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Real-time, Autonomous Biosurveillance for Vector-borne Viral Pathogens

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U.S. DEPARTMENT OF ENERGY

UC DAVIS UNIVERSITY OF CALIFORNIA

Project Overview

- Overall goal is to develop and field-test an *autonomous sensor* to detect presence of mosquito-borne viruses (West Nile, etc) with near-real-time capabilites (daily reports)
- Data from sensors will be integrated into BSVE along with mapping & visualization software and predictive models.
- 3-year effort started January 2014 (2 months in)
- Partnership between Sandia National Laboratories, Livermore, CA…
  - Systems engineering, microfluidic assays, statistical modeling expertise
- …and UC Davis Center for Vectorborne Diseases (CVEC)
  - Virology, entomology, and ecology of vectorborne disease
  - BSL-3 laboratory facility and insectary
  - Viral modeling and forecasting expertise
  - Integrated with public health and vector control districts in CA

Background on Arboviruses

- "Arbovirus" = Arthropod-Borne Virus, *i.e.* viruses transmitted by mosquitoes, fleas, ticks, flies, *etc*.
- Major burden on public health & agriculture worldwide
  - Dengue virus is probably the most significant globally (up to 400 million infections/yr)
  - West Nile virus is currently the most famous in United States

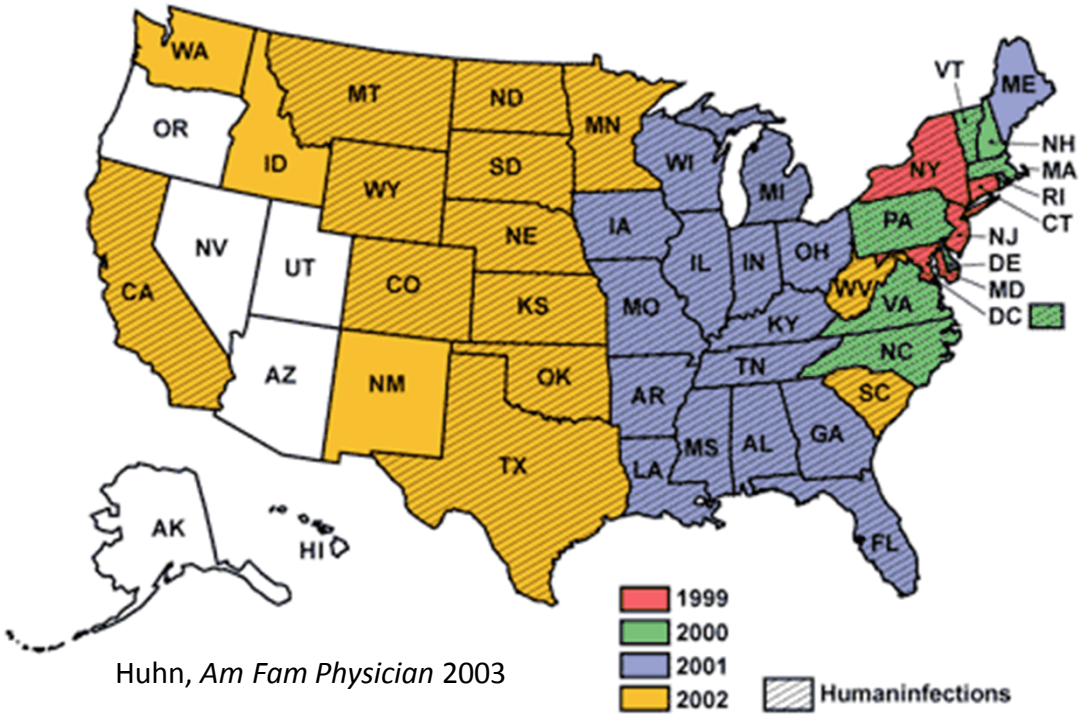
- Military entomologists routinely monitor vector-borne disease threats to protect personnel overseas.
  - Walter Reed identified mosquitoes as vector of Yellow Fever (1901)

Aedes aegypti, the vector of Dengue, yellow fever, Chikungunya, and other arboviruses

Arboviruses – A dynamic threat

- The rapid global spread of West Nile within a few years in the 1990s exemplifies how quickly these viruses can emerge (or re-emerge) and shift geographic boundaries.
  - Assisted by natural and man-made factors including bird migrations, climate change, global trade and transportation, shifts in land use & irrigation
- Dengue, Chikungunya, Rift Valley Fever all have competent vectors in US and present possibilities for new incursion into US.

Spread of West Nile Virus
in USA, 1999-2002



Huhn, *Am Fam Physician* 2003

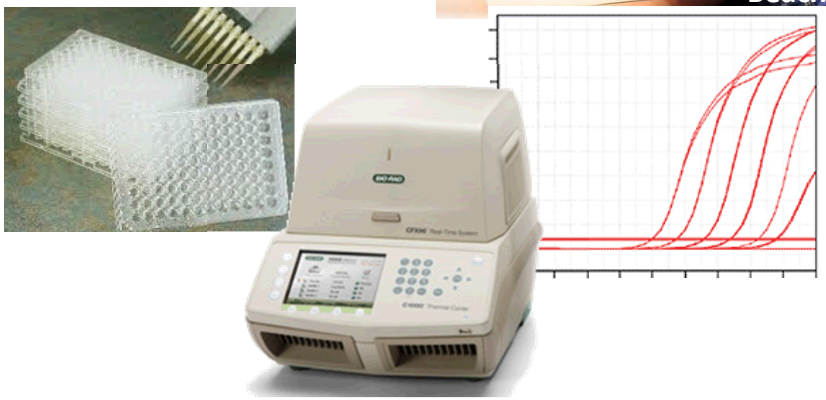
Field surveillance for arboviruses

- Mosquito collection is the primary source of physical data on viral incidence
- Low-tech collection of mosquitoes, skilled manual labor to sort & pool insects, and sensitive laboratory tests for viral RNA (RT-PCR, $20/sample)

Vector surveillance

Photo: Fairfax County, VA via Flickr


Photo: Palm Beach Post



1-2 weeks later

Adulticide spraying

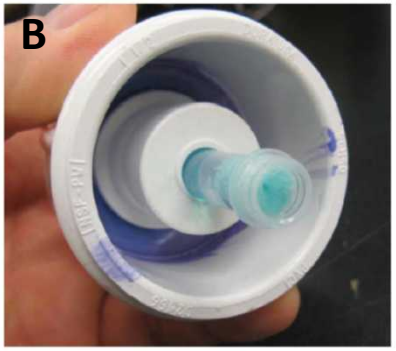

Photo: Huffington Post Alamy


Photo: Lafayette City Parish Gov

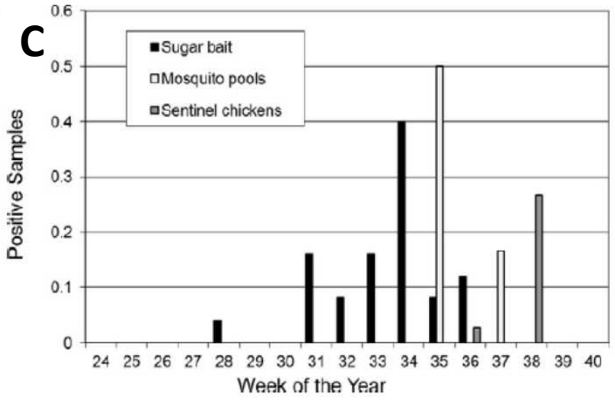
Sugar-feeding for detecting arboviruses

- Collecting, sorting, counting, grinding insects is labor-intensive
- All mosquitoes at all stages of life feed on sugar (only females seek blood)
- Alternative approach: attract mosquitoes to feed on a sugar bait, and leave behind "samples" of saliva containing viruses
- Demonstrated in field for detection of viral RNA in Australia and California.
  - Passive baits placed in field for 1 week, collected, and returned to the laboratory for RT-PCR detection of viruses.
  - Open questions include stability of viral RNA in field, and best method of attracting mosquitoes to baits.
  - But sugar baits in Coachella Valley showed positive results *earlier* than trapped mosquitoes or sentinel chickens in same locations.

