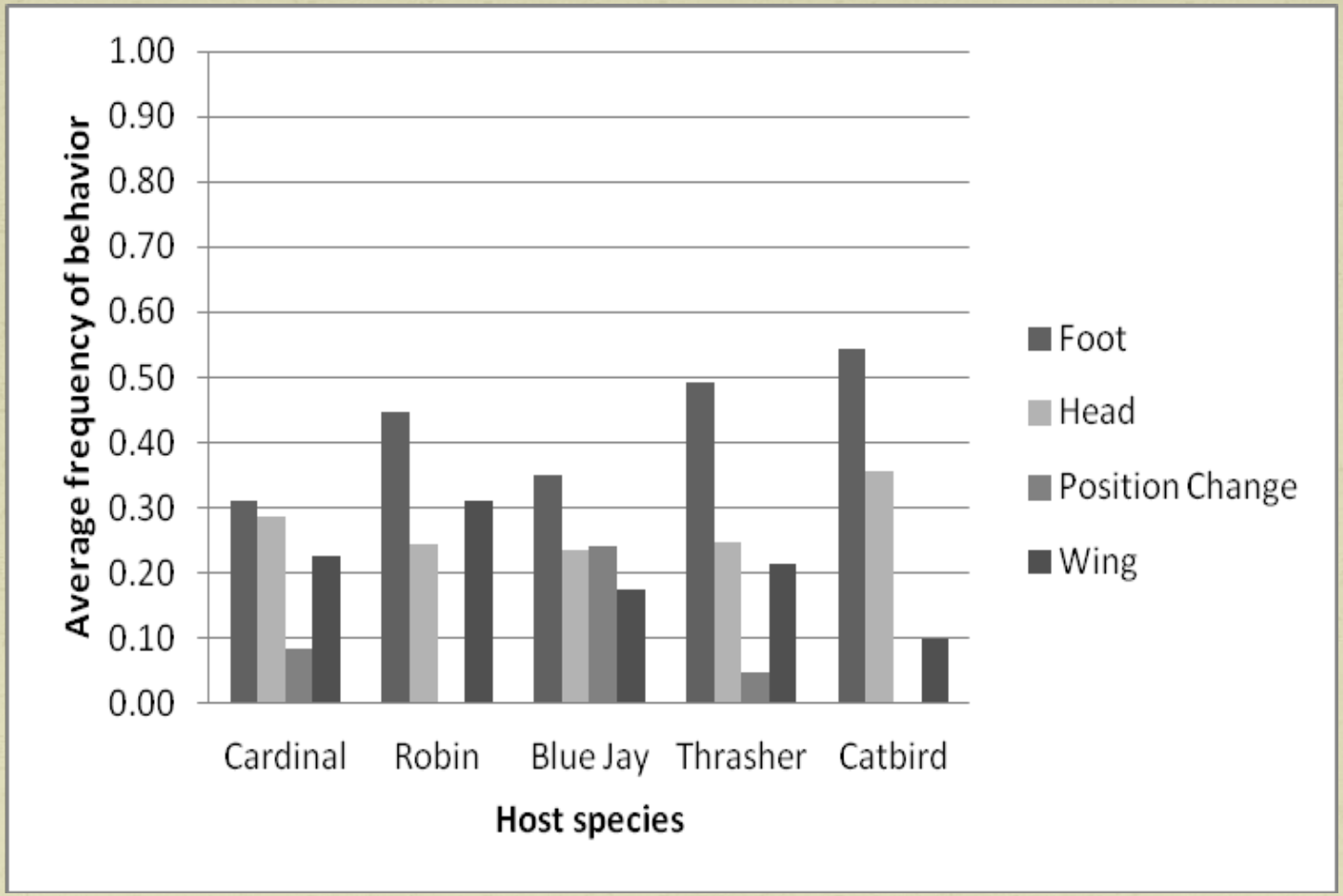
*

Probability that a mosquito obtains a bloodmeal from a Northern Cardinal



- ***Cardinal blood sources differed significantly from 50% when the experiment contained a Robin or a Jay***

