Hawaiian hoary bat ecology project: status update

Hawai'i Cooperative Studies Unit – UH Hilo
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U.S. Geological Survey – Pacific Island Ecosystems Research Center
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K. Courtot, J. Hoeh, K. Montoya-Aiona, T. Zinn



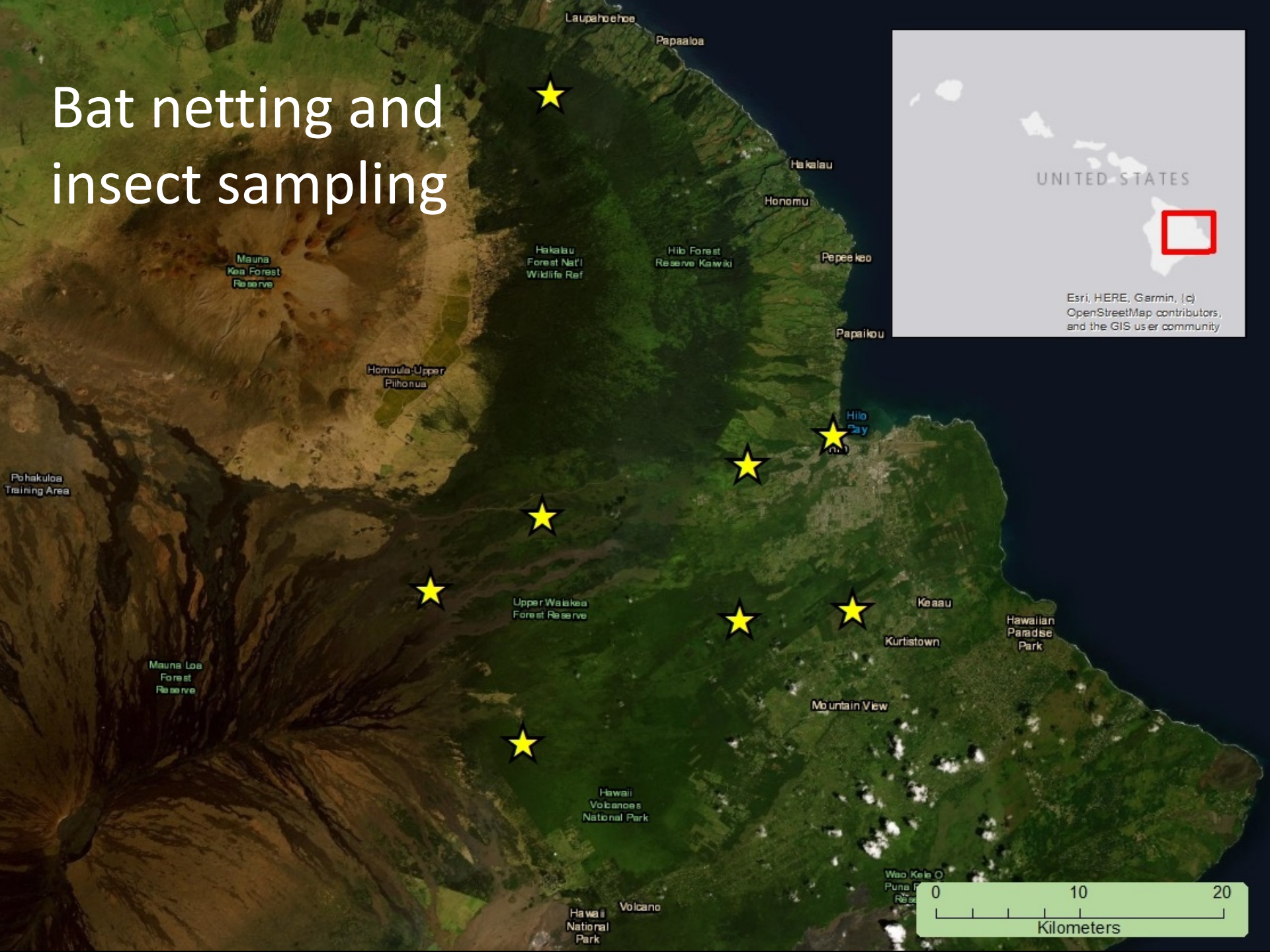
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Hawai'i Cooperative Studies Unit
University of Hawai'i at Hilo

Research topics

- Mist-netting effort, bat capture and tracking
- Roost ecology
- Diet and metabarcoding
- Population genetics and genetic sexing

Bat netting and insect sampling



Capture effort

- 125 net nights, capturing 65 bats in 2018–2019
  - 2,163 net hours, 0.03 bats/net hour
- Insect light trapping with 2 sheets on 50 nights
  - Concurrent with netting 49 nights
  - Concurrent with 25 guano samples







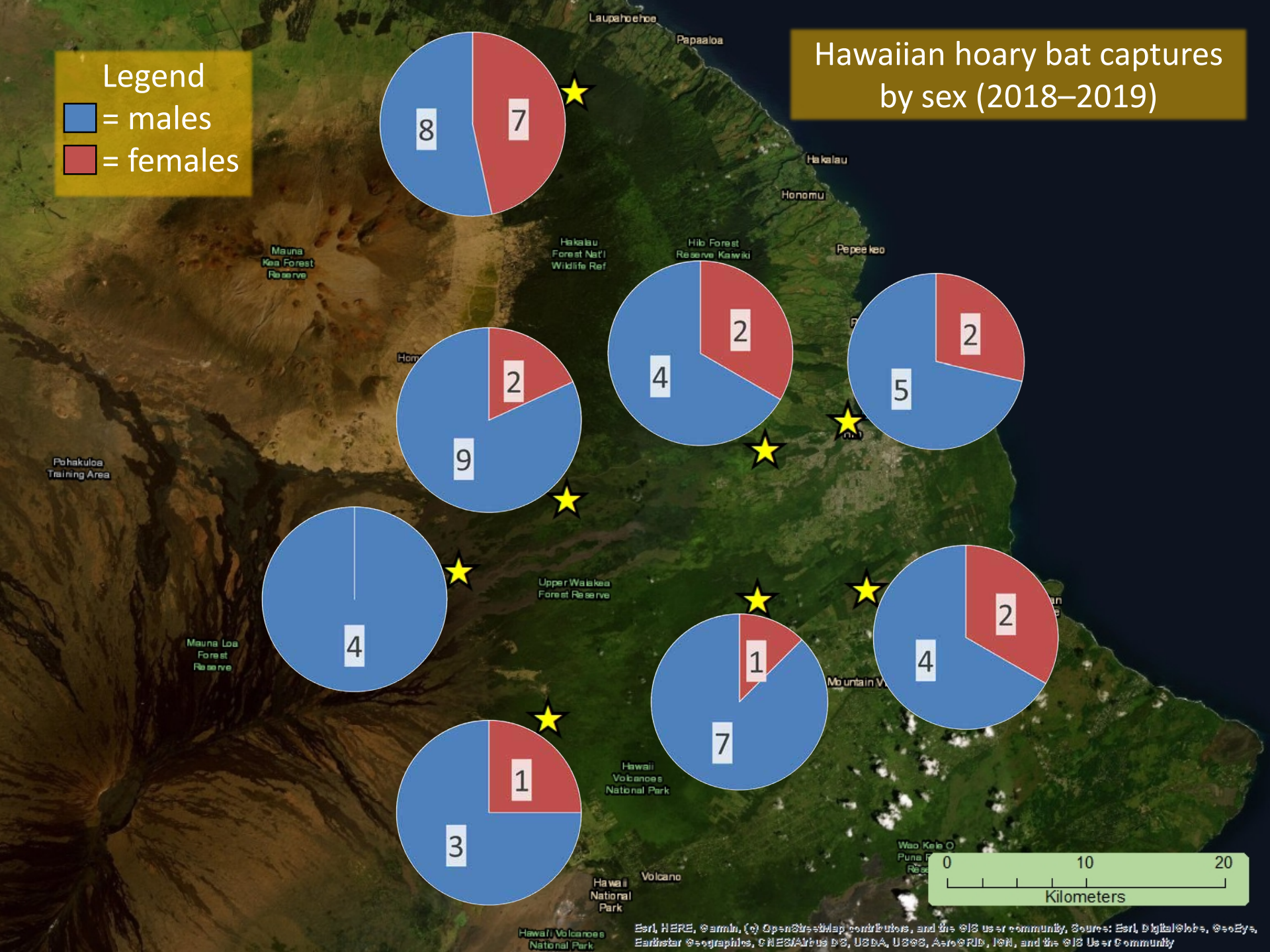
Samples (2018 – 2019)

Samples	Female			Male			Total
	Adult	Juvenile	Total	Adult	Juvenile	Total	
Guano	13	0	13	28	1	29	42
Tissue	16	1	17	47	1	48	65
Fur	17	1	18	48	1	49	67
Wing photos	8	1	9	33	0	33	42

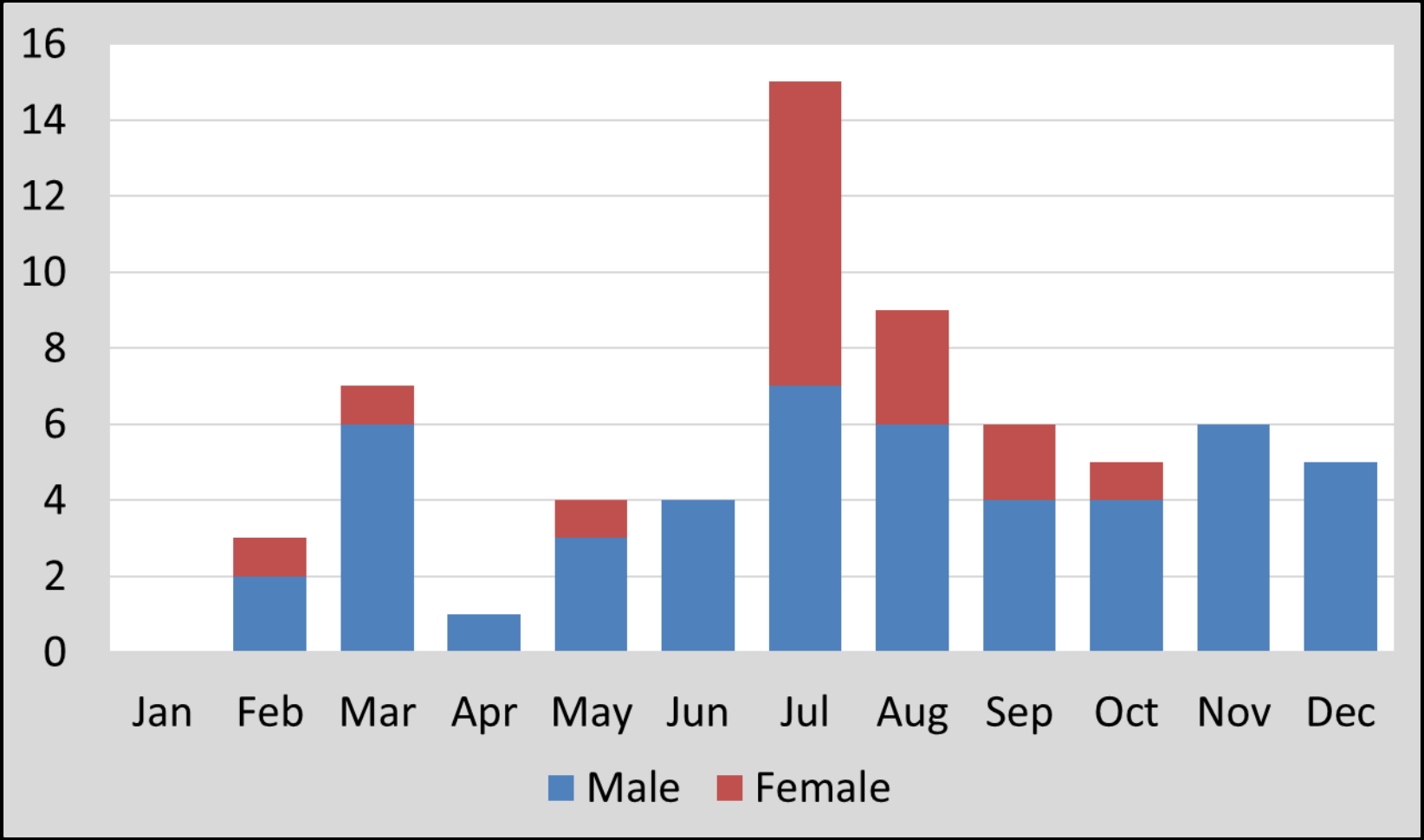
Standard metrics collected from all bats: capture time, mass (g), forearm length (mm), age, sex, reproductive status, band color pattern

Recaptures
(2018 – Feb 2020)

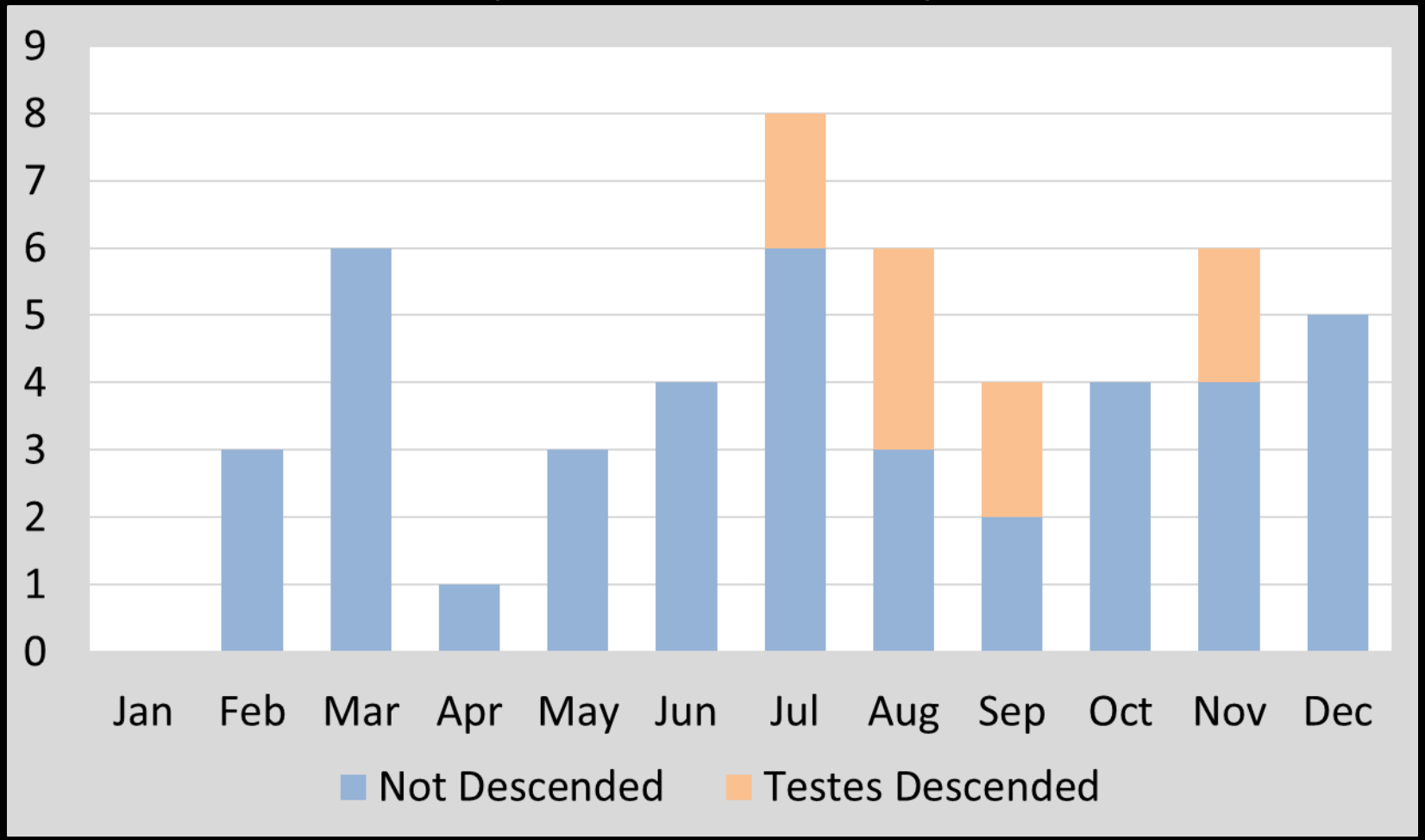
Bat ID	First Capture	Second Capture	Days between capture	Mass
Female	Jul-2018 Post-Lactating	May-2019 Non-reproductive	300	19.5 / 19.5
Male	Dec-2018 Not descended	Feb-2019 Not descended	50	15.75 / 15
Male	Mar-2019 Not descended	Jul-2019 Not descended	118	15.5 / 17.5
Female	Jul-2019 Lactating	Jul-2019 Lactating	6	19.5 / 19.5
Male	Mar-2019 Not descended	Feb-2020 Not descended	331	16 / 16


Hawaiian hoary bat captures by sex (2018–2019)
Legend
= males
= females
8
7
2
4
2
5
2
9
4
2
4
1
7
1
3
Laupahoehoe
Papaaloa
Hakalau
Honomu
Pepeekeo
Mauna Kea Forest Reserve
Hakalau Forest Nat'l Wildlife Ref
Hilo Forest Reserve Kaiwiki
Pohakuloa Training Area
Upper Waiakea Forest Reserve
Mauna Loa Forest Reserve
Hawaii Volcanoes National Park
Volcano
Hawaii National Park
0
10
20
Kilometers
Esri, HERE, Garmin, (c) OpenStreetMap contributors, and the GIS user community, Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