CO_2 baited



Floral scent / No CO_2



Previous trials of viral RNA detection by sugar feeding mosquitoes (B,C by our collaborator William Reisen at UC Davis for WNV surveillance in Coachella Valley)

Our proposed enhancement

- Automate the sugar-feeding surveillance technique *via* a "smart trap" that sits in the field for 1 week (up to 1 month) and autonomously:
  - Presents a fresh bait to attract mosquitoes each evening…
  - and performs assays the following morning for viral RNA
  - scores the assays as positive or negative for a panel of viruses
  - And wirelessly transmits the data back to a monitoring station.
- Data on viral incidence as function of time and place can be a data source into the BSVE.

To make an impact…

- Smart traps need to be cheap, reliable, and rugged
  - Deploy a spatial grid of traps to create viral incidence maps
  - Easily portable with low footprint and low power requirements – shouldn't require a car battery, or a laboratory presence
- Assays need to be cheap, reliable, sensitive
- Sugar-feeding data needs to be understood *vs* conventional surveillance

Project Objectives / Tasks

1. Develop single-use assay cartridges to detect a panel of arboviruses historically endemic in California: West Nile Virus (WNV), St. Louis Encephalitis Virus (SLEV), Western Equine Encephalitis Virus (WEEV)
   - Verify assay performance *in vitro*, and then for detection of viruses after sugar-feeding by laboratory-infected mosquitoes
2. Build fieldable integrated trap system to perform sugar-feeding and viral detection, for up to one month of autonomous operation in the field
   - Small number (16) prototypes
3. Test complete system (trap hardware and assays) in Coachella Valley, CA concurrent with conventional vector surveillance (traps & sentinel chickens)
4. Develop strategies/designs for scaling up trap and assay device manufacturing
5. Develop protocols and architecture to archive assay data and integrate into BSVE
6. Develop protocols to map and model viral incidence from integrated data sets (trap data plus environmental data).

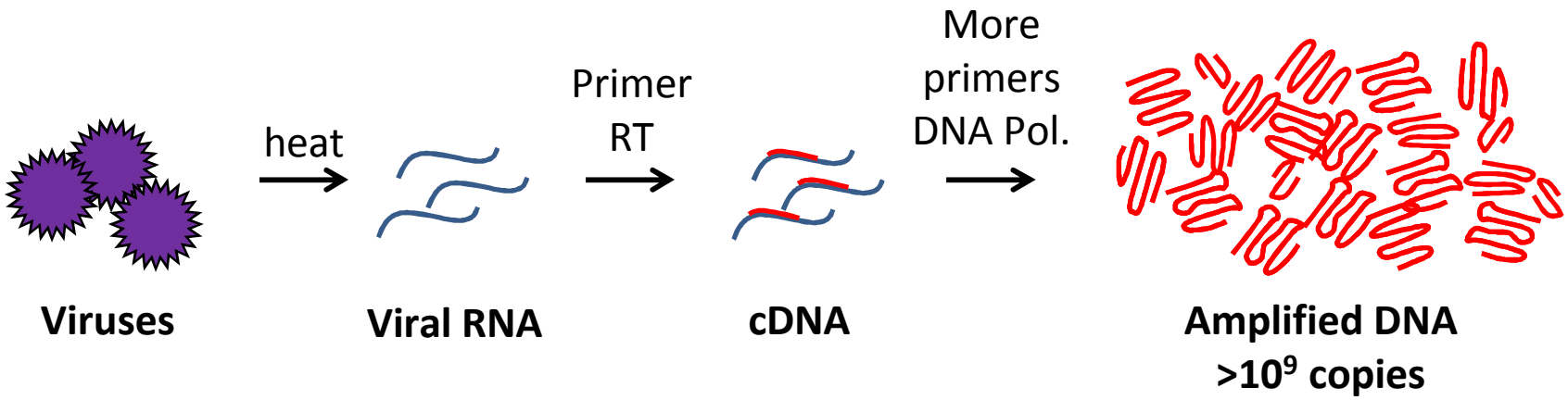
Task 1 - Viral detection assay

- In a single feeding, an infected mosquito may deposit 10^2-10^4 PFU of virus
- This is too little to detect directly or by immunoassay (e.g. “dipstick”/LFA)
- Nucleic acid amplification is the only practical way to detect such small amounts.
- Need to transduce a large signal from a small amount of viral RNA.

- In the laboratory: RT-PCR is “gold standard” technique
  - Well-standardized, well-characterized, great sensitivity, etc.
  - But requires nicely purified samples, and a lot of power to cycle temperature
  - Requirement for sample prep (RNA extraction) adds to instrumental complexity

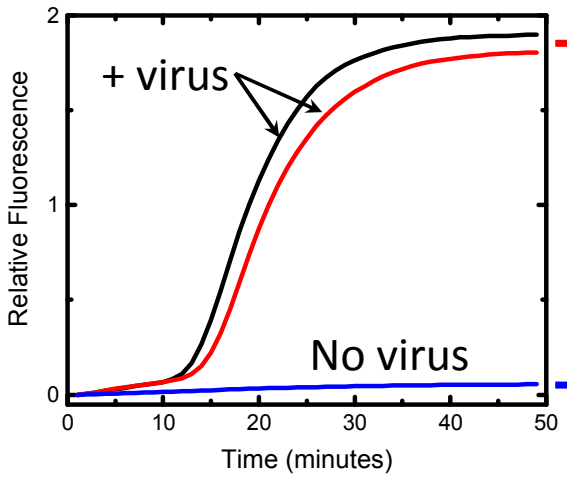
- In the field / low-resource settings: RT-LAMP is an alternative
  - Best-known isothermal nucleic acid amplification chemistry; not locked down by a single supplier, demonstrated in low-resource settings for HIV, TB, parasitic diseases, relatively tolerant to crude samples.