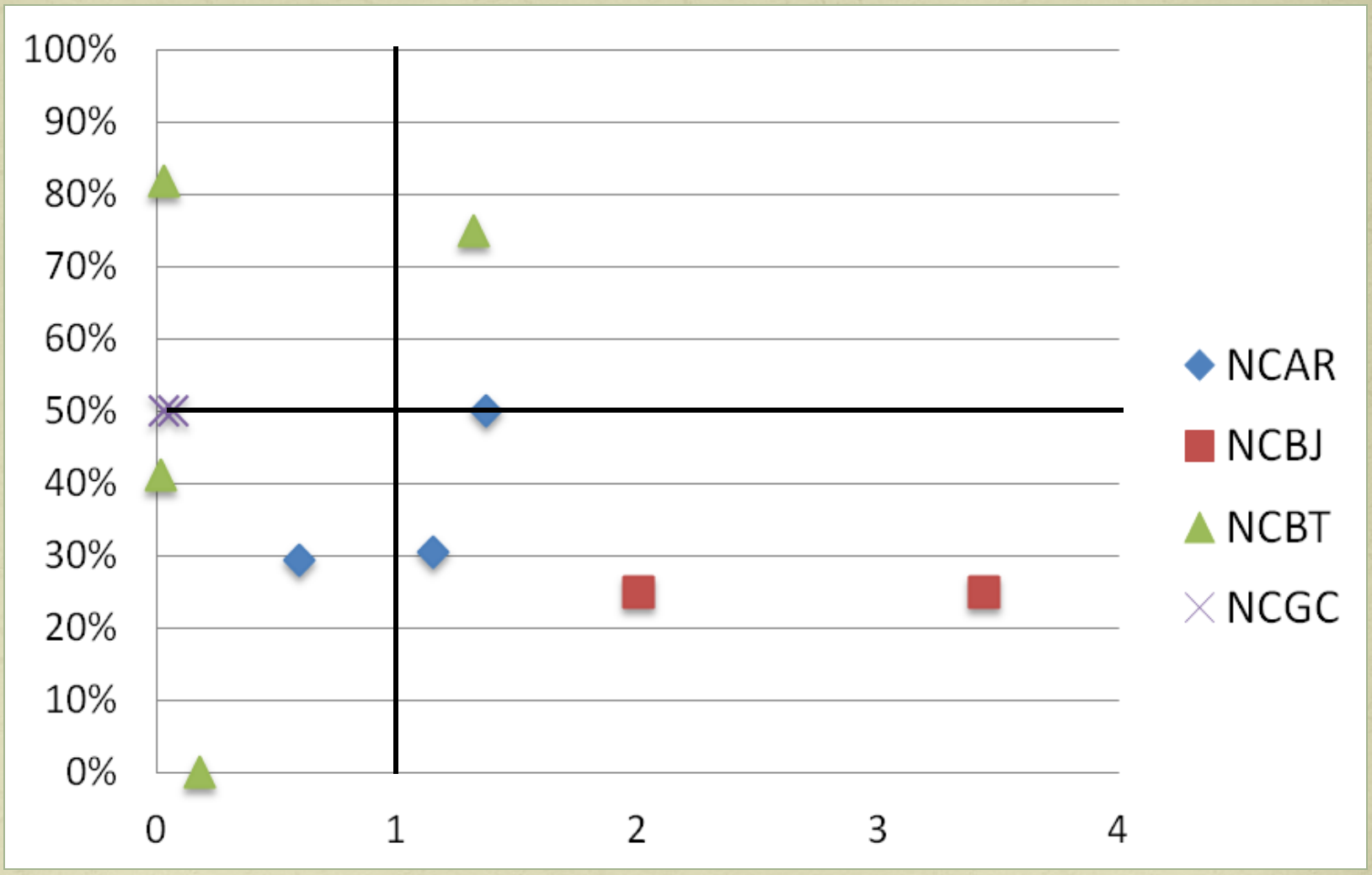
Host Defensive Behaviors



18 hours of video – 1 hr/bird for 2011 EXPS
30 min/ bird for 2012 EXPS

Data presented not corrected for time differences

Host defensive behaviors and mosquito blood feeding success



Conclusions

- **Mosquito blood feeding success was neither enhanced nor diminished with varying host combinations**
  - Compared to Cardinal – Cardinal control experiments

- **Mosquitoes significantly underfed on Northern Cardinals when paired with a Robin or Jay**
  - Potential preference for Robins OR Jays
  - Confirms patterns observed in *Cx. Pipiens pipiens* prefernce studies