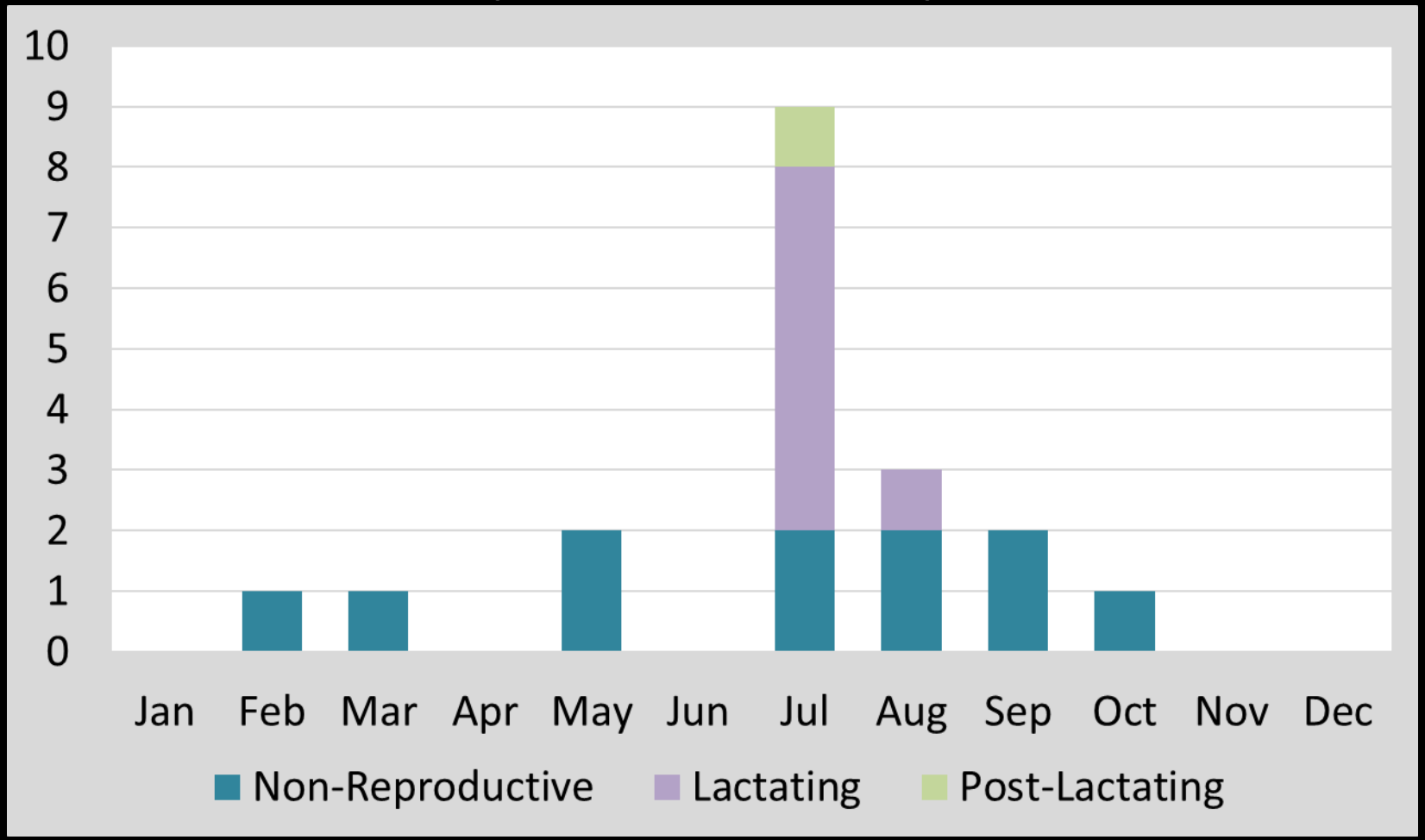
Sex of Hawaiian hoary bats by Month (2018 – 2019)



Reproductive condition in male Hawaiian hoary bats (2018 – 2019)



Reproductive condition in female Hawaiian hoary bats (2018 – 2019)



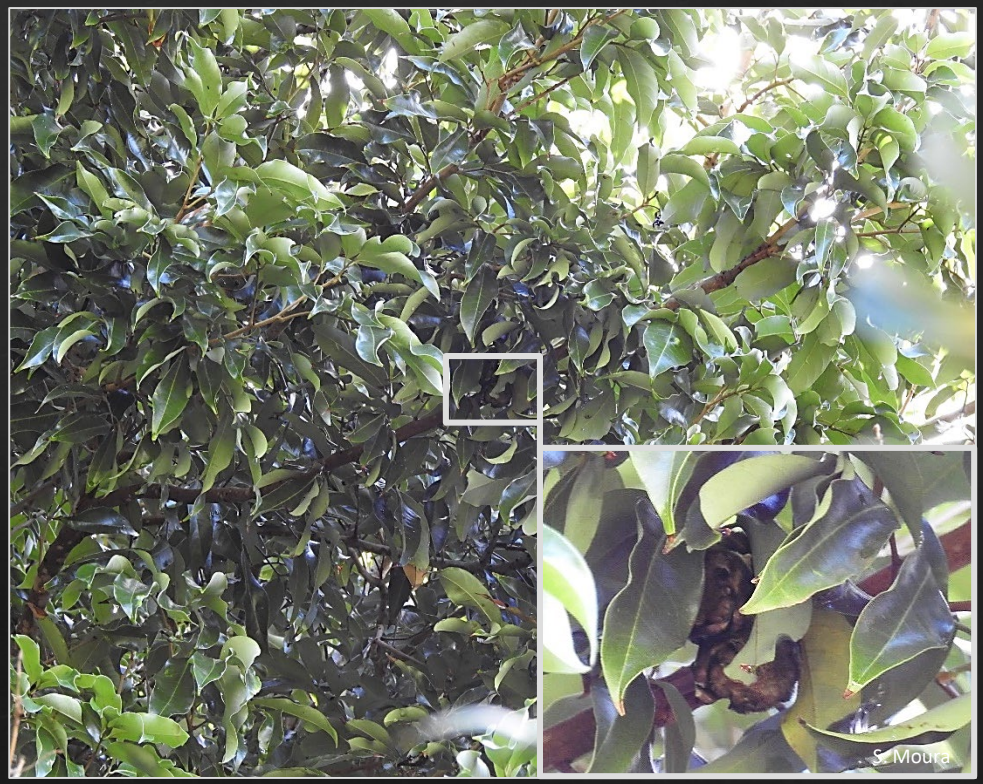
Roost ecology of the Hawaiian hoary bat



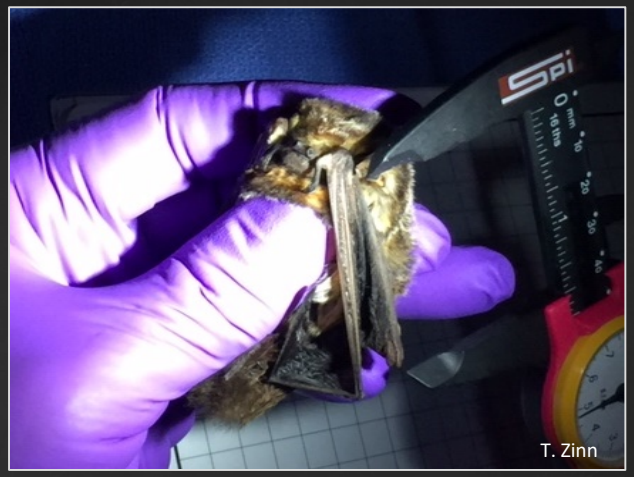
K. Montoya-Aiona

Objectives

- Identify and characterize roost
  - locations, habitat
  - tree attributes
  - fidelity



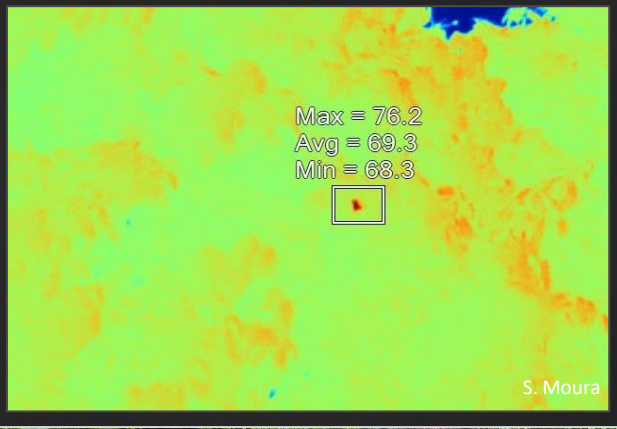



T. Zinn


T. Zinn


T. Zinn


M. Gorresen


Max = 76.2
Avg = 69.3
Min = 68.3
S. Moura

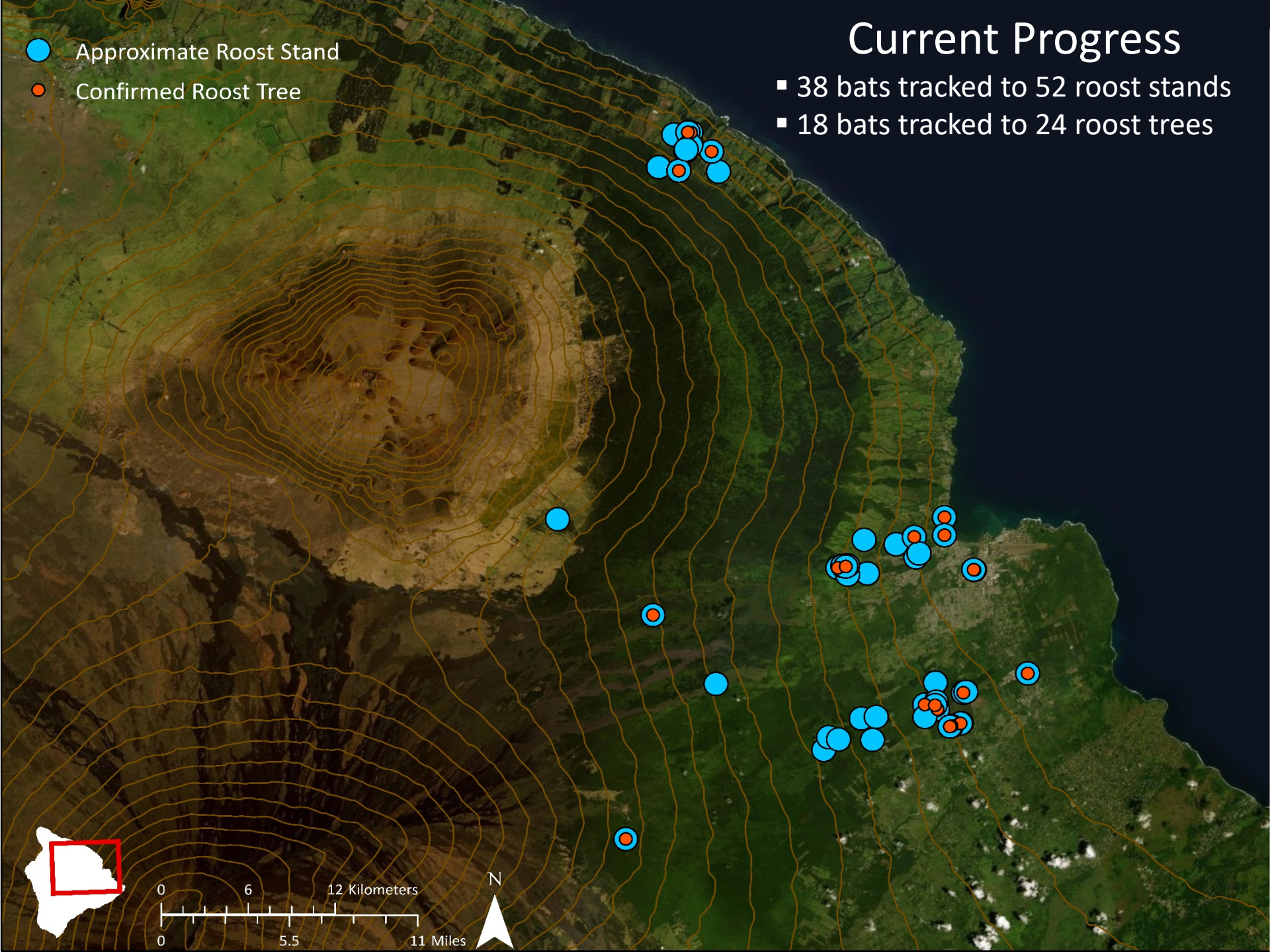

M. Gorresen


USGS
USGS
K. Courtot


S. Casler

Roost Tracking Effort

- 486 tracking events
  - 486 tracking events x 2 personnel x 8 hrs / 52 roosts
  - = 150 personnel hrs / roost
- 38 bats tracked to 52 roost stands
  - 28 male; 10 female; 37 adult; 1 juvenile
- 18 bats tracked to 24 roost trees
  - 11 male; 7 female; 16 adult; 1 juvenile
  - Bats visible at 14 perches within 24 roost trees


Approximate Roost Stand
Confirmed Roost Tree
Current Progress
38 bats tracked to 52 roost stands
18 bats tracked to 24 roost trees
0
6
12 Kilometers
0
5.5
11 Miles
N



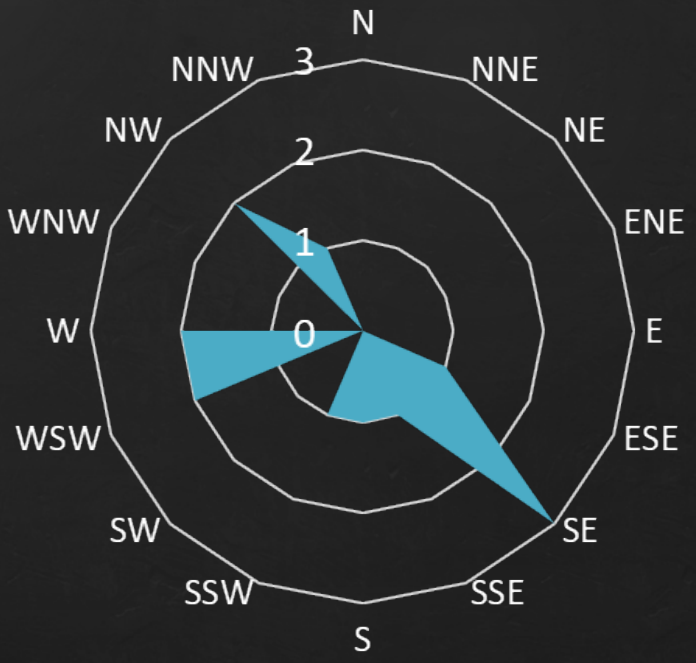
J. Jeffrey

Day Roost Tree Species

- ʻŌhiʻa (*Metrosideros polymorpha*)
- Melochia (*Melochia umbellata*)
- Lychee (*Litchi chinensis*)
- Mango (*Mangifera indica*)
- Ironwood (*Casuarina equisetifolia***)**
- Gunpowder (*Trema orientalis*)
- Australian toon (*Toona ciliata*)
- Eucalyptus (*Eucalyptus* spp.)
- Paperbark (*Melaleuca quinquenervia*)

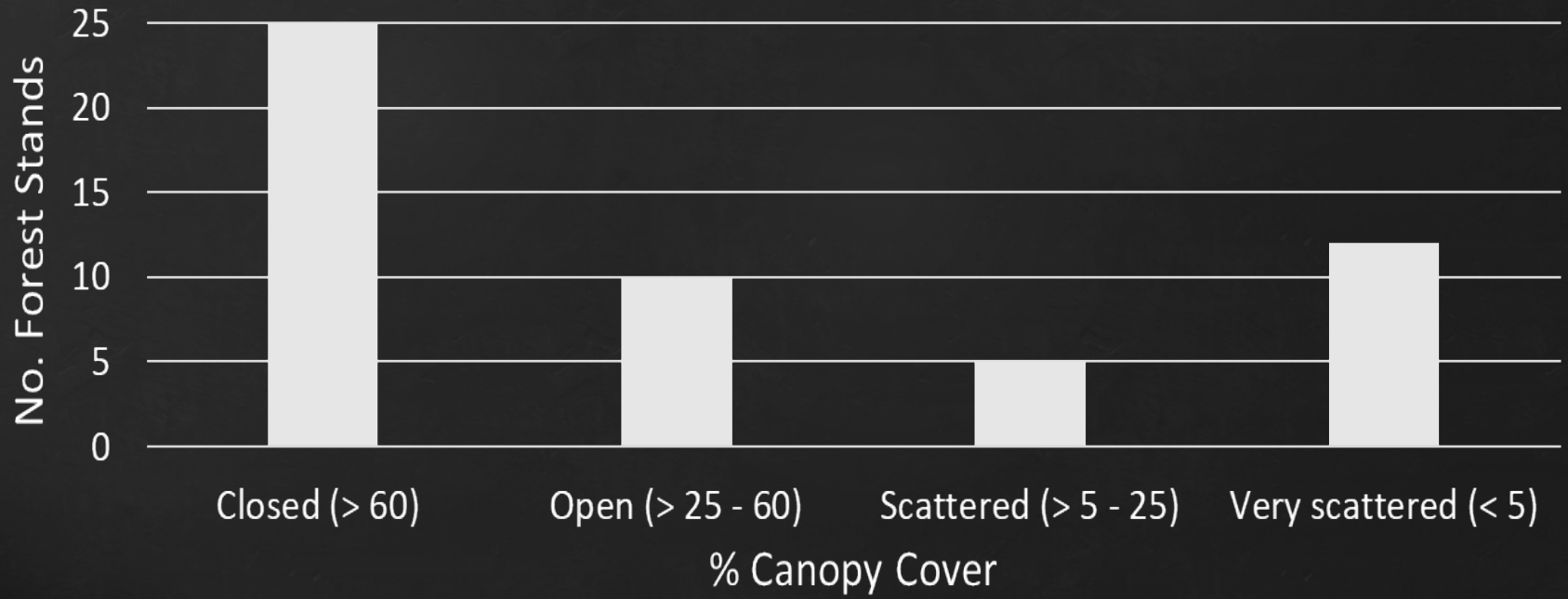
Day Roost Tree Metrics

- Tree height: 9 - 56 m (mean = 21)
- Tree canopy cover: 3 - 97% (mean = 43%)
- Perch height: 5 - 23 m (mean = 14)
- Perch canopy cover: 4 - 99% (mean = 48%)
- Perch aspect: southward & westward; 110° to 334°