Task 1 - RT- LAMP RNA detection overview

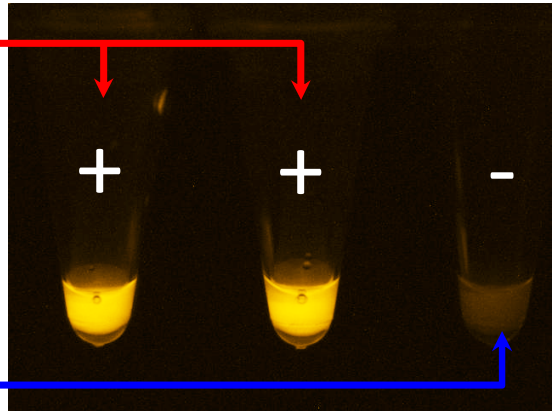


- 2 enzymes, single tube, single continuous process, single temperature (63 °C)
- Viruses become "leaky" at 63 °C – don't absolutely need to extract RNA
- DNA stain becomes brightly fluorescent as DNA is amplified

"Real time" fluorescence

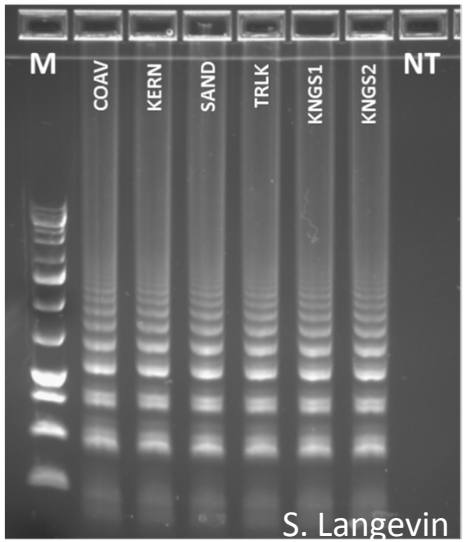


Endpoint fluorescence



or

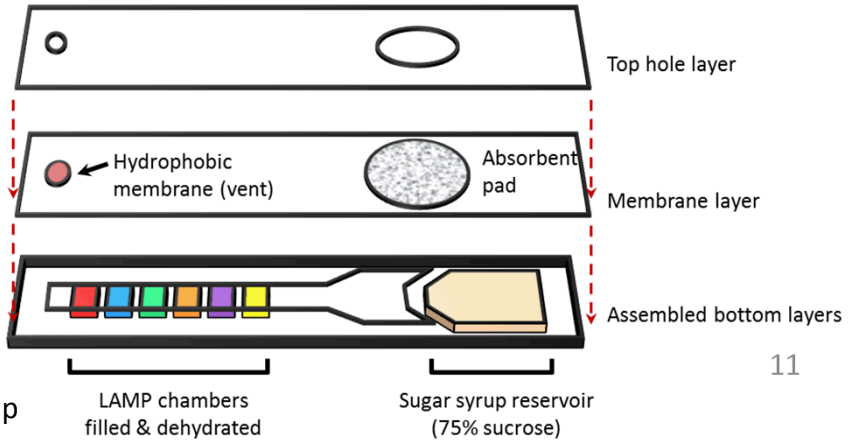
RT-LAMP for WNV in mosquito extracts
Endpoint gel analysis



Task 1 – Microfluidic Assay development

- Aim: Develop integrated microfluidic viral assay and mosquito bait
- Tasks:
  - Verify bench-scale RT-LAMP assays for viral panel (WNV, SLEV, WEEV + control)
  - Develop integrated microfluidic bait RT-LAMP assay device
  - Verify assay performance with live, sugar-feeding mosquitoes in a laboratory setting
  - Develop protocol to dehydrate assay reagents and test stability at ambient and elevated temperatures
- Outputs:
  - Metrics of sensitivity (detection limit) and specificity for each target
  - Threshold signals to score "positive" or "negative" for each target, or "assay failure" if positive or negative amplification controls are indistinguishable

- Deliverables: a single-use, integrated device comprising a sugar bait and stabilized RT-LAMP assays for detecting WNV, SLEV, and WEEV from sugar-feeding mosquitoes, with positive and negative amplification controls

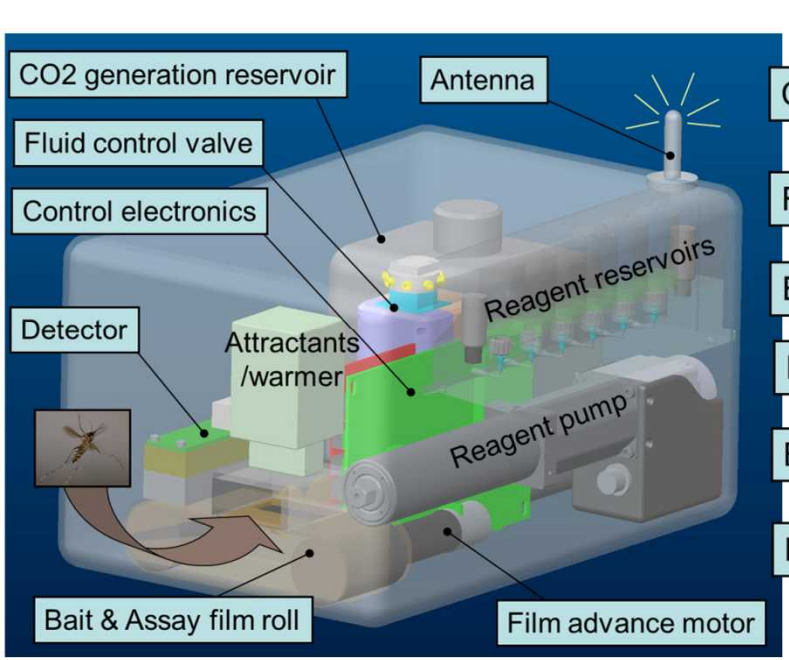


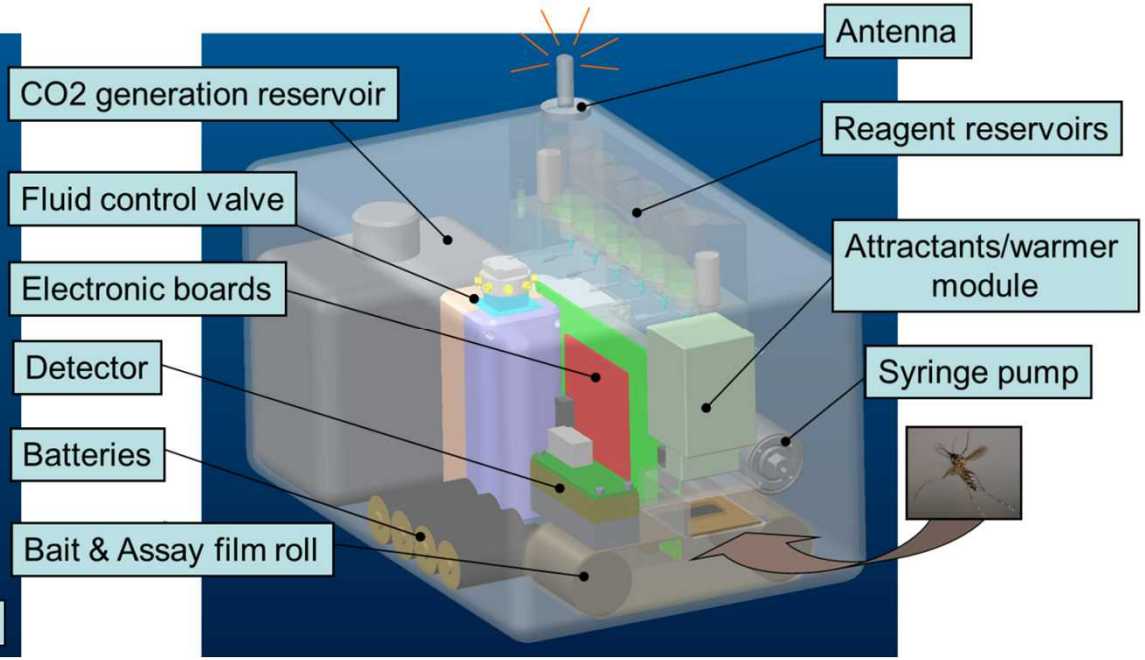
Conceptual design of integrated microfluidic assay strip