Conclusions

- **Hosts varied in their defensive behaviors**
  - All birds tend to protect their feet/legs and head
  - No difference in the number of defensive behaviors observed
- **There was no relationship between a Cardinal's defensive behavior and mosquito blood feeding success on a Cardinal**
  - Defensive behavior data limited
  - Potential behavioral differences between Cardinals and Robins/Jays

Summary

- **Experiments present evidence that:**
  - ***Cx. quinquefasciatus* blood feeding success is variable**
  - ***Cx. quinquefasciatus* has no innate preference for Northern Cardinals**
- **What is driving mosquito host feeding patterns in the Southeast?**

Acknowledgements

- Main Collaborators
  - Paula Marcet
  - Danny Mead
  - Uriel Kitron
  - Gonzalo Vazquez Prokopec
- Lab Members
  - Rebecca Levine
  - Donal Bisanzio
  - Christopher Hoover


Questions?

Future Directions

Transmission of West Nile virus (WNV)

- West Nile virus
  - Family *Flaviviridae*
  - member of the Japanese Encephalitis Antigenic Complex
  - Vector-borne disease
    - Vector – Mosquitoes, mainly *Culex spp.* (North America) – **chronic infection**
    - Reservoir – Birds, mainly *Passerines* (song birds) – **acute infection**
    - Mammals/Humans considered dead-end hosts

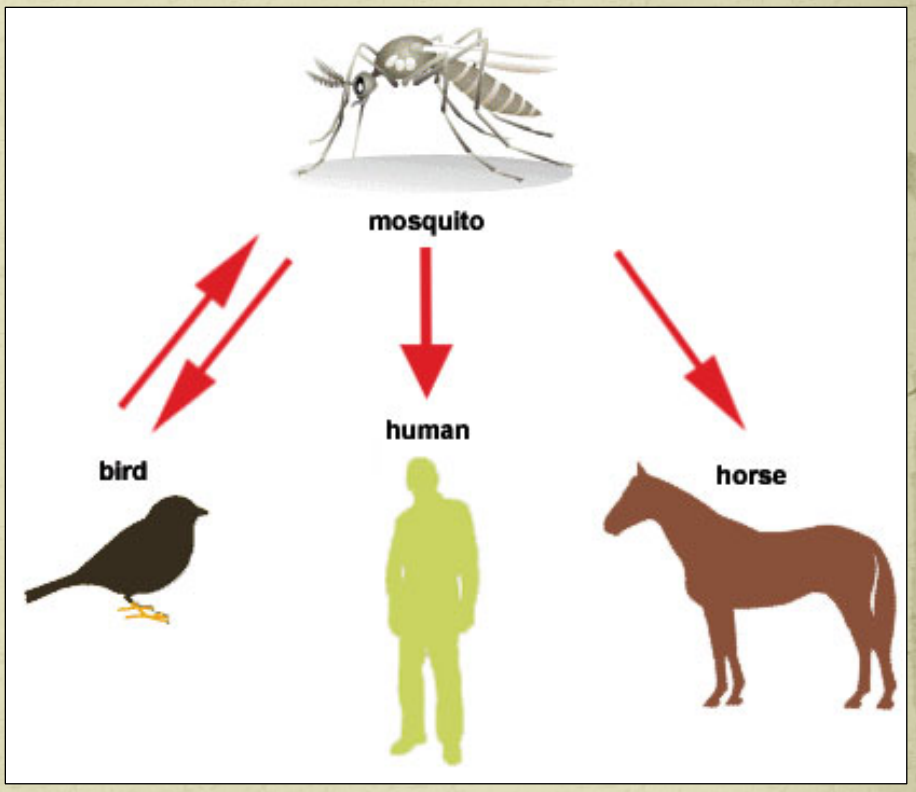


Photo credit: City of Berkley - Environmental Health

WNV in North America

- First infections detected New York, NY in 1999
- Subsequent spread through the contiguous US
  - Reaches California by 2004
  - Circa 2013 – detected in southern Canada, Caribbean, Central and South Americas