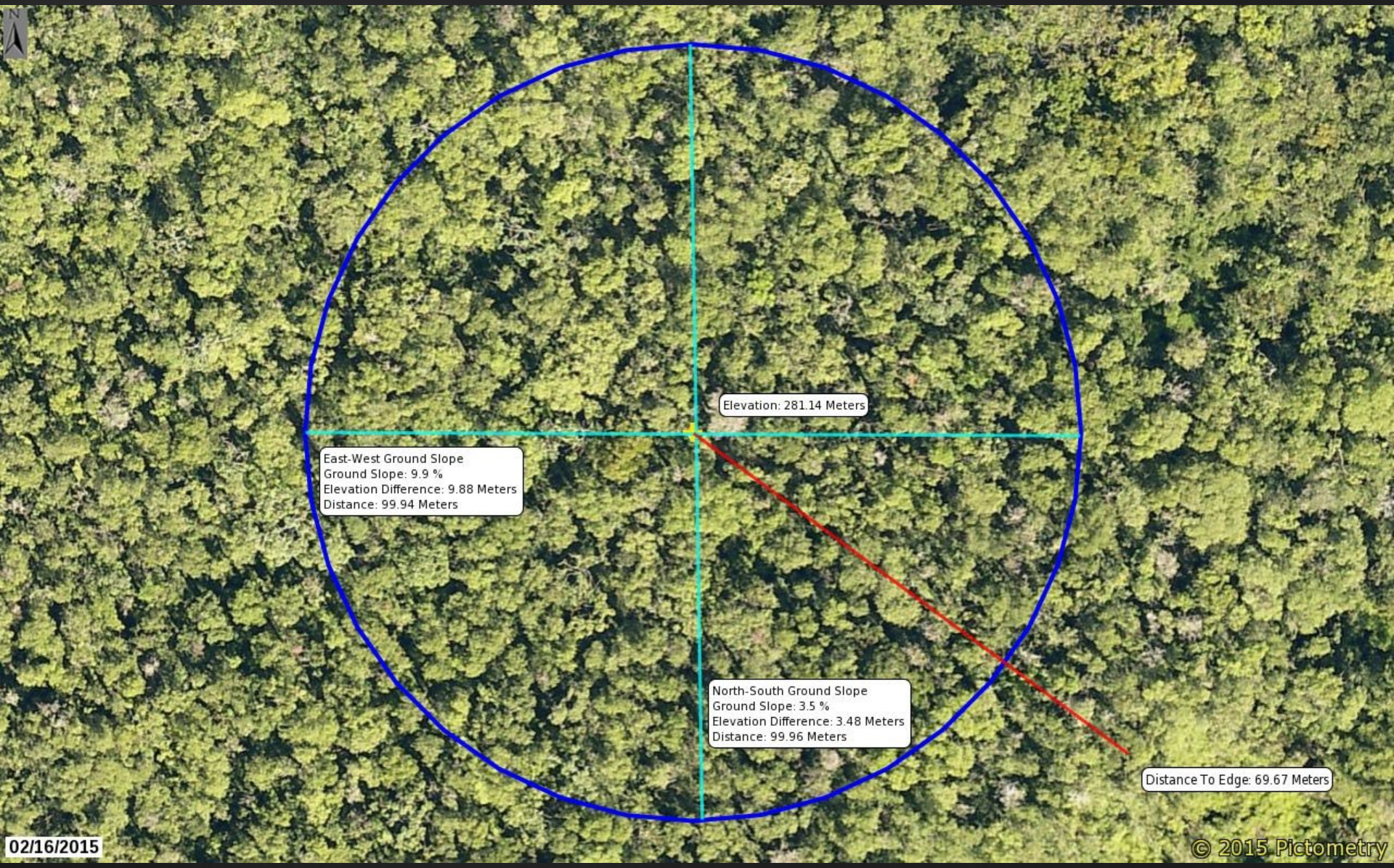
Day Roost Stand Metrics

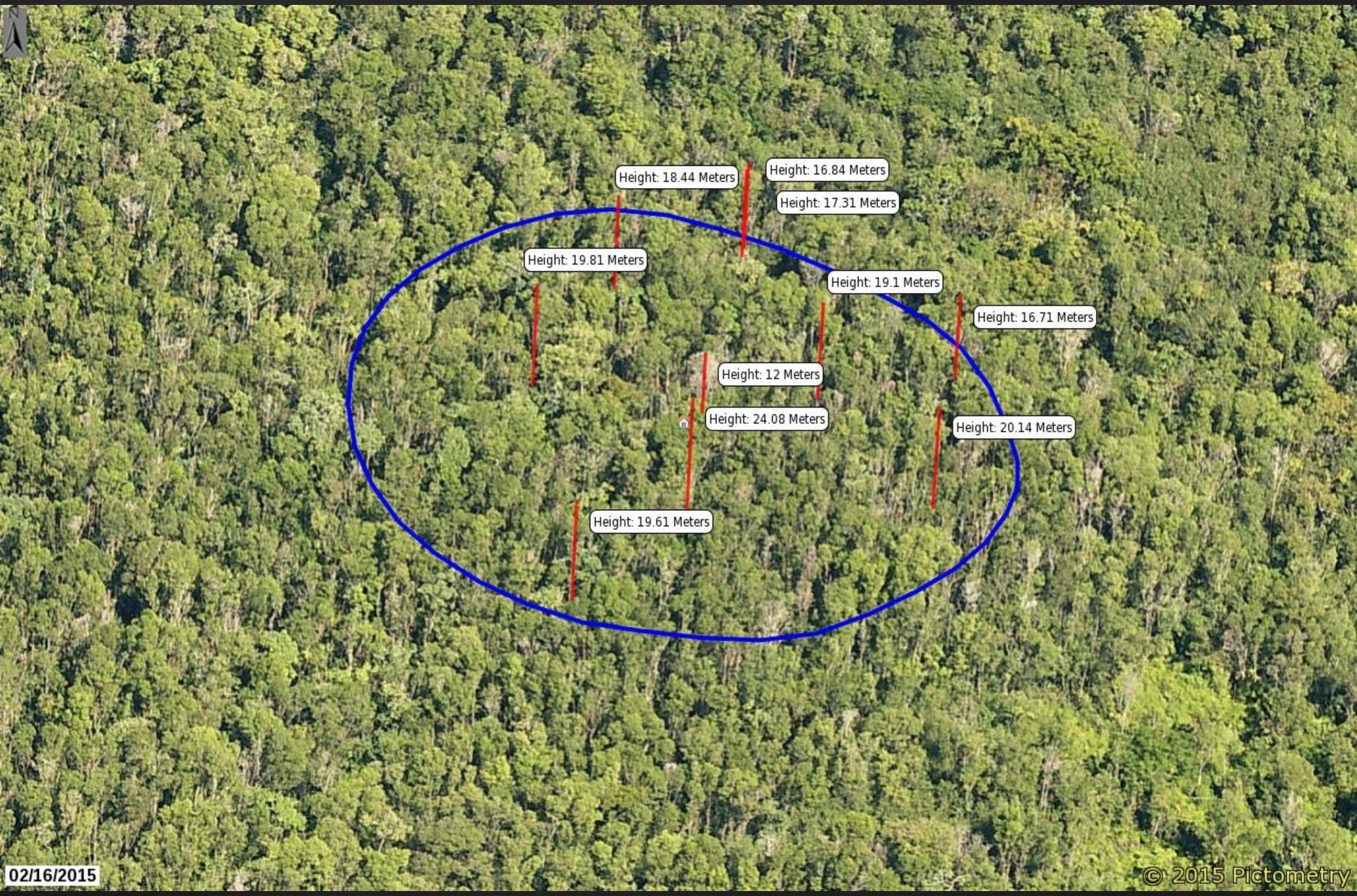
- Mean forest stand canopy height 11 - 44 m (mean = 24)
- Forest stand canopy cover classification (n = 52)

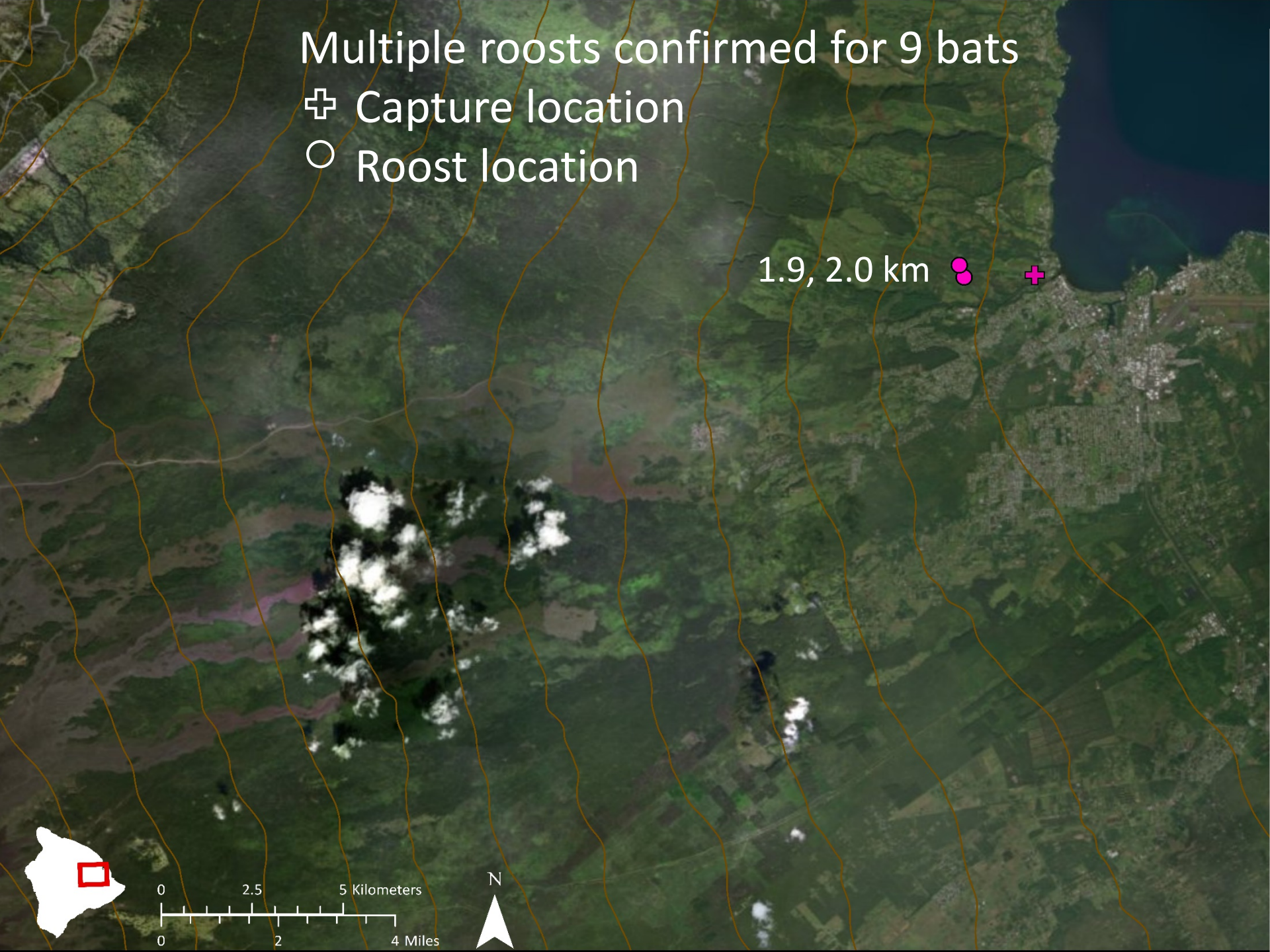


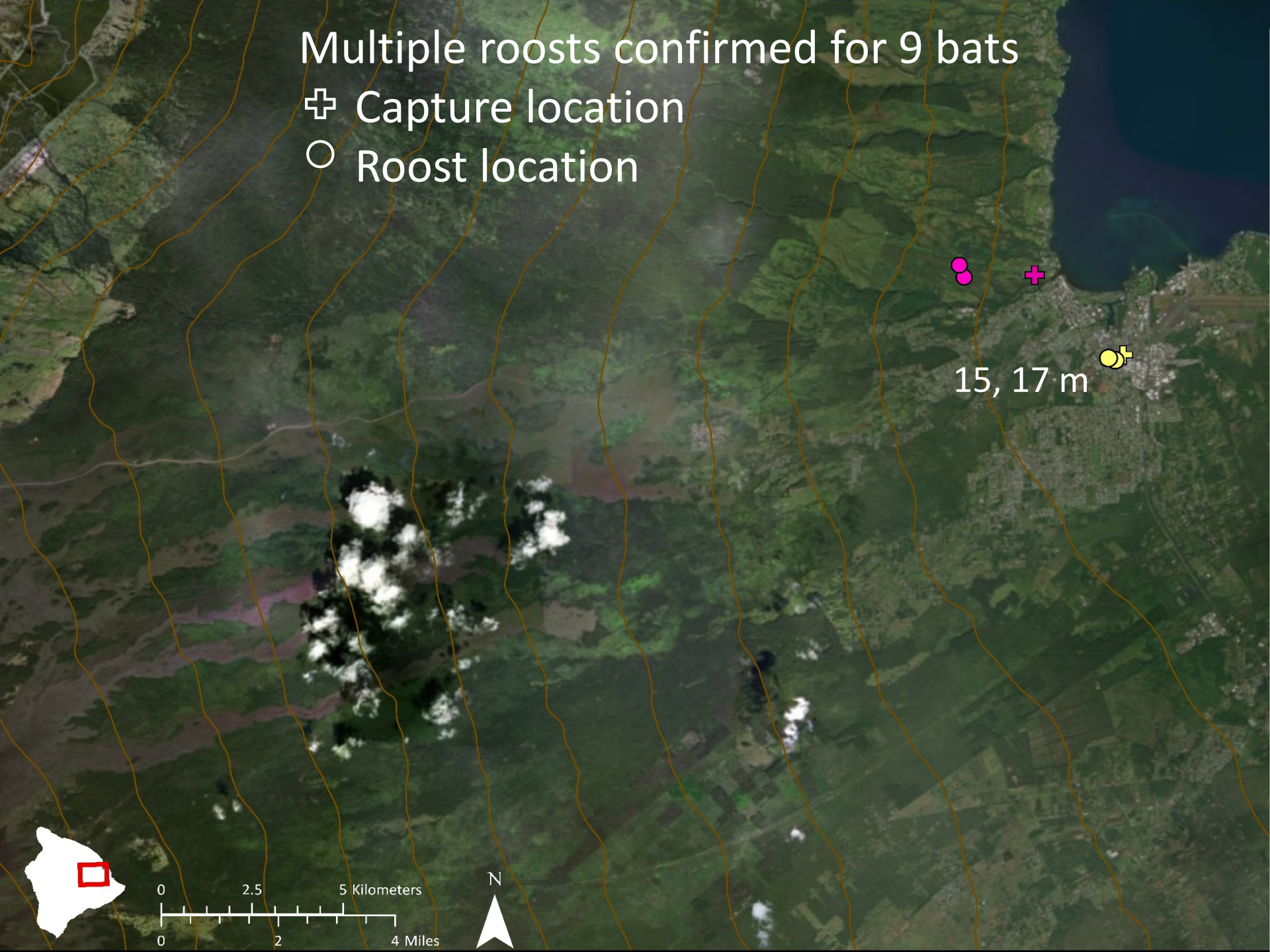

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© 2019 Microsoft Corporation © 2019 DigitalGlobe ©CNES (2019) Distribution Airbus DS