Task 2: Design and build integrated smart trap system

- Aim: Develop fieldable prototype hardware capable of autonomously presenting sugar baits, attracting mosquitoes to feed, running and interpreting viral assays, and transmitting assay results (viral incidence) once per day
- Tasks:
  - Design and fabricate electronics to control the smart trap (microprocessor, PCB, *etc*.)
  - Integrate microfluidic assay into smart trap
  - Build assay hardware subsystems (heater, temperature controller, fluorescence detector, environmental sensors).
  - Build trap fluidics (reagent delivery)
  - Build attractant subsystems (CO_2 generation and chemical attractants)
  - Establish a power management strategy for integrated system
  - Build integrated smart trap system
  - Test integrated system outdoors
- Outputs:
  - System reports on status (self-checks), assay results, local environmental data once/day *via* text message
- Deliverables: 5 prototype traps and performance data/failure modes for subsystems in outdoor trials

Smart Trap concept designs





Approximate dimensions: 5 x 6 x 9 inches

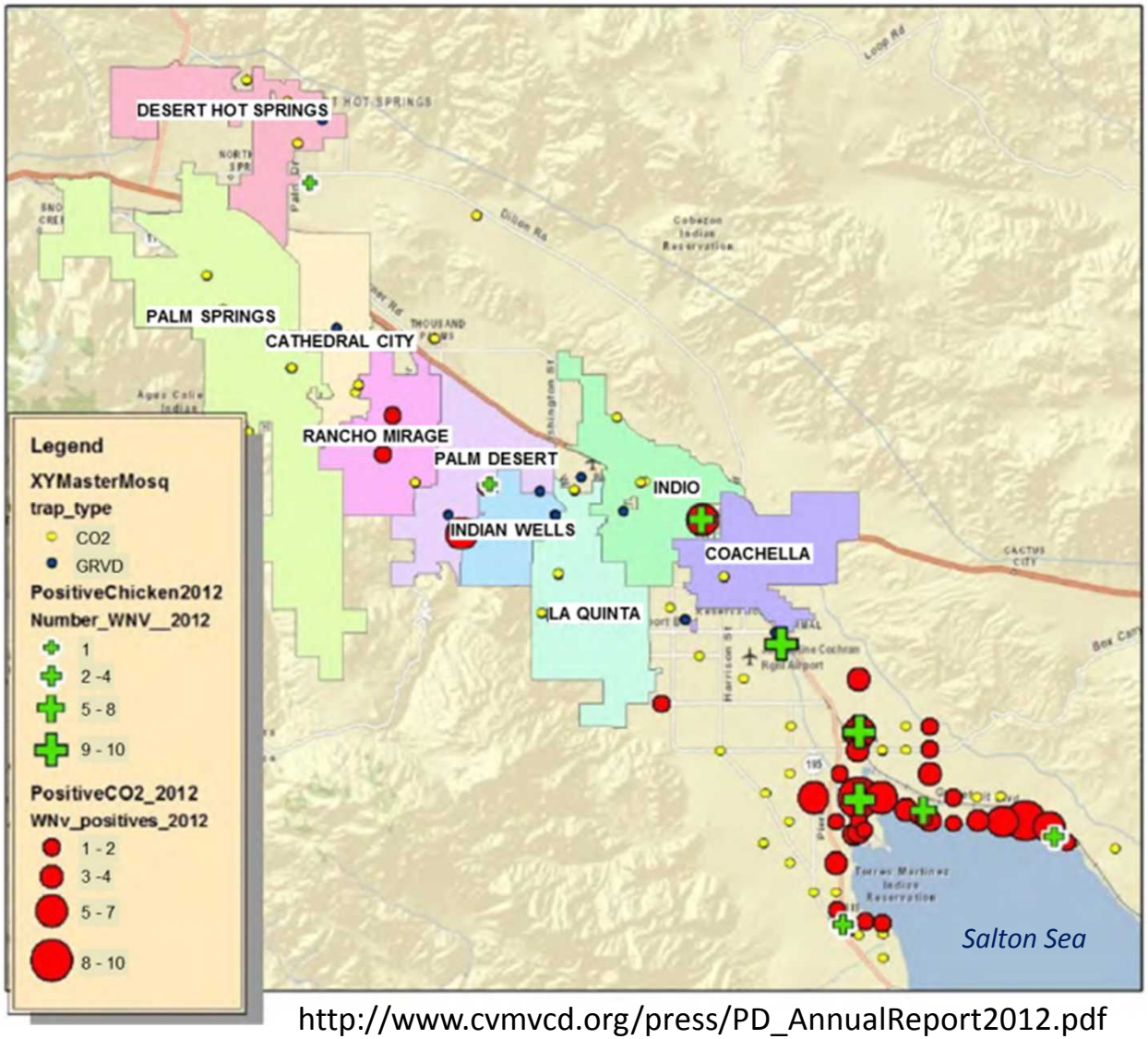
Initial prototypes: $3-5K each

Mass production target <$500

Task 3: Field test smart trap in an “arbovirus hotspot”

- Aim: To obtain validation data for the smart trap system in a field comparison with concurrent “conventional” surveillance techniques in Coachella Valley, an arid region with varied land use (residential, irrigated agriculture, desert)
- Tasks:
  - Manufacture 11 additional prototype traps and assay devices
  - Deploy traps in Coachella valley for one-month trial, near locations where trapping and/or sentinel chickens are currently in use.
  - Inspect hardware following test.
  - Analyze smart trap and conventional surveillance data.
- Output: Viral incidence at 16 locations in Coachella Valley over a one-month period
- Deliverables: Performance data for an extended deployment of the smart trap system, validated against conventional “gold standard” surveillance techniques.

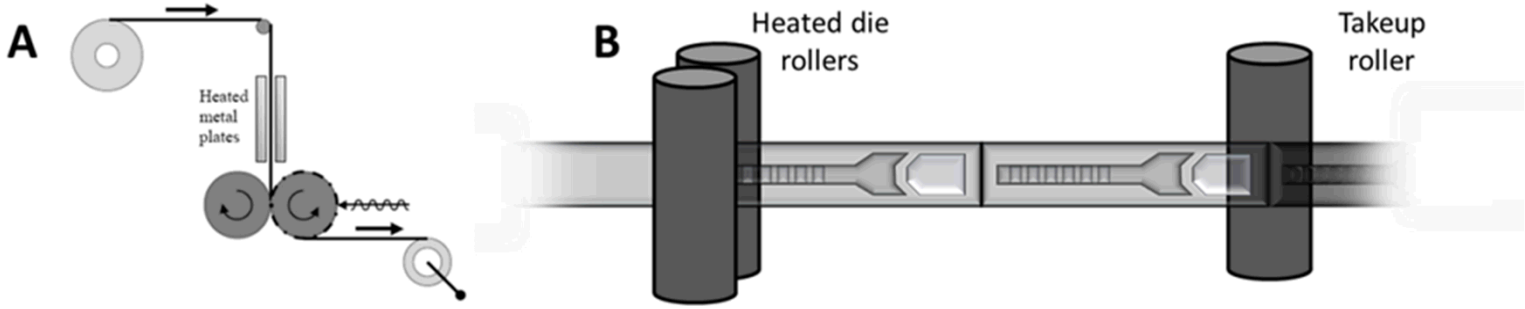
Coachella Valley WNV Surveillance, 2012



http://www.cvmvcd.org/press/PD_AnnualReport2012.pdf

Task 4: Second-generation smart-trap

- Aim: To simplify the design of the smart trap and assays to enable scalable manufacturing
- Tasks:
  - Simplify the fluidic delivery system for reagents & CO_2 generation
  - Integrate additional components (chemical attractants, assay wash buffer) onto the assay device
  - Re-evaluate electronic components for mass production of printed circuit board
  - Develop a strategy for mass production of assays (*e.g.* hot embossing or injection molding, *vs.* laboratory-assembled laser-cut laminates)
  - Identify possible partners for technology transfer/mass production
- Deliverables: Design for second-generation trap and proof-of-concept data for simplified fluid delivery and integrated assay