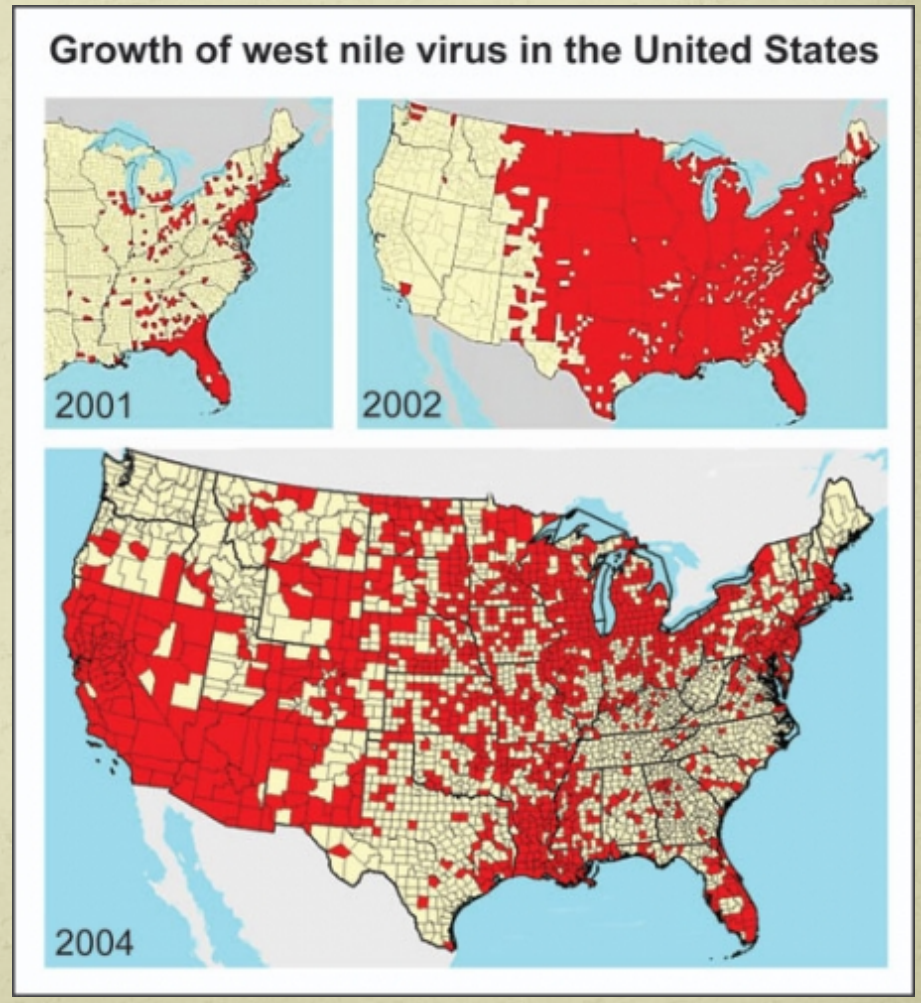


Photo Credit: Centers for Disease Control and Prevention

"*Cx. nigripalpus* tend to eventually engorge on the most tolerant host of those to which they are exposed, irrespective of host size."

Edman, J. D., L. A. Webber, et al. (1974). "EFFECT OF HOST DEFENSES ON FEEDING PATTERN OF CULEX-NIGRIPALPUS WHEN OFFERED A CHOICE OF BLOOD SOURCES." Journal of Parasitology **60**(5): 874-883