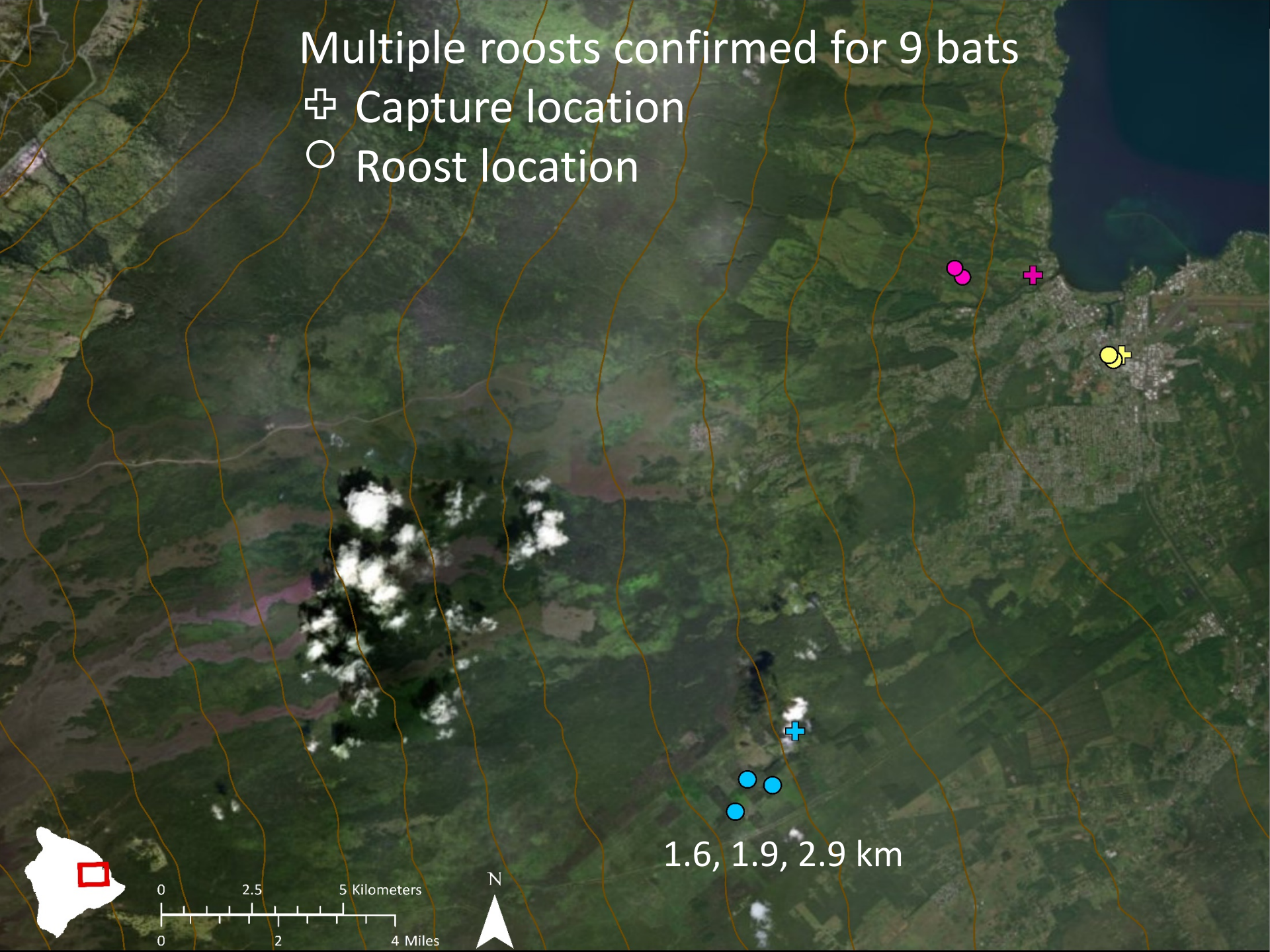

N
02/16/2015
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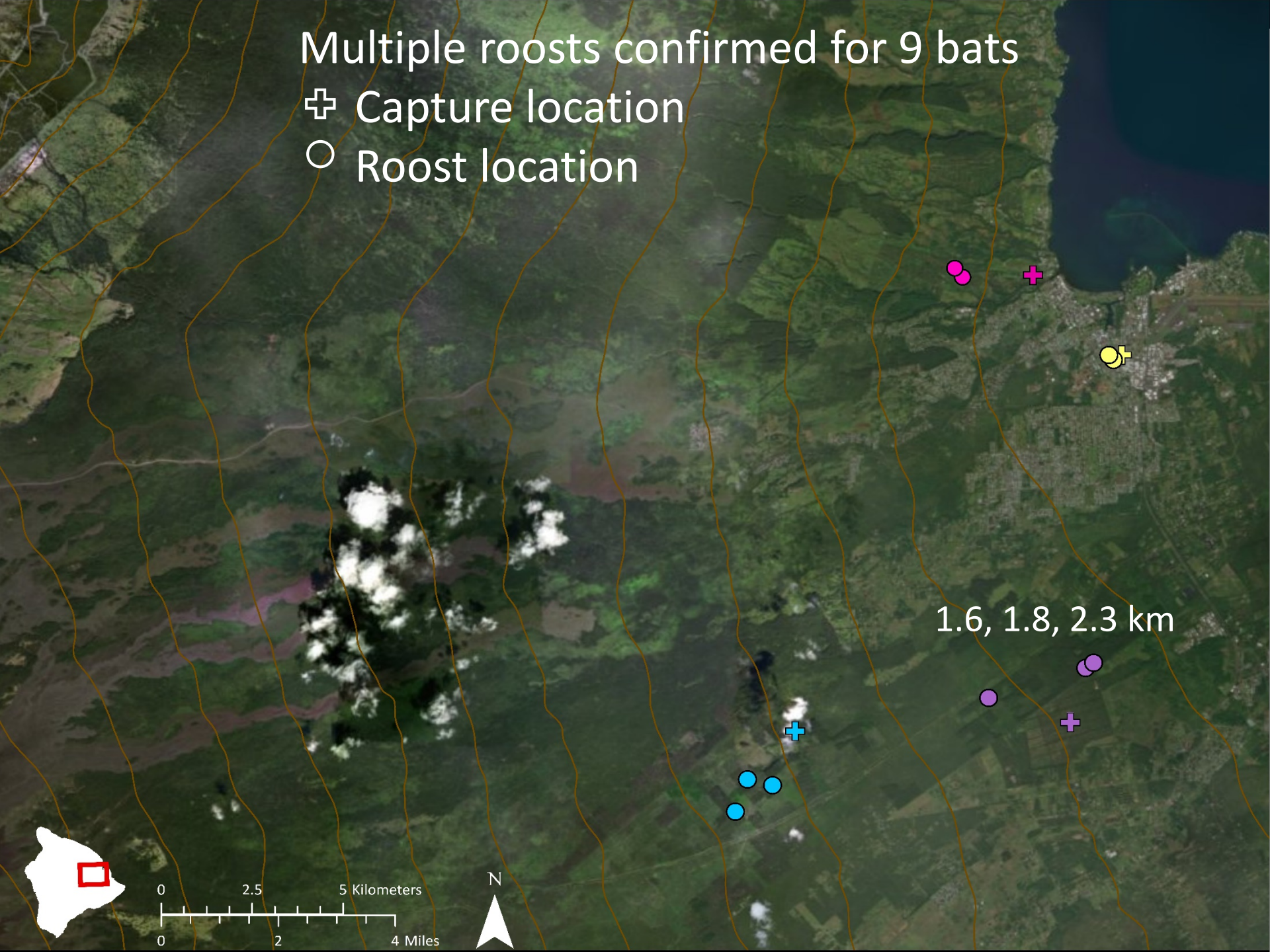

Elevation: 281.14 Meters
East-West Ground Slope
Ground Slope: 9.9 %
Elevation Difference: 9.88 Meters
Distance: 99.94 Meters
North-South Ground Slope
Ground Slope: 3.5 %
Elevation Difference: 3.48 Meters
Distance: 99.96 Meters
Distance To Edge: 69.67 Meters
02/16/2015
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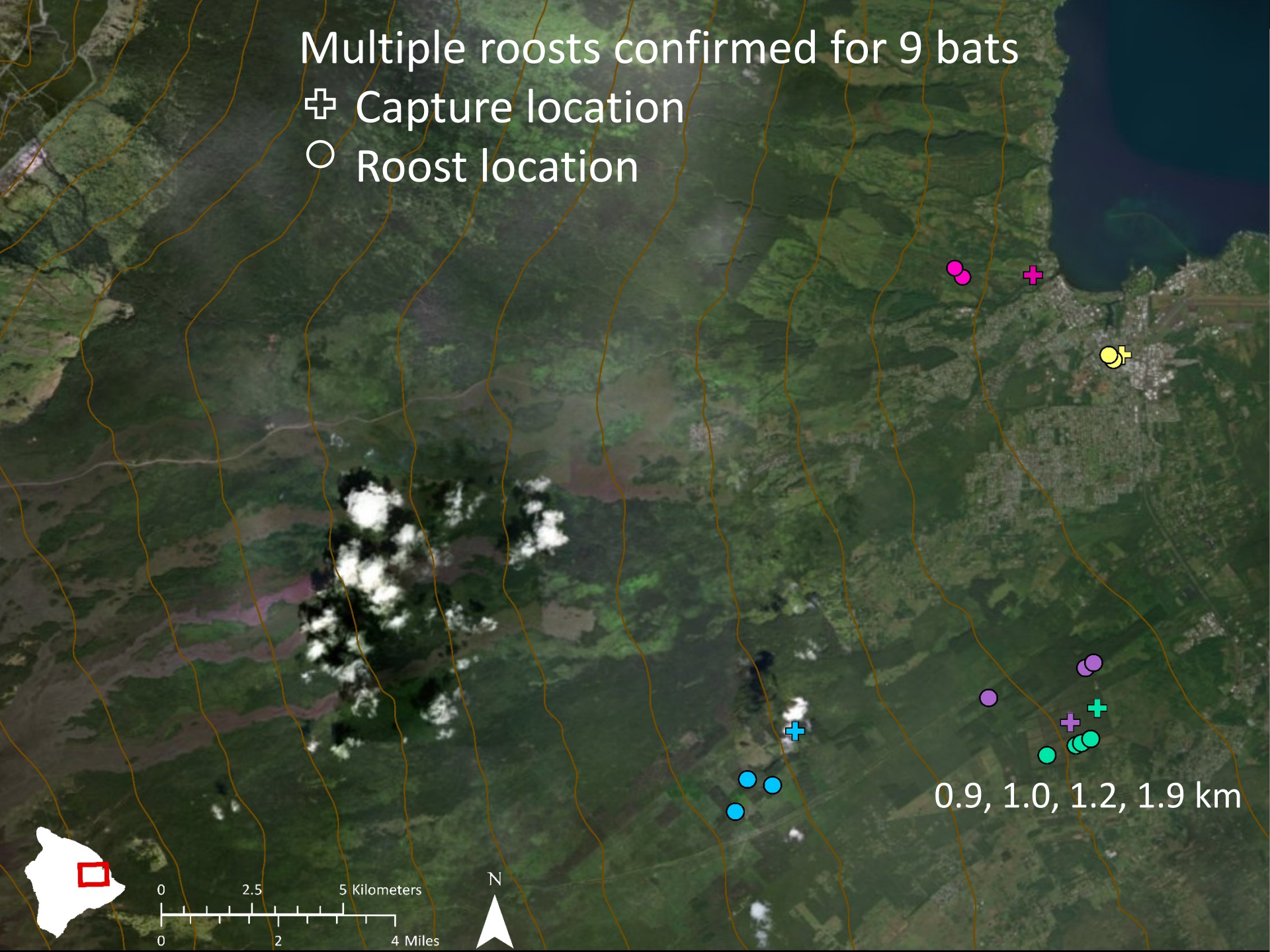

Height: 18.44 Meters
Height: 16.84 Meters
Height: 17.31 Meters
Height: 19.81 Meters
Height: 19.1 Meters
Height: 16.71 Meters
Height: 12 Meters
Height: 24.08 Meters
Height: 20.14 Meters
Height: 19.61 Meters
02/16/2015
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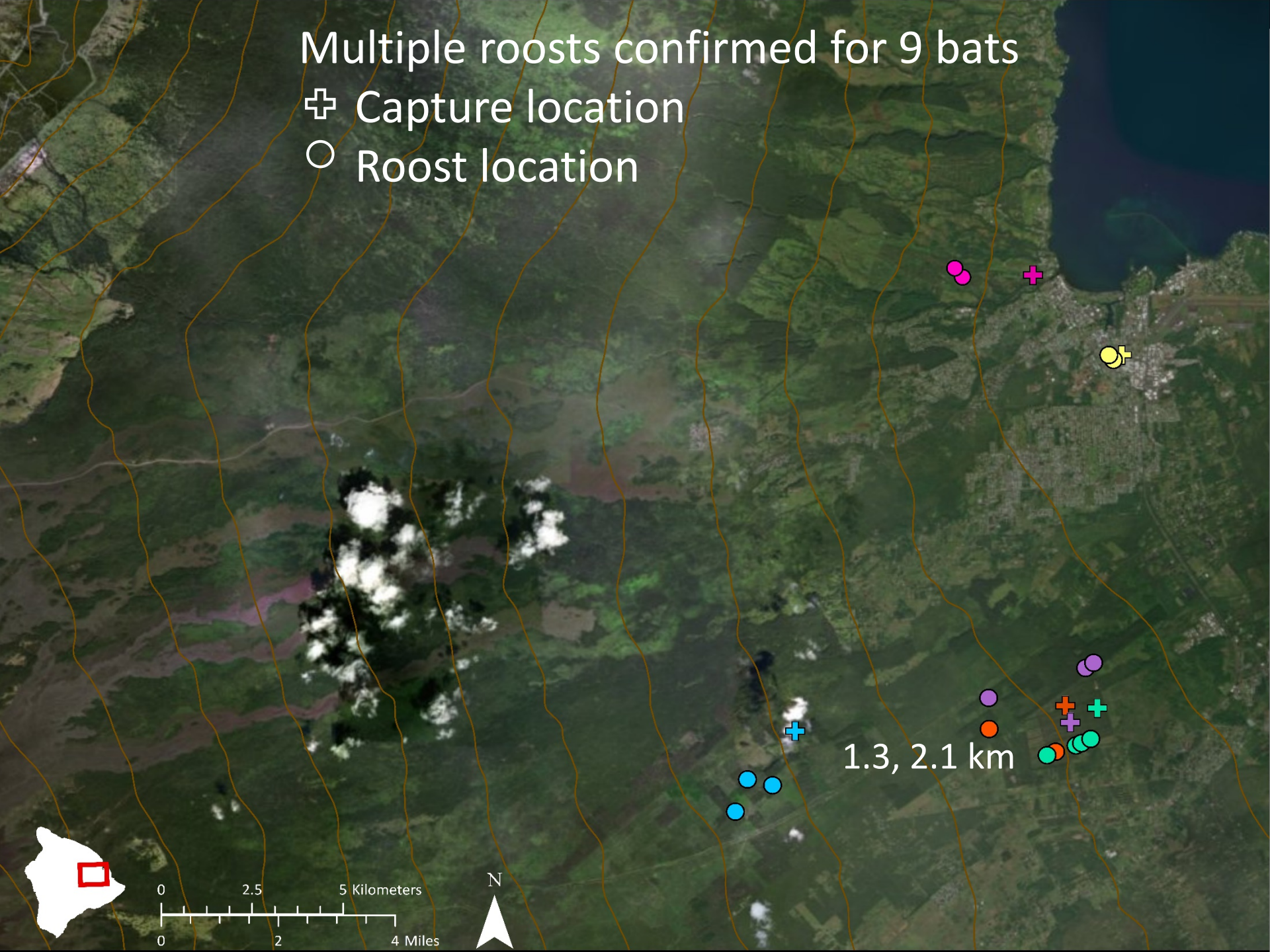

Multiple roosts confirmed for 9 bats
Capture location
Roost location
1.9, 2.0 km
0
2.5
5 Kilometers
0
2
4 Miles
N

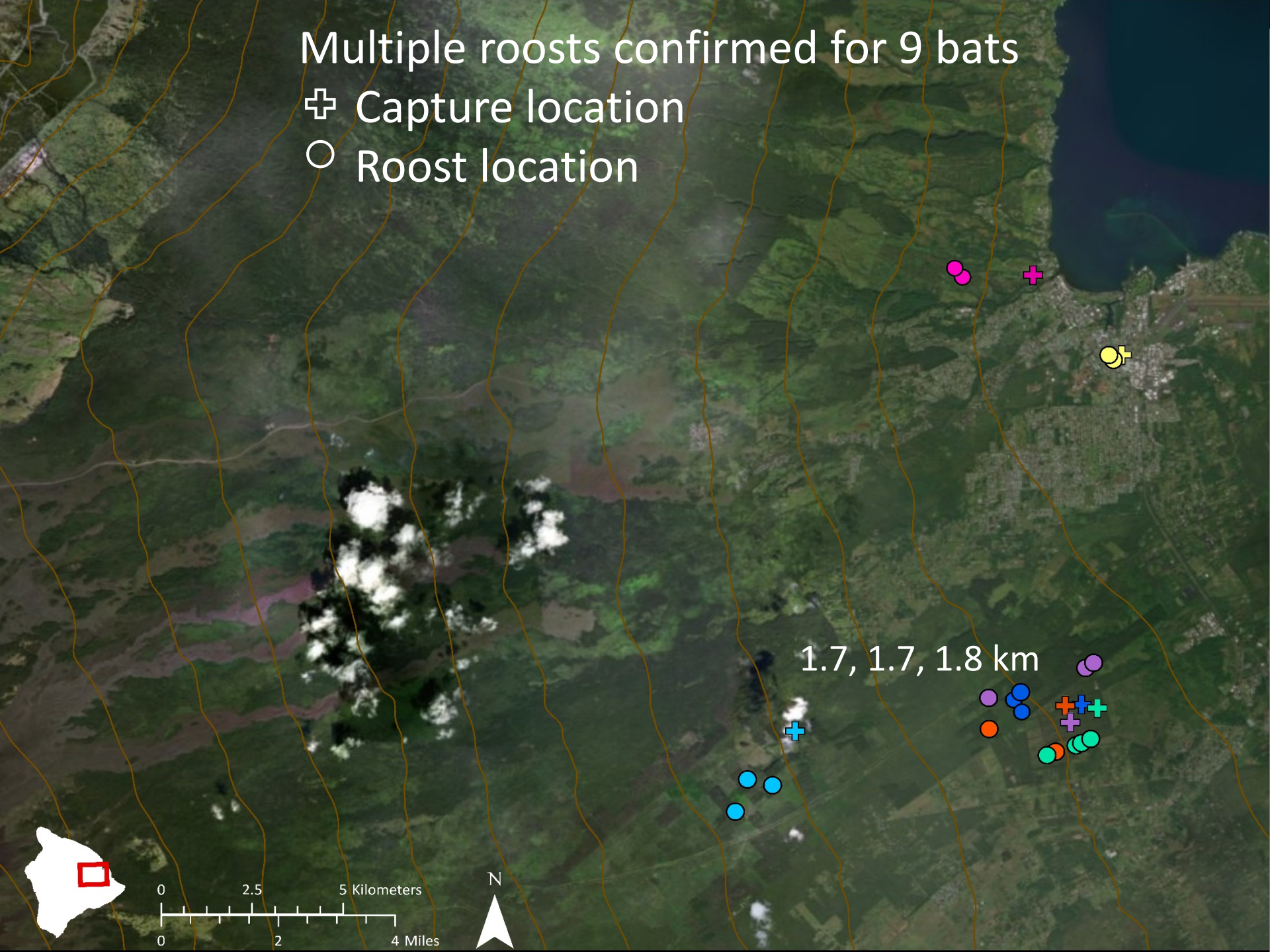

Multiple roosts confirmed for 9 bats
Capture location
Roost location
15, 17 m
0
2.5
5 Kilometers
0
2
4 Miles
N