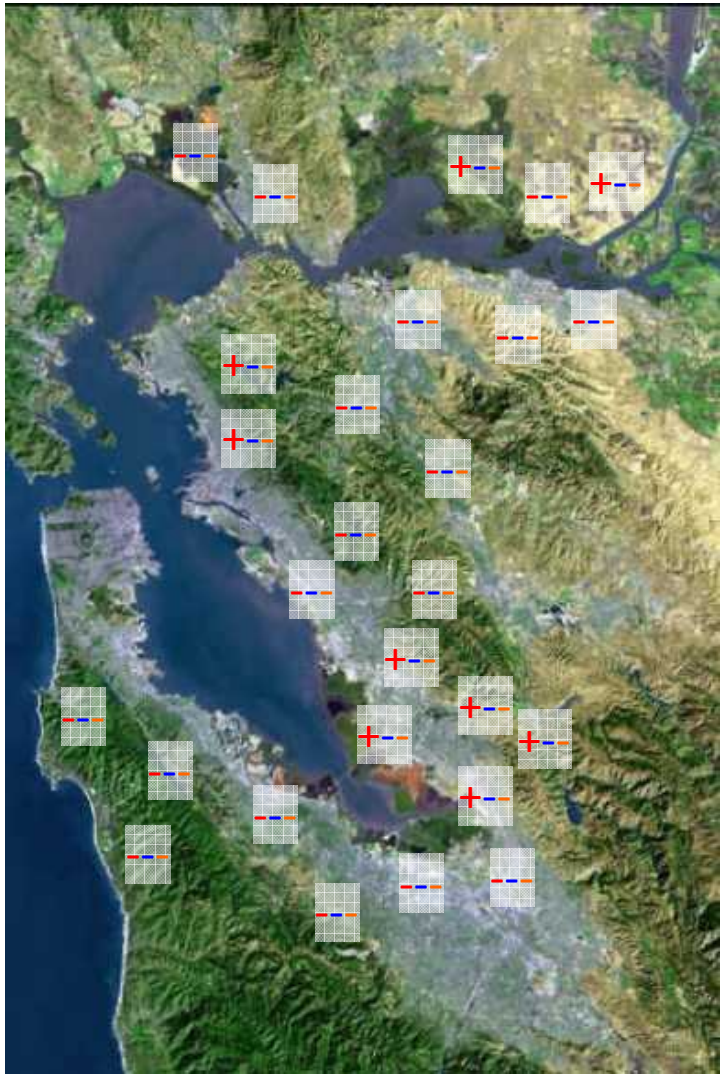


Roll-to-roll embossing for large-scale manufacture of laminated plastic assay devices

Task 5 – Computational infrastructure for collecting and processing data

- Aim: Develop the AWS infrastructure for collecting & processing sensor data
- Tasks
  - Develop scripts (Python) to convert SMS messages to XML files and upload into AWS's SimpleDB
  - Implement AWS website with grails and deploy via Beanstalk
  - Construct AMI with mapping models and R scripts
- Outputs
  - Translucent WNV incidence maps overlaid on Google satellite map over test site
  - Pins at sensor locations, colored by incidence (daily reports)
  - Binary time-series plots of detection (detection/no-detection as a function of time)
- Deliverables: Computer system with SMS->XML convertors, AMI with mapping scripts

Viral incidence map - concept



WNV + -

WEEV + -

SLEV + -

Task 5 – Smart trap network

Smart traps in field communicate to relay trap

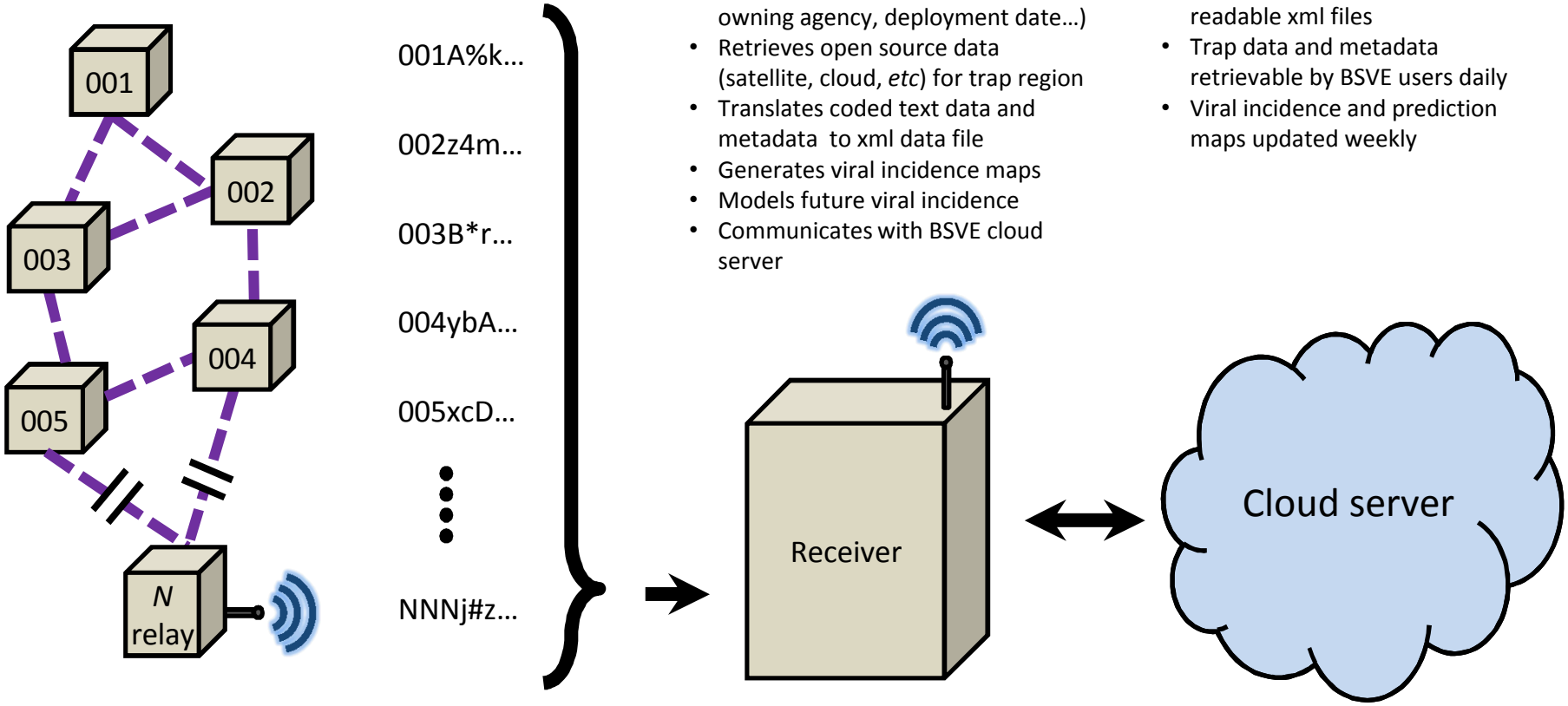
Relay compiles and sends trap data and metadata as SMS text messages