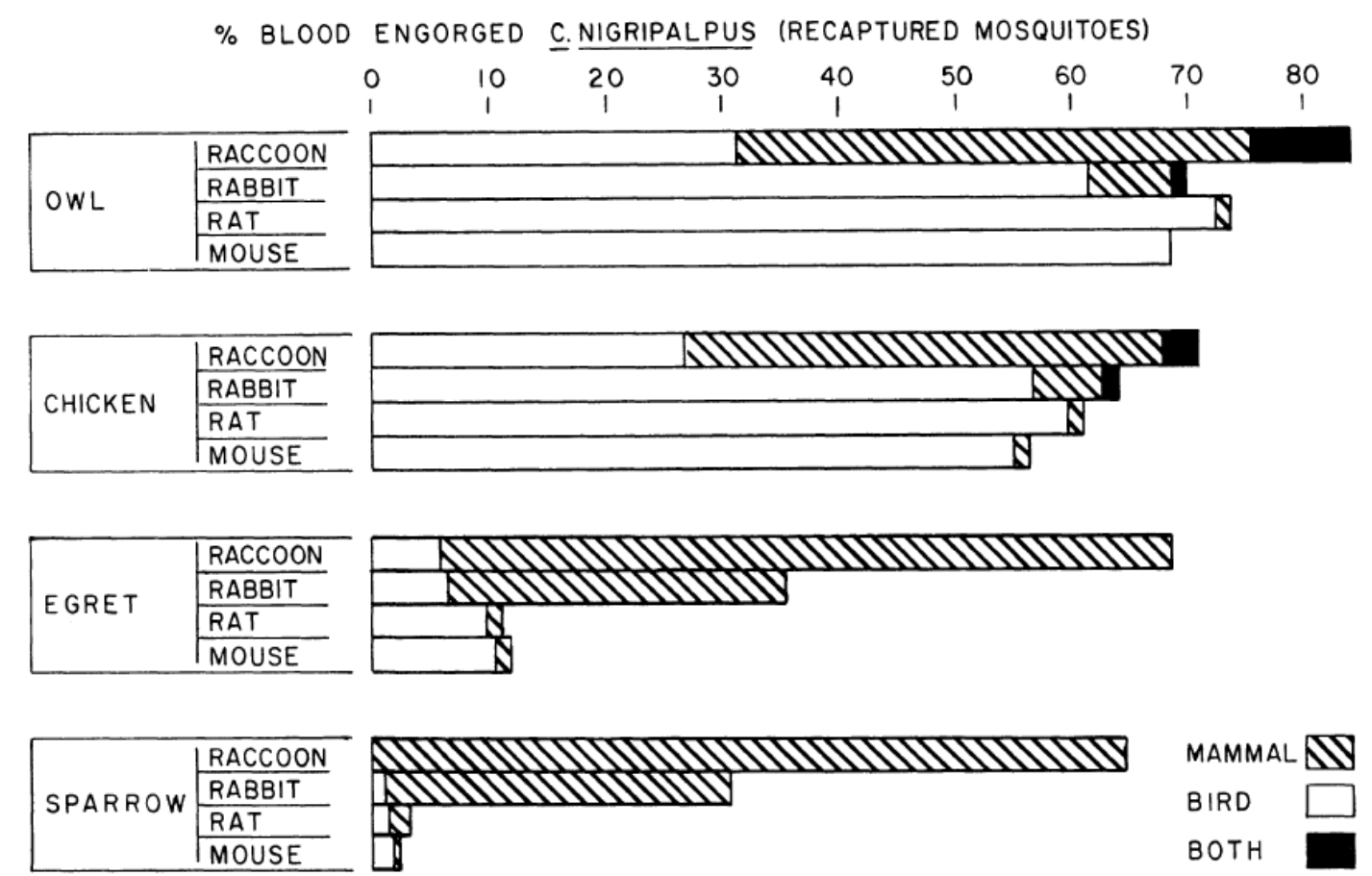
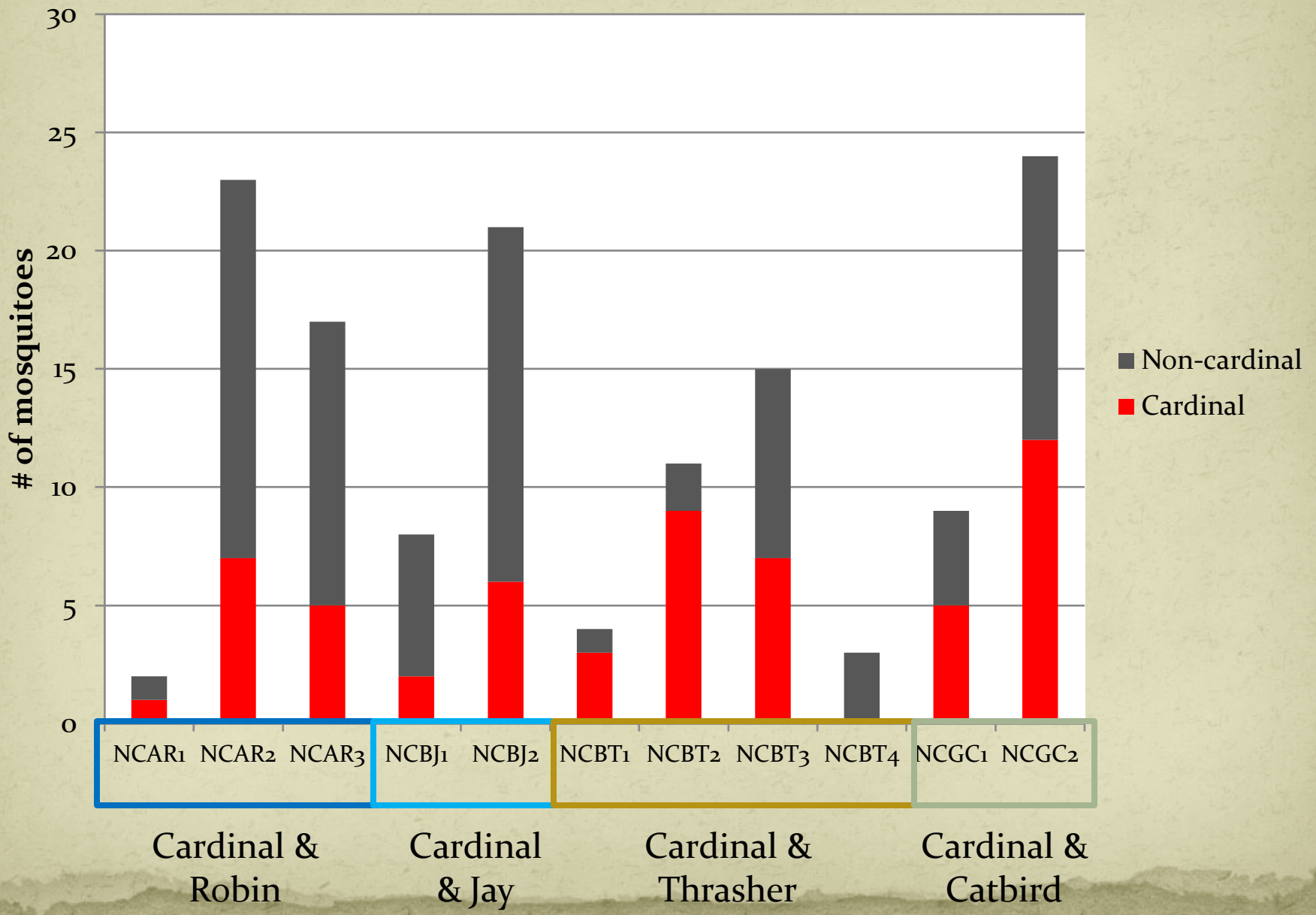