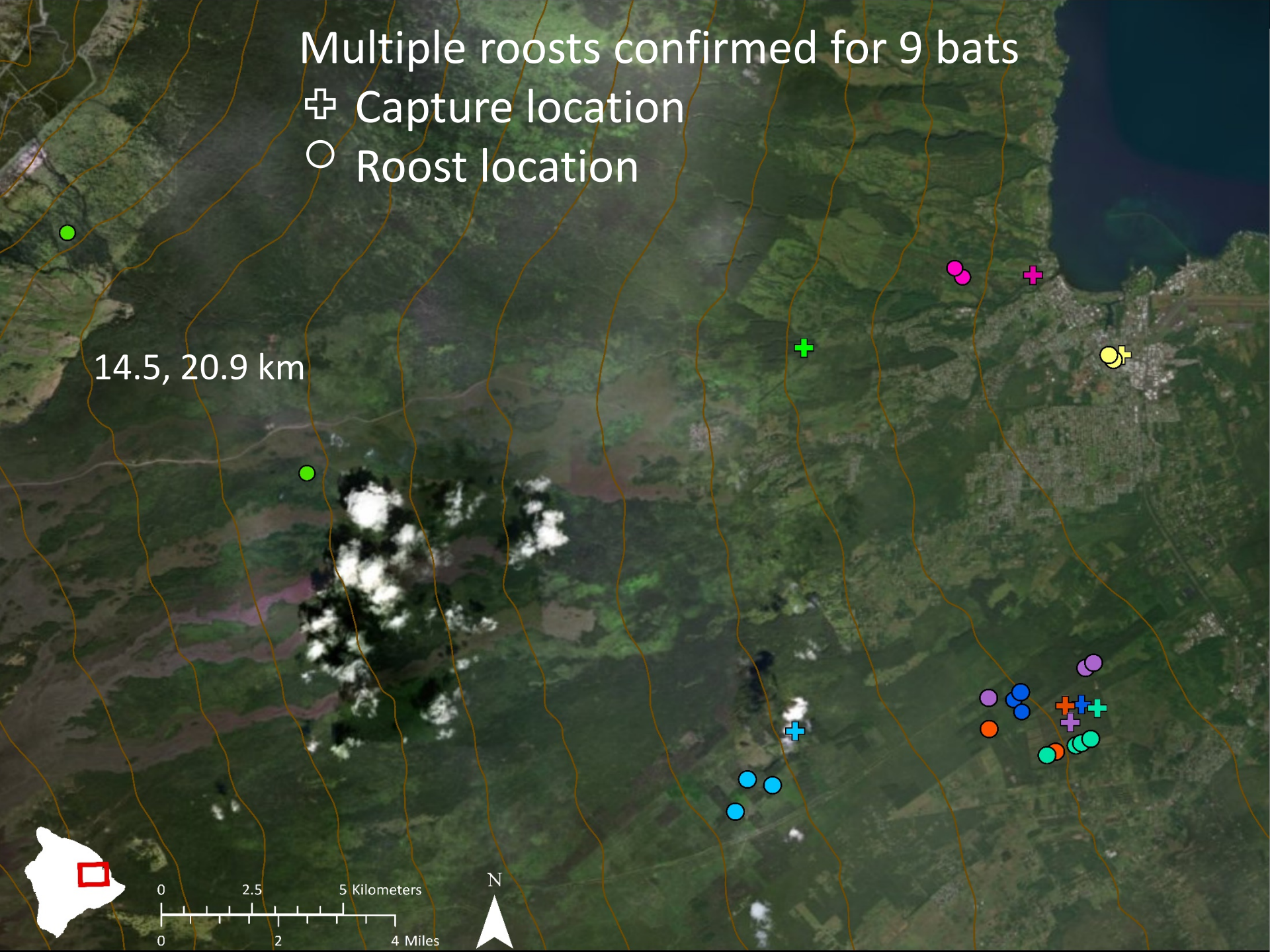

Multiple roosts confirmed for 9 bats
Capture location
Roost location
1.6, 1.9, 2.9 km
0
2.5
5 Kilometers
0
2
4 Miles
N

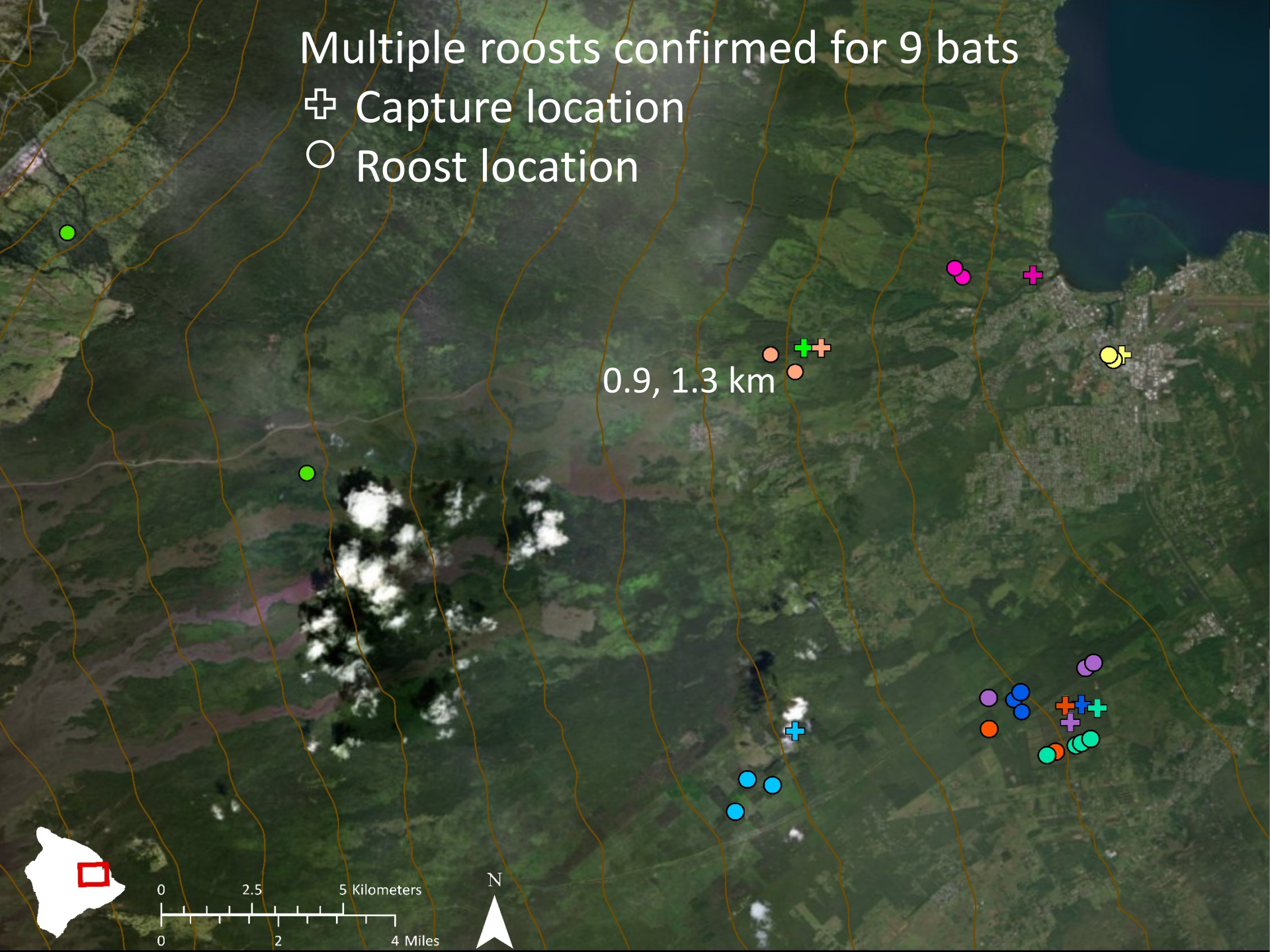

Multiple roosts confirmed for 9 bats
Capture location
Roost location
1.6, 1.8, 2.3 km
0
2.5
5 Kilometers
0
2
4 Miles
N


Multiple roosts confirmed for 9 bats
Capture location
Roost location
0.9, 1.0, 1.2, 1.9 km
0
2.5
5 Kilometers
0
2
4 Miles
N


Multiple roosts confirmed for 9 bats
Capture location
Roost location
1.3, 2.1 km
0
2.5
5 Kilometers
0
2
4 Miles
N


Multiple roosts confirmed for 9 bats
Capture location
Roost location
1.7, 1.7, 1.8 km
0
2.5
5 Kilometers
0
2
4 Miles
N


Multiple roosts confirmed for 9 bats
Capture location
Roost location
14.5, 20.9 km
0
2.5
5 Kilometers
0
2
4 Miles
N


Multiple roosts confirmed for 9 bats
Capture location
Roost location
0.9, 1.3 km
0
2.5
5 Kilometers
0
2
4 Miles
N

Maternity Roosts

- 3 maternity roosts monitored in 2019 reproductive season
  - 1 bat with 1 pup

    Urban habitat, Lychee
  - 1 bat with 2 pups

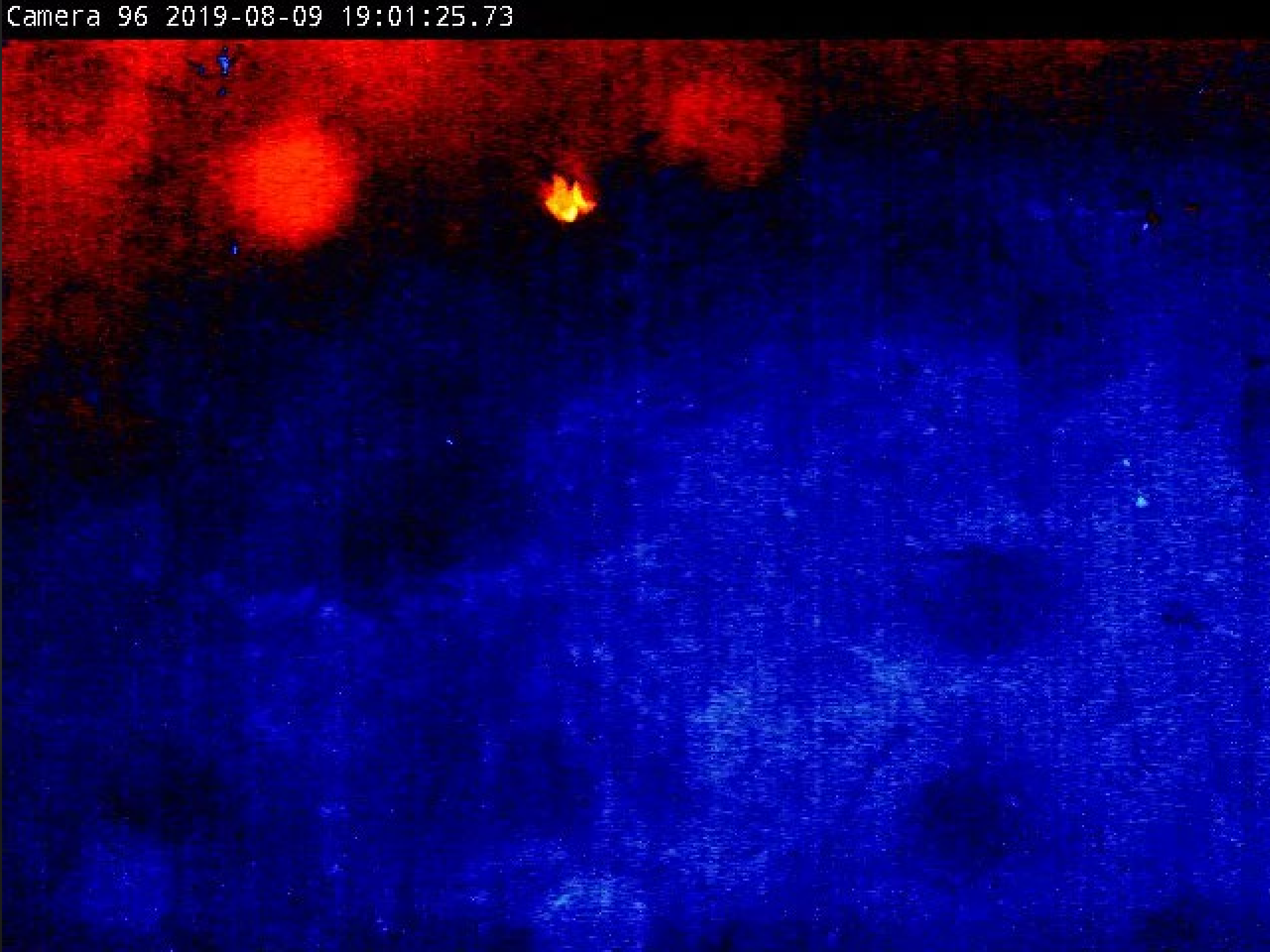
    Mixed native/non-native plantation, Eucalyptus sp.
  - 1 bat with 1 pup

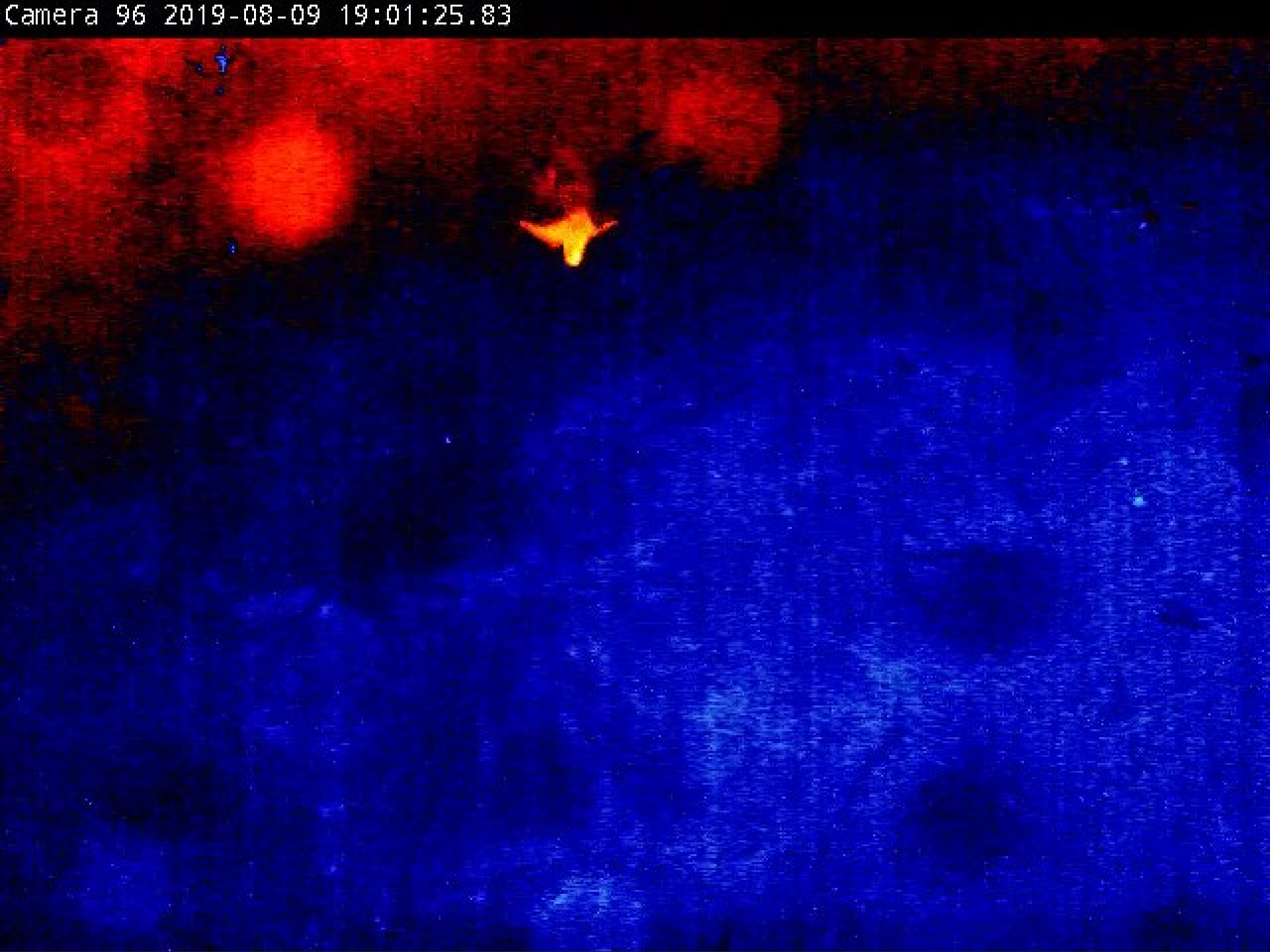
    Non-native agriculture/plantation habitat, Paperbark