Receiver machine

- Retrieves additional trap metadata (GPS coordinates, assay panel, owning agency, deployment date…)
- Retrieves open source data (satellite, cloud, *etc*) for trap region
- Translates coded text data and metadata to xml data file
- Generates viral incidence maps
- Models future viral incidence
- Communicates with BSVE cloud server

BSVE

- Accepts data from receiver in the form of human- and machine-readable xml files
- Trap data and metadata retrievable by BSVE users daily
- Viral incidence and prediction maps updated weekly



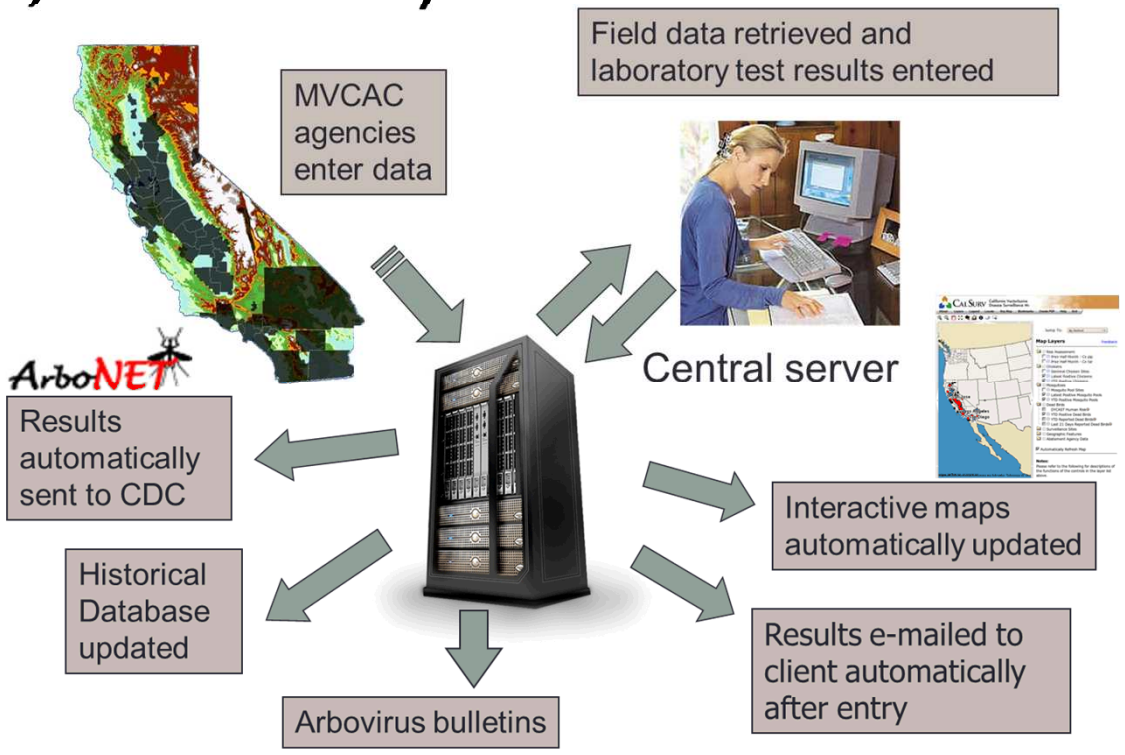
Integration of smart trap network into BSVE. Communication between the traps and the cloud is mediated by an intermediate “receiver”.

Task 6 – Mapping methods and models for WNV incidence from sensor data

- Aim: Develop a way to interpolate WNV incidence data from a finite number of sensors to full site (using meteorological & land-use covariates)
- Tasks
  - Identify which of the 2 spatial models (kriging versus point-process) works best for our site
  - Identify the right covariates for our site and encode in a logistic model
  - Compare our WNV incidence model predictions (from smart trap data) with data from other reports(CDPH-WNV, sentinel chicken etc)
- Outputs
  - WNV incidence mapped on a grid, on a daily, weekly, monthly and annual basis, archived in SimpleDB
- Deliverables
  - The spatial and covariates model, and R implementation

Model to predict & map viral "hot spots" from time series of smart trap incidence data

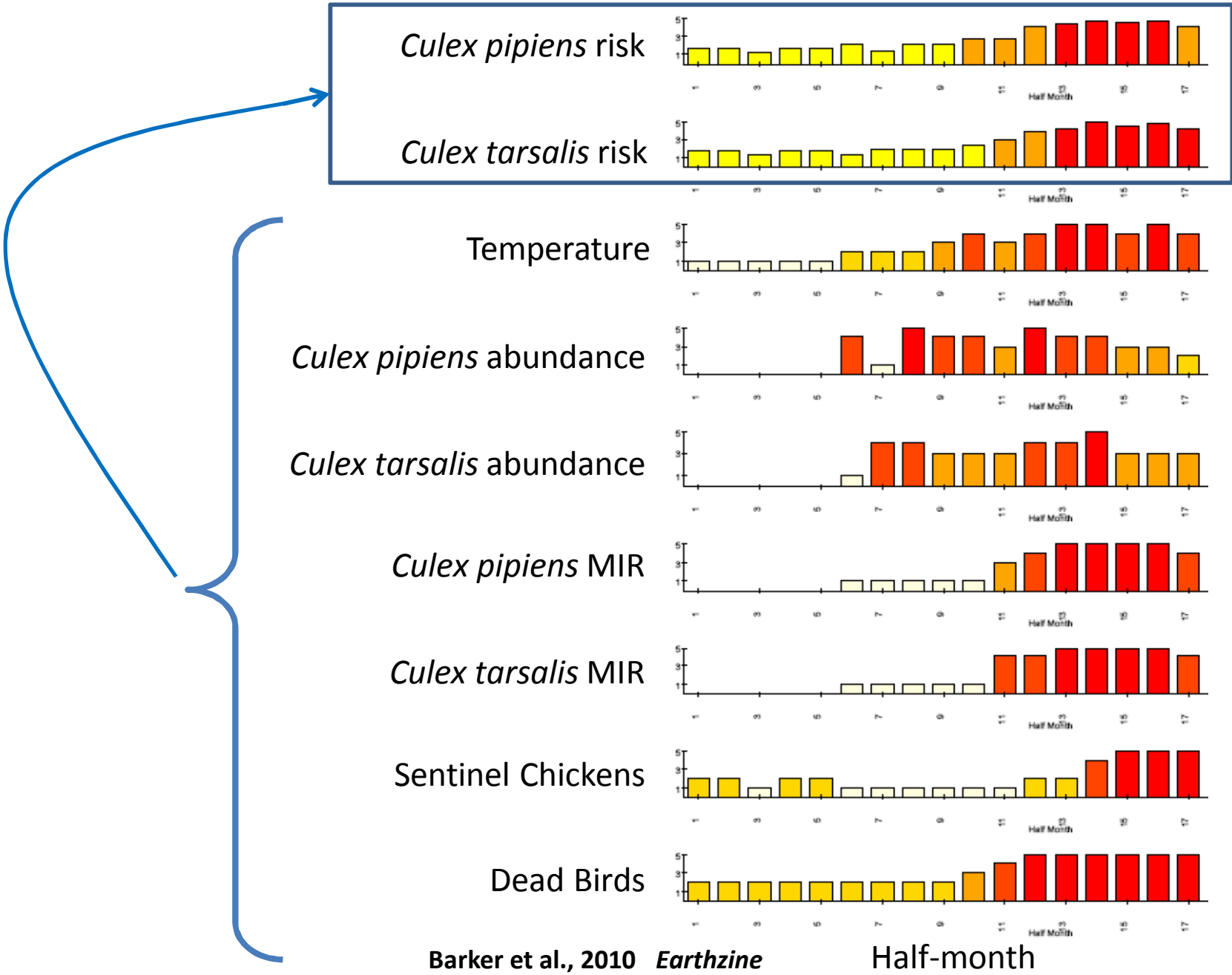
Rapid Arbovirus Data Acquisition: CalSurv Gateway (C. Barker, UC Davis)