FIGURE 1. Blood meal sources of *C. nigripalpus* simultaneously exposed to different paired combinations of birds and mammals.

Mosquito blood meal sources by replicate for 2011 & 2012 experiments



Defensive Behaviors Results

Movement	Northern Cardinal	American Robin	Blue Jay	Brown Thrasher	Gray Catbird	TOTAL
Foot	140 (39%)	31 (46%)	15 (33%)	300 (32%)	298 (71%)	784 (43%)
Wing	85 (24%)	20 (30%)	8 (17%)	464 (50%)	26 (6%)	603 (33%)
Head	113 (32%)	16 (24%)	11 (24%)	151 (16%)	96 (23%)	387 (21%)
Position Change	19 (5%)	0	12 (26%)	17 (2%)	0	48 (3%)
TOTAL	357	67	46	932	420	1822

What's next?

- Is it preference or feeding success?
  - Frequency of contact an important variable
  - Proposal (*currently under IACUC review*)
    - Manipulate ratios of experimental species
    - 2 species tests:
      - 1:1, 2:1, and 3:1 ratios
    - 3 species tests:
      - 1:1:1, 2:1:1 ratios
    - 4 species tests:
      - 1:1:1:1 ratios

What's next ? (cont'd)

- What about other potential hosts?
  - Squirrels!! (*currently under IACUC review*)
  - Easy to capture & share similar habitat with experimental avian hosts
  - Incorporate into current experimental design:
    - 1:1 avian to mammal ratio
    - 2:1 avian to mammal ratio & vv
    - 3:1 avian to mammal ratio and vv

How to test the 'Dilution Effect'?

Experiments are host-central & not directly testing preference

- Usually 1 epidemiologically important vector
- Focused on 3-4 bird species
- Exp purposes' vary:
  - defensive behaviors
  - Preference - w/o feeding
  - Feeding success – w/o quantifying host defenses

Incorporating Diversity into EXPs

- Vector-borne diseases: biodiversity a two-way street
  - approx. 60 mosquito spp. & 300 avian spp. annually reported to CDC with WNV infections (entire USA)

- How can we incorporate:
  - Multiple vectors
  - Multiple avian hosts
  - Multiple non-avian hosts?