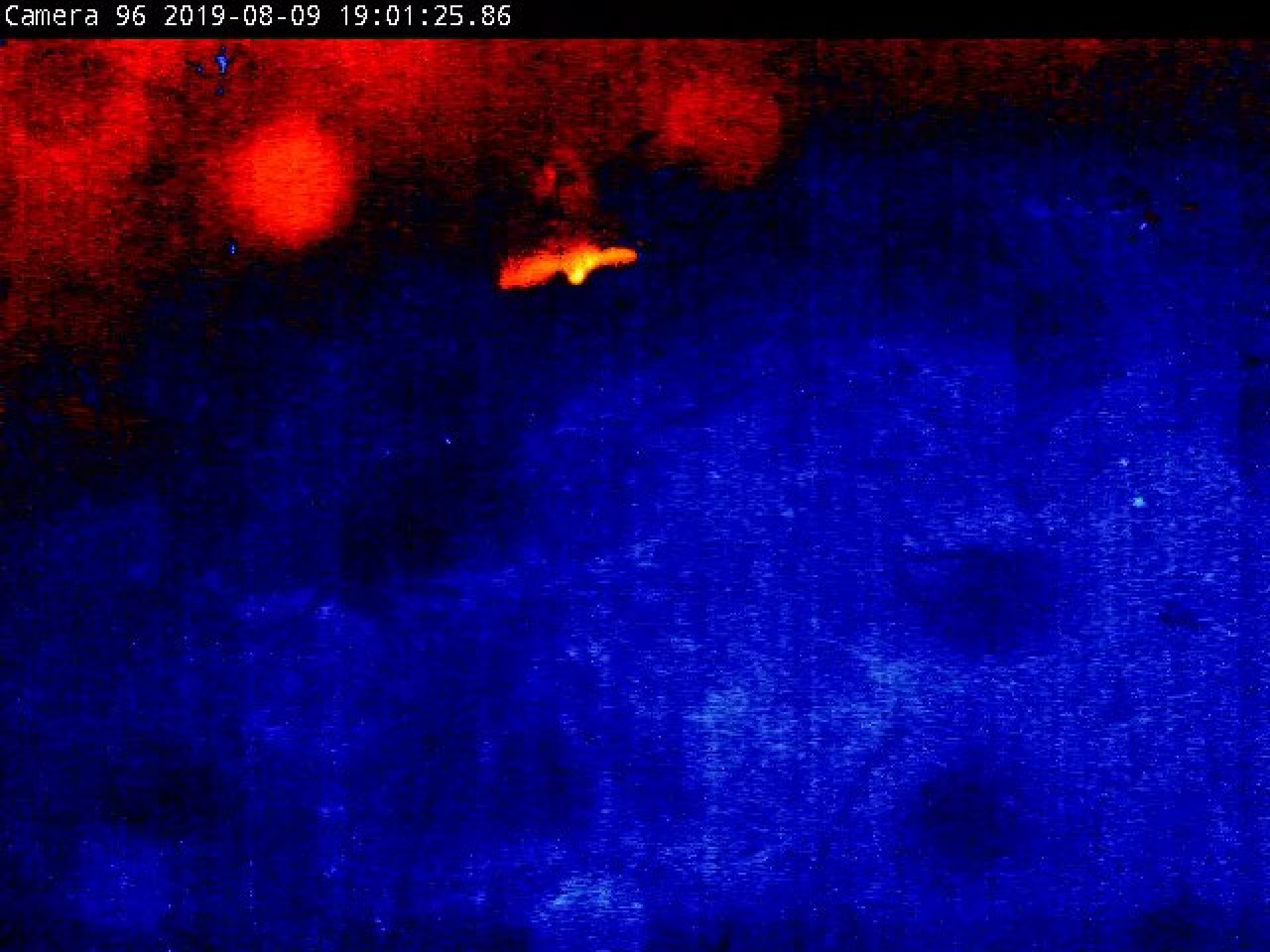




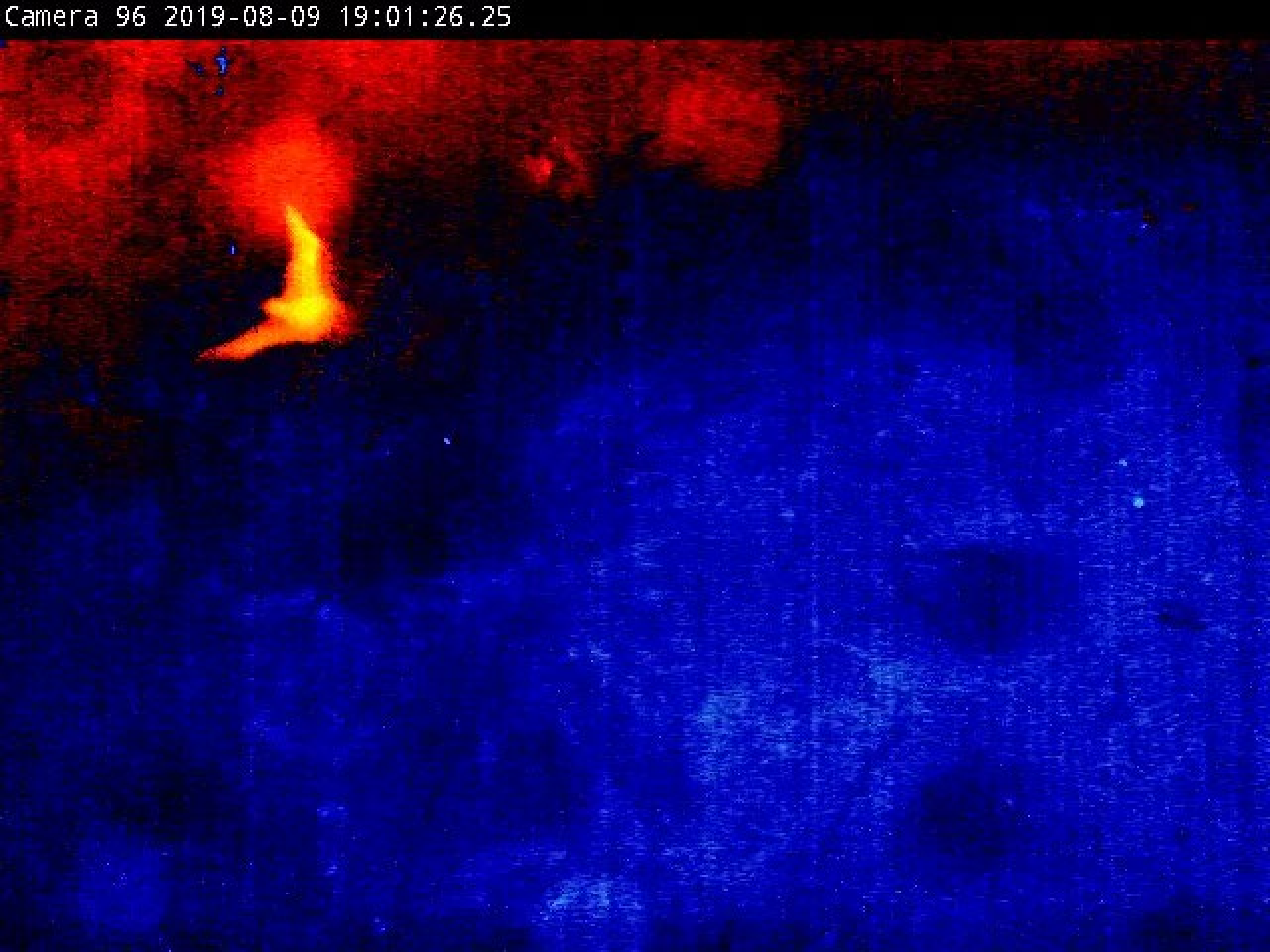


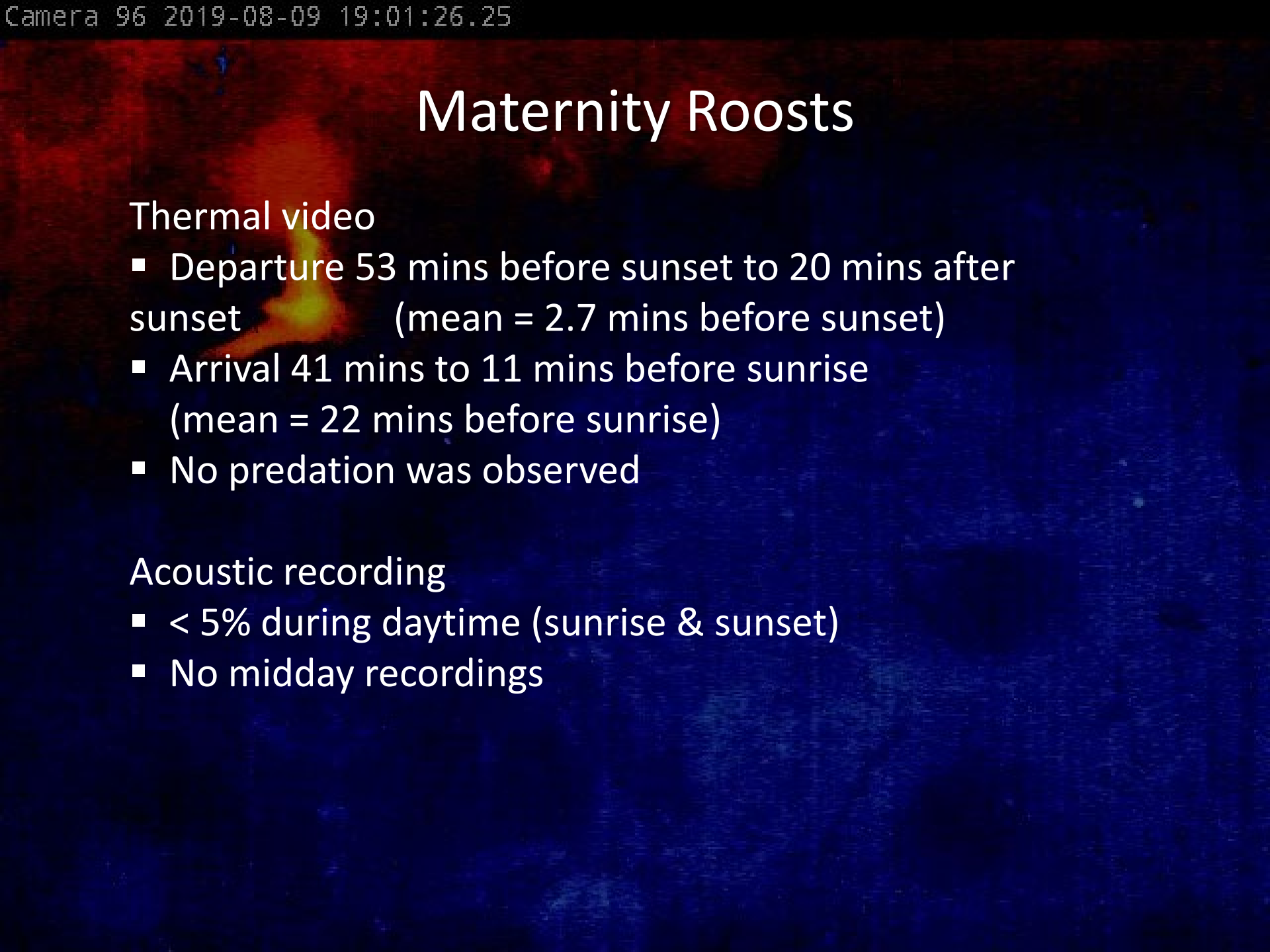

Camera 96 2019-08-09 19:01:25.73


Camera 96 2019-08-09 19:01:25.83


Camera 96 2019-08-09 19:01:25.86


Camera 96 2019-08-09 19:01:26.02


Camera 96 2019-08-09 19:01:26.25



Maternity Roosts

Thermal video

- Departure 53 mins before sunset to 20 mins after sunset (mean = 2.7 mins before sunset)
- Arrival 41 mins to 11 mins before sunrise (mean = 22 mins before sunrise)
- No predation was observed

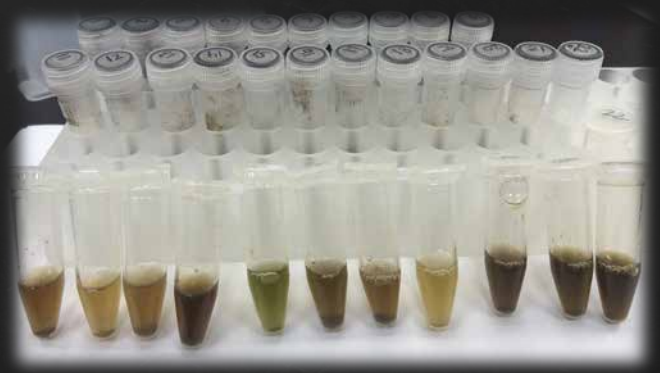
Acoustic recording

- < 5% during daytime (sunrise & sunset)
- No midday recordings

Diet metabarcoding of Hawaiian hoary bats

- Metabarcoding methodology
- Current progress from Hawaii Island study
- Bat prey availability and diet example
- Results: Waihou Bat Restoration Site, Maui (2019)
- Future directions for bat diet research





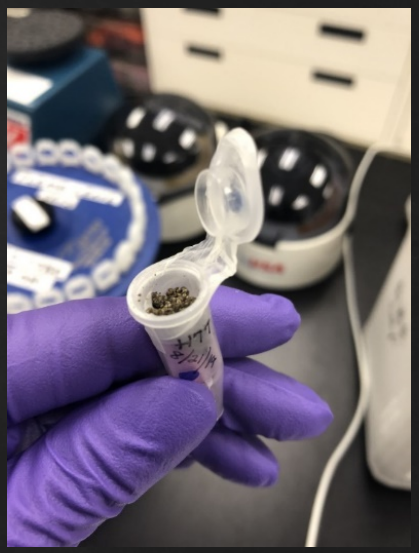
Objectives

- Bat diet composition and insect prey availability
  - Bat diet - age, sex, reproductive season, foraging habitat type
  - Prey availability – season, foraging habitat type
  - Metabarcoding to compare bat diet contents to available prey

- Prey-host plant associations
  - Link insects found in bat diet to host plants used in habitat restoration

What is metabarcoding?

- Extraction of environmental DNA from gut or feces → high-throughput sequencing of taxonomically informative markers

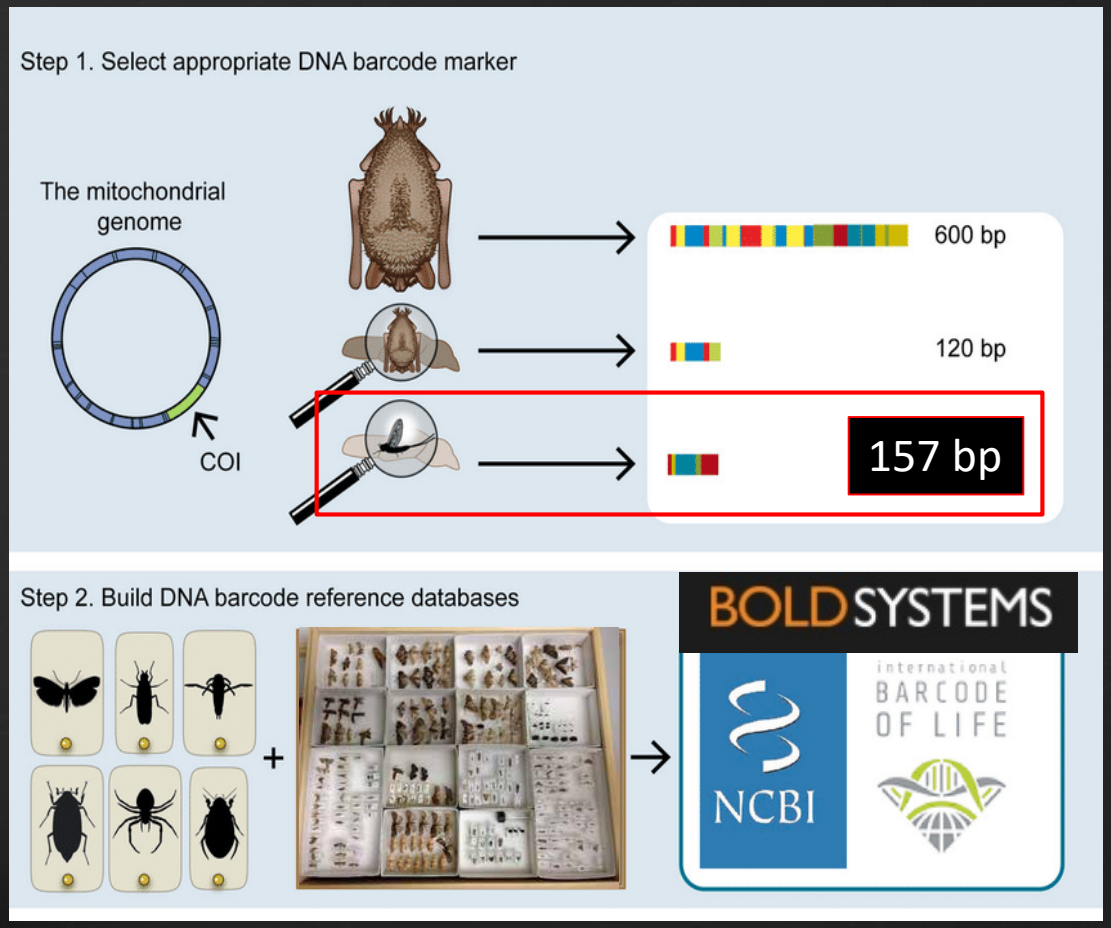


- Advantages
  - taxonomic species level prey identification
  - process many samples simultaneously

- Requires knowledge of
  - the ecological system under study – what prey are available for bats to consume?
  - analytical procedures used to process samples
    - What genetic markers will detect prey items in guano?
    - Is local insect fauna genetically characterized to properly identify prey contents?