Goals:

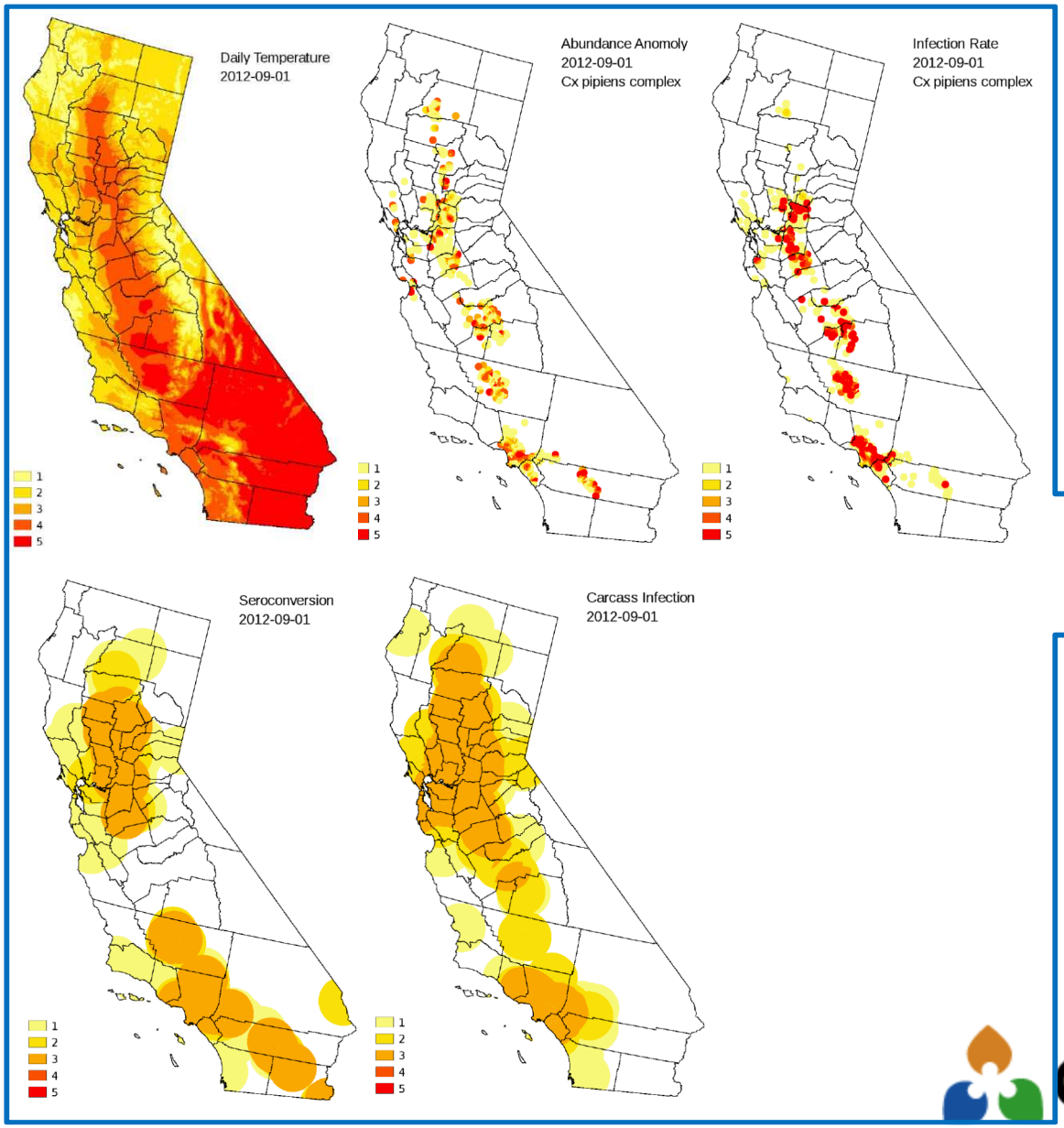
- Securely store and manage California's surveillance data
- Avoid redundant data entry
- Automate reporting to user agencies
- Provide useful tools for decision support for vector control

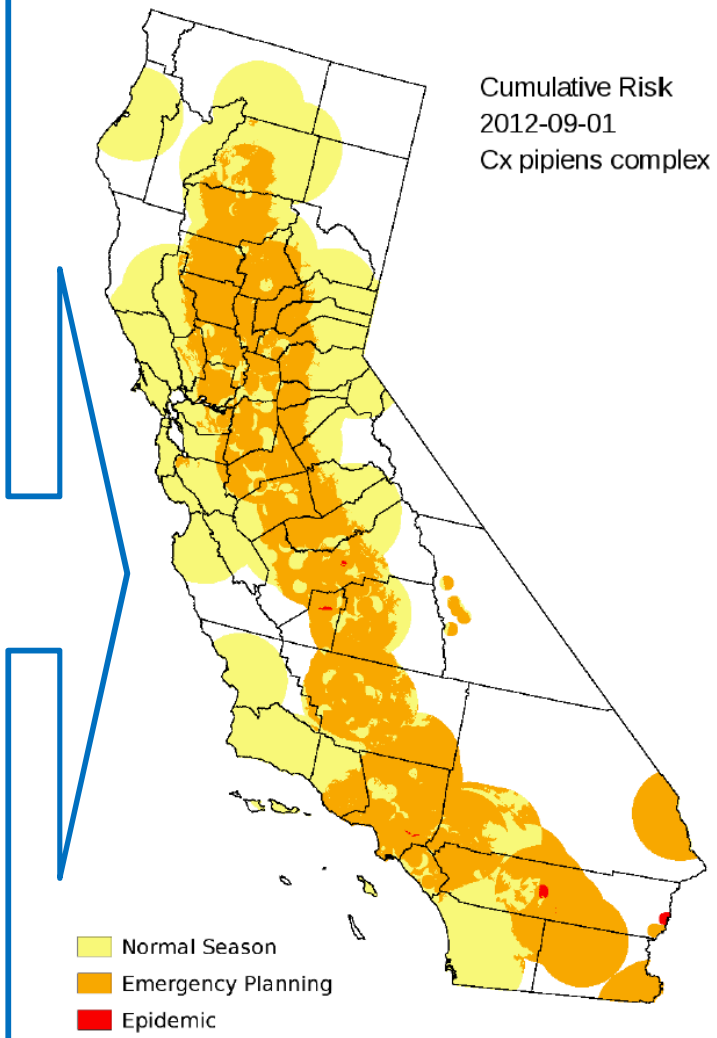
CA Mosquito-borne Virus Surveillance & Response Plan