Alberdi et al. 2018

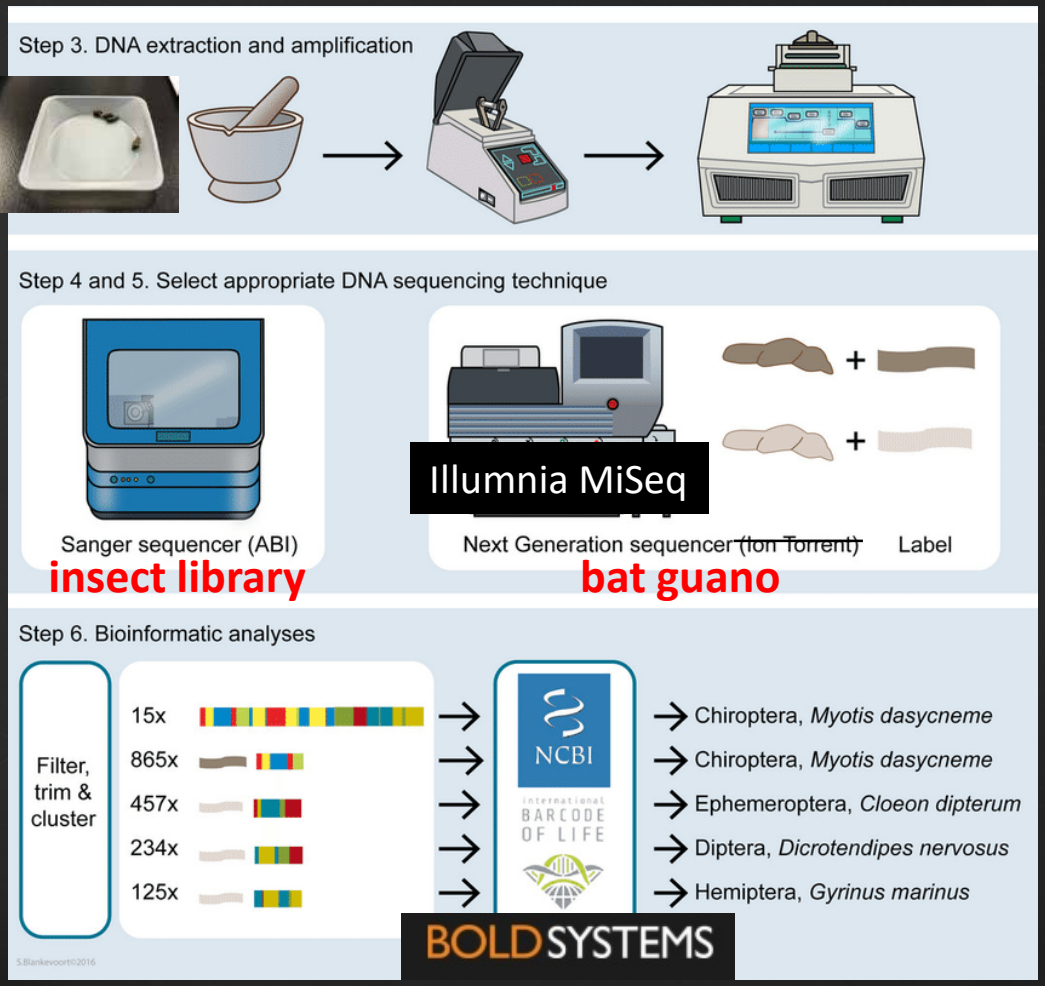
Metabarcoding methods



- Marker: CO1 “Zeale” arthropod specific primers
- Good for identifying Lepidoptera, Coleoptera
- Hawaiian insects not well represented on public barcode databases
- Building local allows higher taxonomic level identification (genus-species)

Haarsma et al. 2016

Metabarcoding methods



- Extract DNA
  - bat guano, local insects
- Sequence
- Analyze diet
  - Operational Taxonomic Units (OTUs) ~diversity~
  - Sequence counts ~relative abundance~
- Identify prey taxa
  - Compare diet to local library, public databases

Haarsma et al. 2016

Current progress

- Bat guano – first year samples analyzed
  - 35 individuals across 8 sites
  - 13 females, 22 males

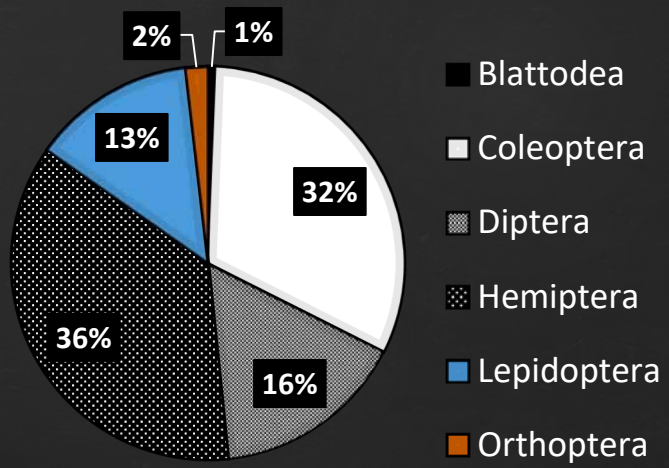


- Local insect prey barcode library
  - Insect selection, DNA extraction starting next quarter
- Insect collection efforts
  - Sorted to order, family – focus on Lepidoptera, Coleoptera, Diptera
- Prey-host plant associations
  - Starting caterpillar collection at sites



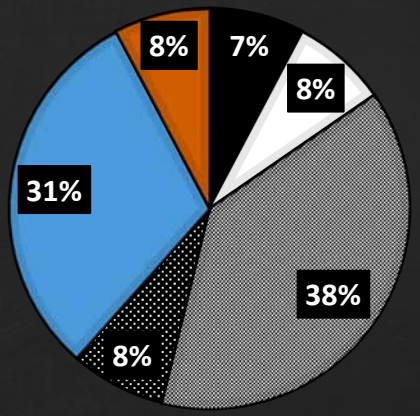
Example of prey and diet of a female bat August, low elevation native/exotic mix habitat

Prey availability (sampled at site)



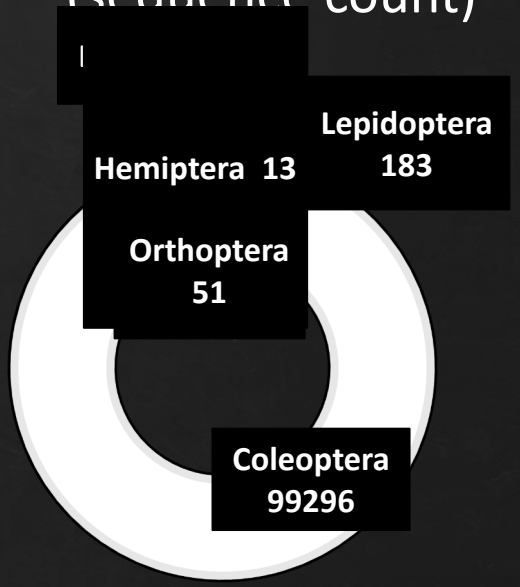
Count of prey insects 2,220

Bat diet diversity (OTUs)



OTUS, 13 unique

Relative abundance in diet (sequence count)



OTU sequence count 99,842

Linking bat diet and prey availability at Waihou, Maui





Hawaiian hoary bat activity, diet, and prey availability at the Waihou mitigation area, Maui

Pinzari et al. 2019. Technical Report HCSU-090

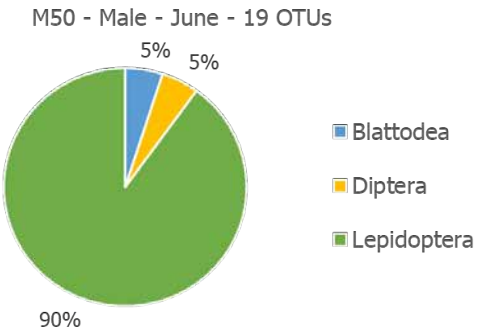
https://doi.org/10.5066/P9U0KRMY

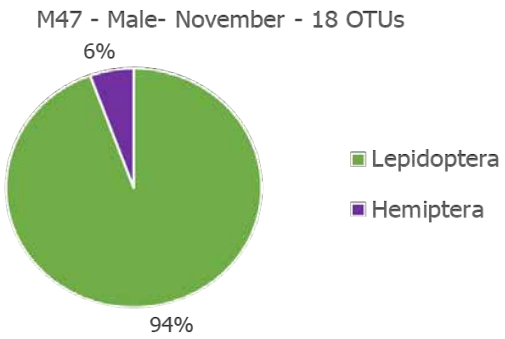


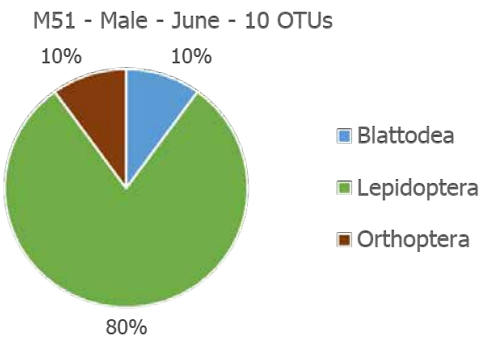
- 42 unique prey taxa found in bat diet (9 bats)
- 7 arthropod orders (Blattodea, Coleoptera, Diptera, Ephemeroptera, Hemiptera, Lepidoptera, Orthoptera)
- 32 families – 18 within Lepidoptera
- 13 species-level identifications
- Bat prey is available at Waihou Restoration Area

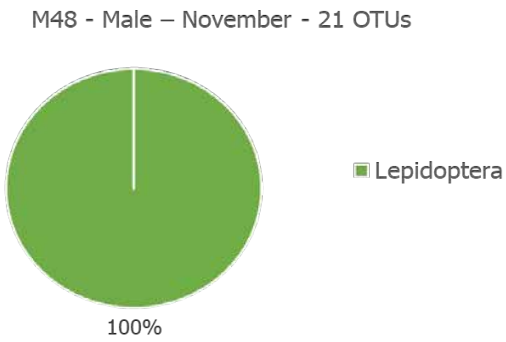
genetic analysis of guano samples affords *greater taxonomic resolution* and *better prey identification* of soft-bodied insects

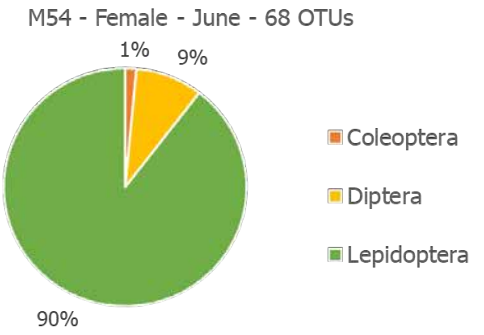
Guano – bat diet diversity

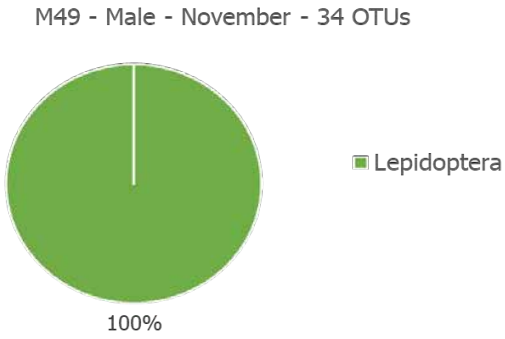






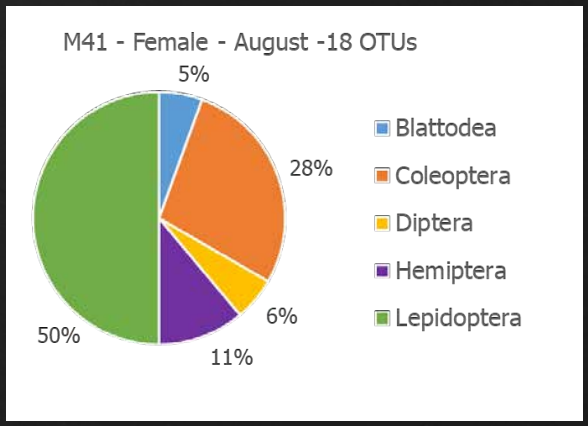






Waihou
high percentage of Lepidoptera, especially November

Single bat from Auwahi Wind Energy, highest diet diversity



2 females:
Coleoptera in diet

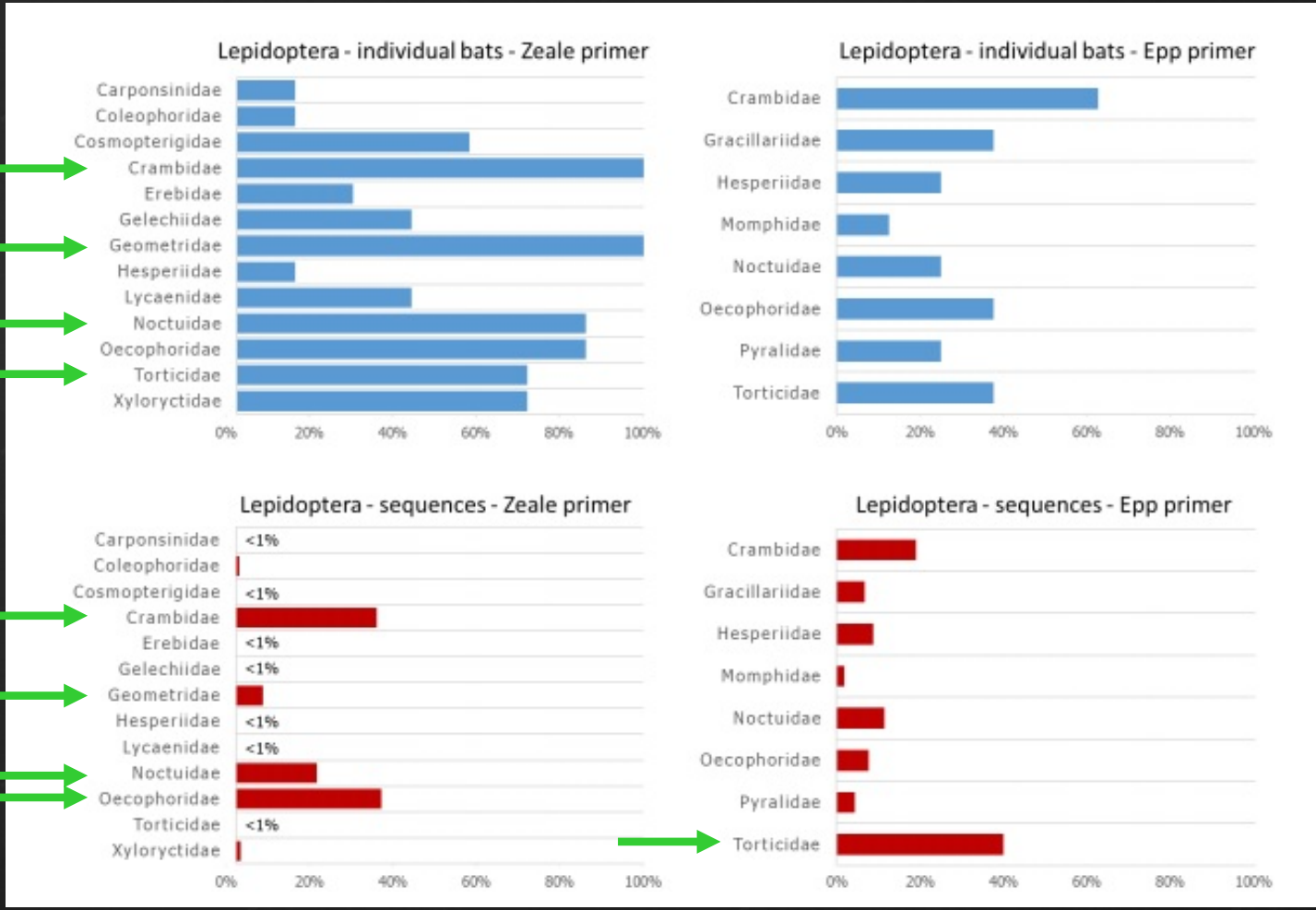
Bat diet diversity & prevalence – Lepidoptera families

Zeale primers

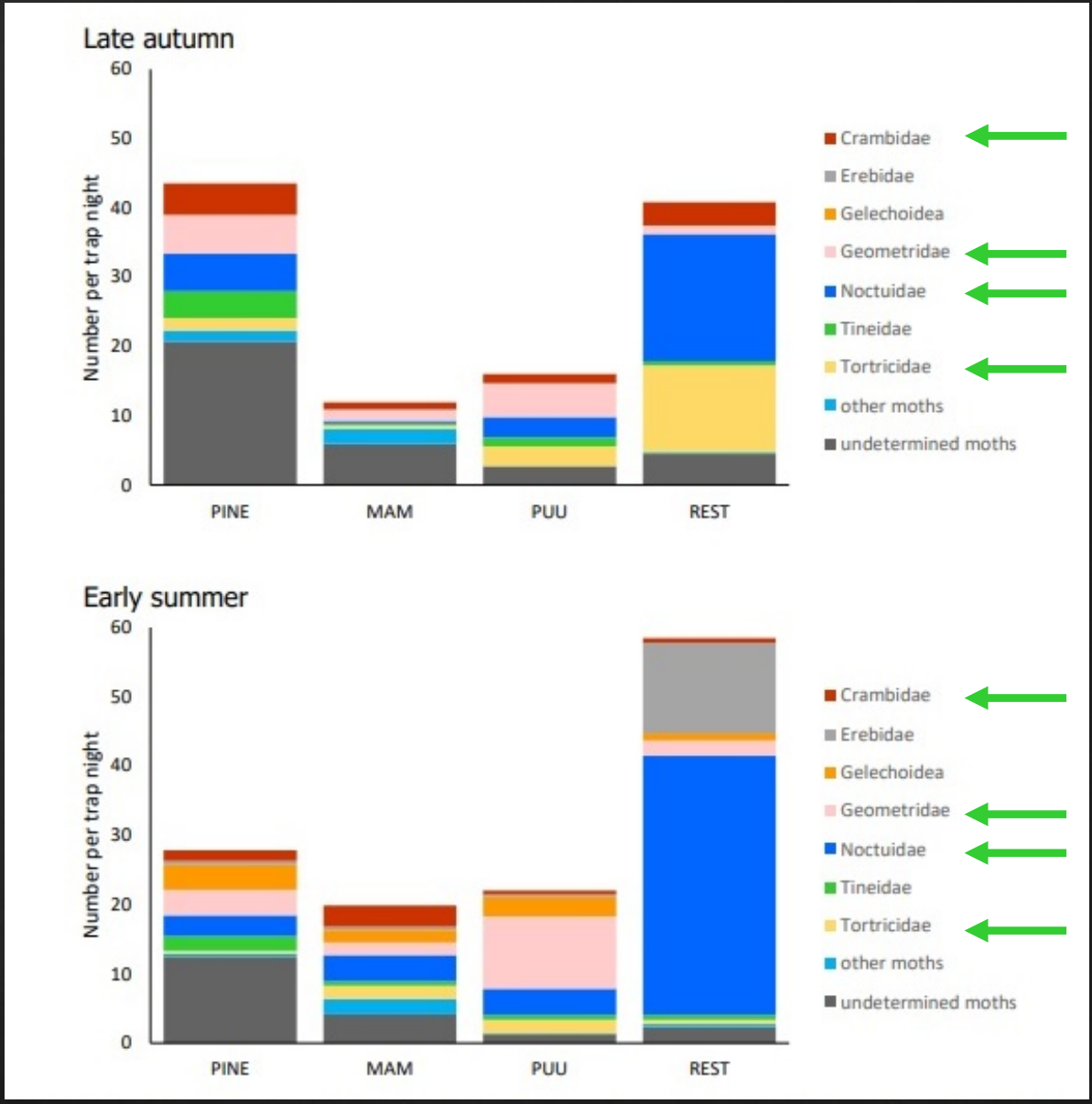
Epp primers

Prevalence (number of individual bats in which families were detected)

Relative abundance (sequence count)



Spatial & temporal prey availability (insect captures)



Waihou insect reference library

Order	Family	Subfamily or Genus and Species	Individuals barcoded
Coleoptera	Hydrophilidae	*Sphaeridium scarabaeoides*	1
	Scarabaeidae †	*Aphodiinae* sp.	1
		Digitonthophagus gazella †	1
	Staphylinidae	*Philonthus* sp.	1
Diptera	Sepsidae	*Sepsis thoracica*	1
Hemiptera	Cydnidae†	*Pangaeus bilineatus*	1
	Lygaeidae	*Lygaeidae* sp.	1
Lepidoptera	Carposinidae †	*Carposina* sp. *	1
	Cosmopterigidae †	*Hyposmocoma* spp. *	5
	Crambidae †	*Omiodes* spp. *	4
		Udea sp.	2
		Uresiphita polygonalis *	1
		unknown sp.	2
	Erebidae †	*Melipotis indomita*	1
		Schrankia sp.	3
	Geometridae †	*Eupithecia* sp. *	2
		Scotorythra spp. *	7
		unknown sp.	1
	Noctuidae †	*Athetis thoracica*	3
		Chrysodeixis eriosoma	1
		Feltia subterranea †	1
		Ophiusa disjungens	1
		Peridroma saucia †	3
		Pseudaletia unipuncta †	1
		Spodoptera exempta	1
		unknown sp.	1
	Tineidae	*Opogona sacchari*	1
	Tortricidae †	*Acleris* spp.	2
		Amorbia emigratella	2
		Crocidosema sp. †	1
		unknown sp.	1
	Sphingidae †	*Hyles lineata*	1
	Xyloryctidae †	*Thyrocopa* sp. *	1

- 57 individuals
  - Lepidoptera
    Coleoptera
    Diptera
    Hemiptera
- Overlap between library and guano contents
  - 11 families (all orders)
  - 9 Lepidoptera families

Consumption of native & non-native species

- Native insect families (18 total)
- Agricultural pests, introduced biocontrol agents
  - *Feltia subterranean (*granulate cutworm*)*
  - *Peridroma saucia (*variegated cutworm*)*
  - *Pseudaletia unipuncta (*army worm*)*
  - *Nezara viridula (*southern green stink bug*)*
  - *Crocidosema lantana (*lantana tortricid moth*)*
  - *Digitonthophagus gazella (*gazelle scarab*)*



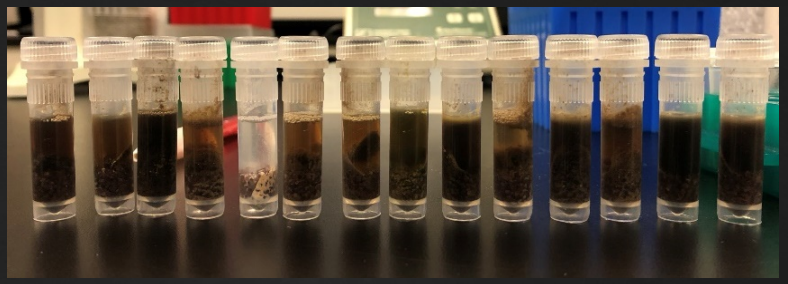



Future bat diet research

- Diet on other islands?
- Diet in other habitats being preserved for bats?
  - Dry forest
  - Wetlands
  - Bat habitat restoration sites







- Collect and store guano in future studies utilizing capture techniques
- Analyze 22 existing samples from USGS guano collection
  - Kauai, Oahu, Hawaii
- Analyze stomach contents of bats found under wind turbines

Population genetics & genetic sexing



Objectives

- Bat conservation genetics & progress
- Completed research results
  - Sex determination of Hawaiian hoary bat carcasses found at wind facilities
- Future research directions on bat demography
- Basic Research
  a) demographic information
  b) genetic variability, population structure, effective population size, population dynamics



Contributes to objectives: diet, prey selection, population modeling



Acoustic surveys show widespread distribution

But...

Abundance

Sex

Bats are difficult to catch, track, locate to roost...

Genetic approach to understanding populations

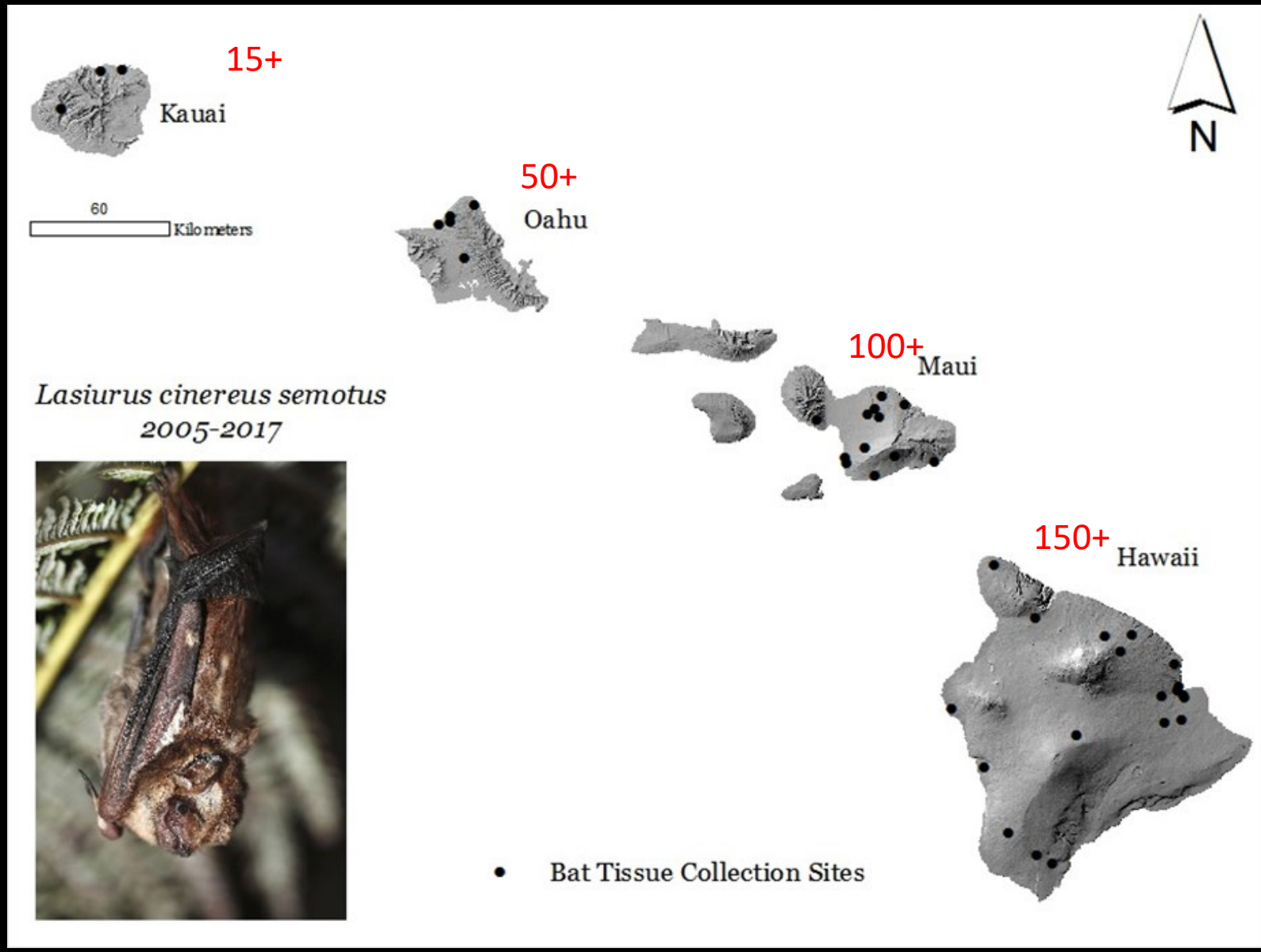
Hawaiian hoary bat conservation genetics tasks



- Collection of bat tissue samples for DNA analyses, sex genotyping
  - Completed
    - ~330 (2005 – present) including representatives from Hawaii, Maui, Oahu, and Kauai
    - ~ 80 samples from wind facilities statewide (missing small %)
- Increase geographic coverage where possible
  - West Kauai – 13 live bat capture September 2019
  - East Maui – increased samples through Auwahi Tier 2 bat research project (11), samples from HT Harvey Maui bat research (20)
  - Molokai, Lanai not sampled



Tissue sampling map



Hawaiian hoary bat conservation genetics tasks

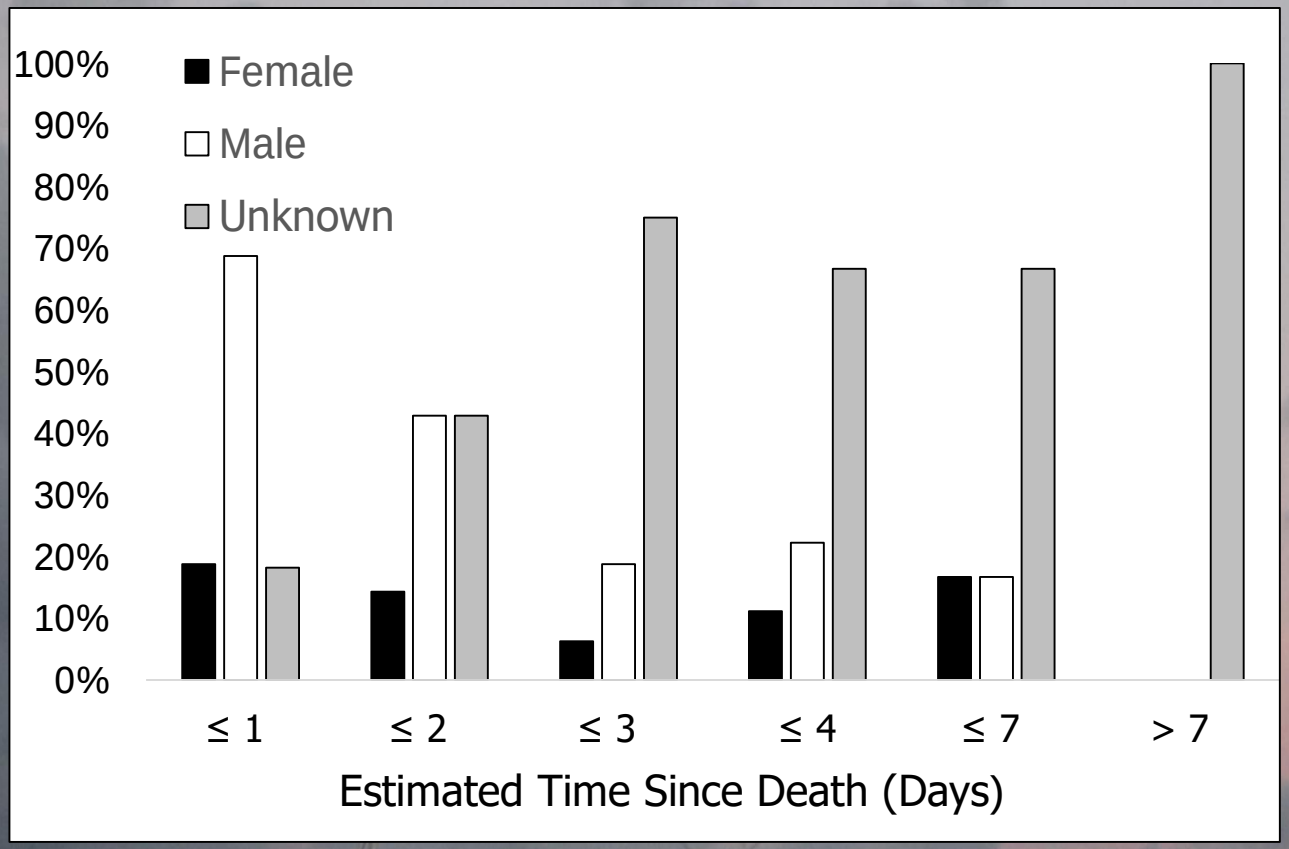


- Mitochondrial DNA COI marker
  - 140 bats sequenced, ~190 in progress
- Nuclear microsatellites for Hawaii, Maui, and Oahu
  - Testing 19 markers published in North American hoary bats – 6 completed
- Single nucleotide polymorphisms (SNPs) ~ collaboration with Virginia Tech
  - First manuscript in submission (23 bats)
    - Genomic diversity, population structure, colonization timing,/phylogeny, genetic signatures of selection & divergence
  - Sequence 24 additional bats
  - Assemble reference genome and generate library of SNP loci

Was this bat female or male?







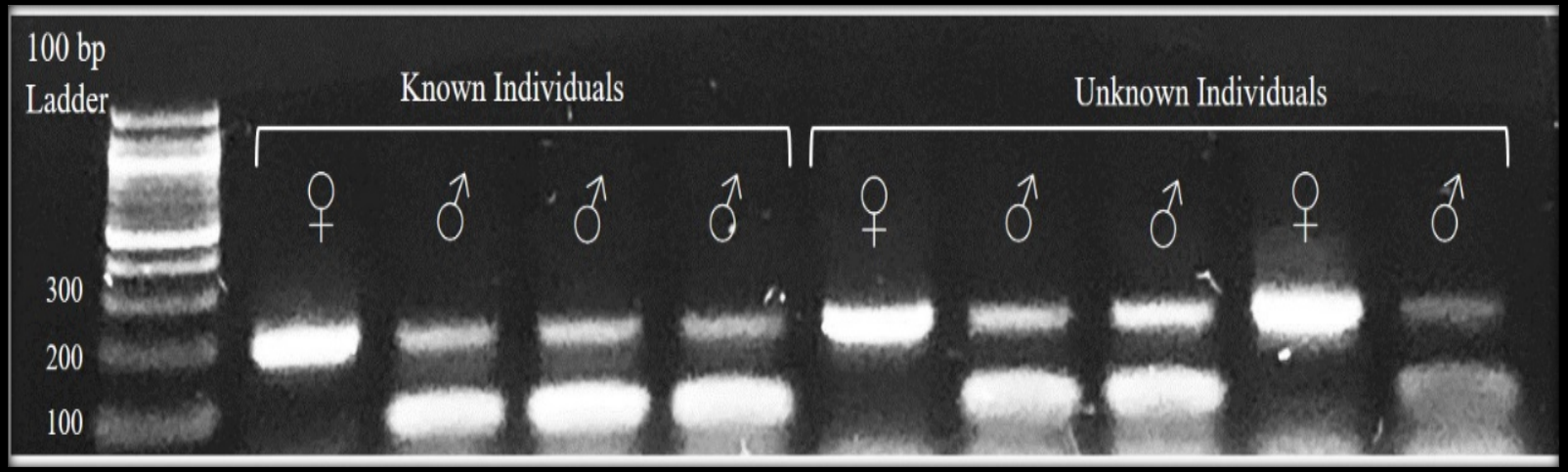
Sexual dimorphism

- Females larger than males
- Overlap in size between juvenile females and adult males



Genetic method for sex determination in bats

- Amplify Zfx and Zfy introns
- Tested multiplex PCR and primer set (Korstian et al. 2013)
- Gel electrophoresis and visualization under UV light
- Males produce two bands (X and Y present)
- Females produce one large band (XX present)



A test of sex specific genetic markers in the Hawaiian hoary bat and relevance to population studies.

HCSU Technical Report TR-085.
http://hdl.handle.net/10790/4375

- Reliable evaluation of sex ratio in subpopulations affected by fatalities
- USGS Data Releases
  https://doi.org/10.5066/P9R7L1NS
  Version 1 (October 2018)
  Version 2 (November 2018)
  Version 3 (April 2019)
  Version 4 (March 2020)



Future directions: Demographics



- Age and reproductive determination in Hawaiian hoary bats
  - Assess level of DNA methylation in tissue, relationship to age
  - Measure age by tooth wear or growth
  - Examine carcass reproductive tracts
  - Current collection: carcasses, museum specimens, tissue samples

- Basic research, demography
  - Data useful for population studies, viability analyses
  - Life span estimation
  - Adults vs. juvenile take at wind facilities
  - Analyze live capture data and reproductive status

Genetic Variability develop/test microsatellite markers specific to Hawaiian hoary bat



- North American Hoary Bat markers may not be informative
  - Thesis research 3/6 markers useful, currently testing 14 more…
- Panel of potential markers generated from SNP data
- Use existing DNA collections to test species specific markers
- Conduct analysis with specific markers
  - Identify populations within islands
  - Male vs female dispersal patterns
  - Within island effective population size estimates

State of Hawaii, Division of Forestry and Wildlife
Wildlife Programs



Mahalo nui loa!



Genetics funding provided by Kawailoa Wind Energy, LLC

Tissue collection assistance: R. Bernard, Y. Castaneda, N. Cortez-Delgado, A. Hubancheva, K. Lahaela, D. Johnston, J. Johnson, A. Miles, K. Montoya-Aiona, G. Parish, R. Moseley, C. Todd, G. Reyes, B. Yuen, V. Zrncic, and T. Zinn.

Carcass sample collection; G. Akau, R. Breeden, J. Charrier, M. Craig, J. Dutton, F. Duvall, S. Franklin, D. Sether, M. Stelmach, M. VanZandt, and T. Work.

A. Veillet at University of Hawai‘i Hilo Core Genomics Facility for laboratory assistance.

Permits (IACUC #04-039-12) through the University of Hawai‘i at Hilo. Biological samples were collected under permits USFWS TE003483-31 and Hawai‘i DLNR-DOFAW WL16-04.

Acknowledgements

- US Geological Survey – PIERC volunteers
  - A. Canady, H. Davies, R. Davis, C. Farrar, D. Gross, M. Holden, V. Mavusana, A. Mendez-Bye, B. Miles, D. Montoya-Aiona, C. Snook, G. Solomon
- Auwahi Wind Energy
  - M. VanZandt, G. Akau, B. Campbell, N. Santos, R. Pederson, J. Galvan
- Ulupalakua Ranch
- University of Tennessee at Knoxville — V. Brown
- State of Hawaii, Dept. Land and Natural Resources, Division of Forestry & Wildlife
- Kaheawa Wind Power II
- Land access by many private landowners, Hawaii County, Dept. Hawaiian Homelands, Hawaii Dept. Land and Natural Resources, USDA-Forest Service-Hawaii Experimental Tropical Forest

USGS science for a changing world

Hawai'i Cooperative Studies Unit, University of Hawai'i at Hilo