Barker et al., 2010 ***Earthzine***

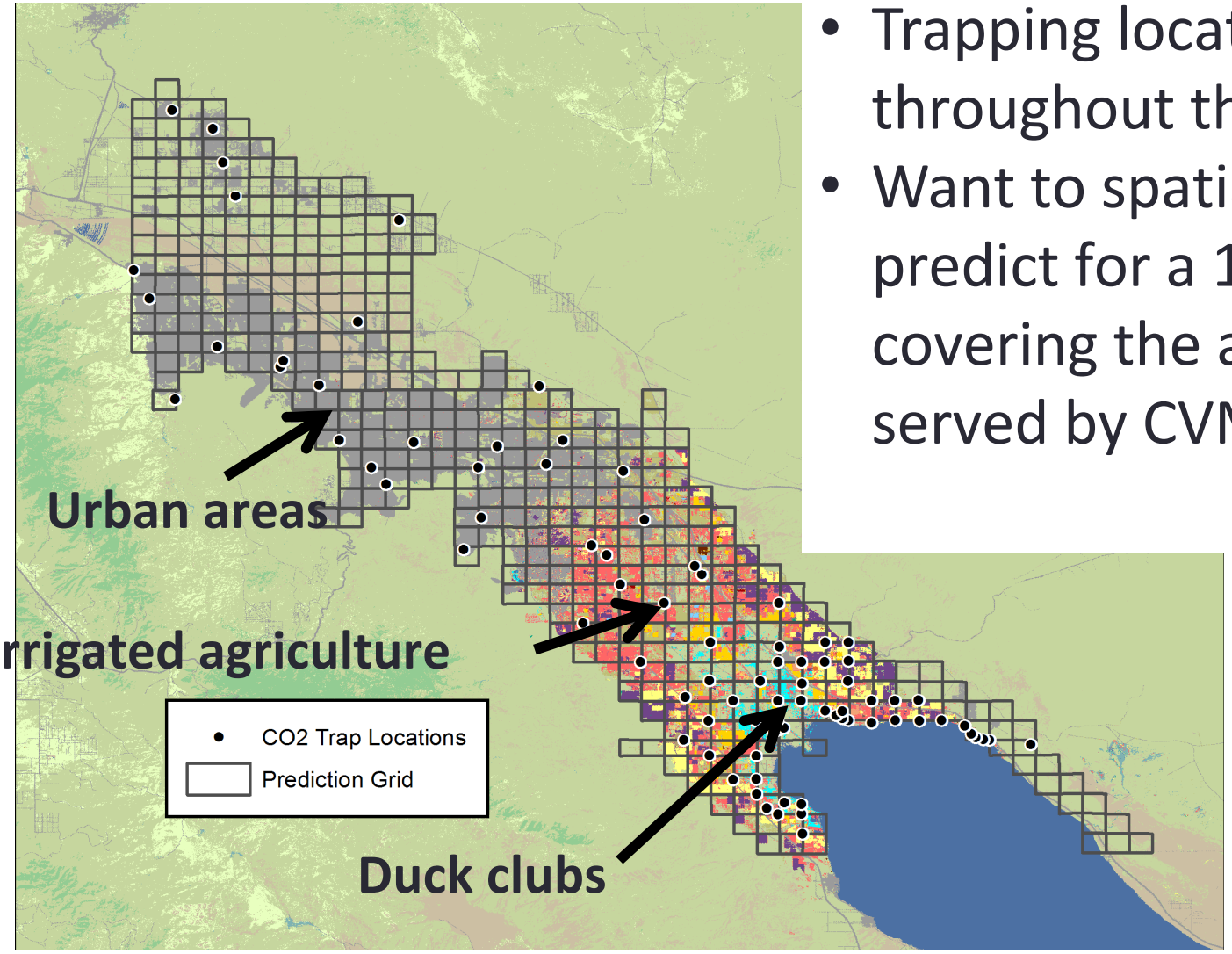
Risk Map: Sep 1, 2013





CalSurv California Vectorborne Disease Surveillance Gateway

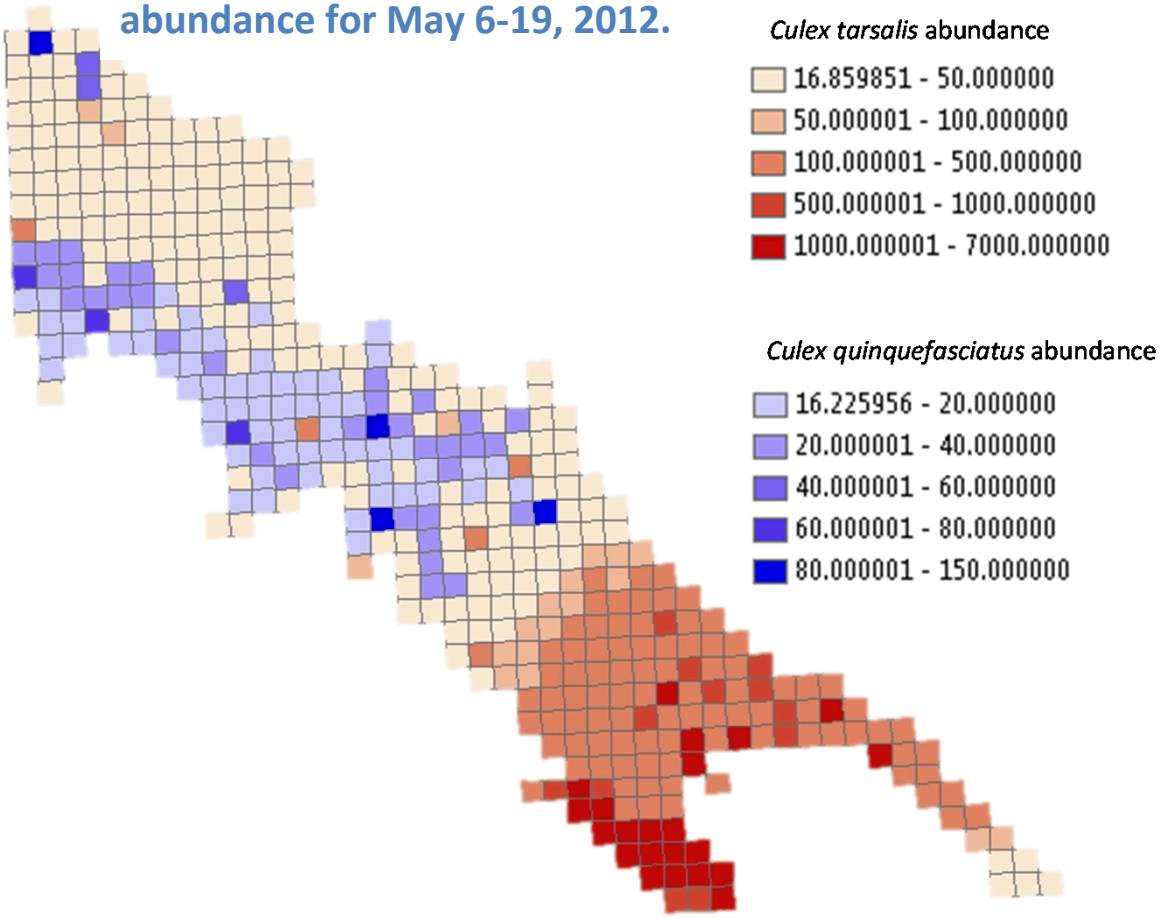
Spatial Framework



- Trapping locations throughout the valley
- Want to spatially predict for a 1-mile grid covering the areas served by CVMVCD

Culex tarsalis in the Coachella Valley

Example showing predicted *Culex* abundance for May 6-19, 2012.



- Models have been validated
- Working to automate data exchange with the CalSurv Gateway
- Will be implemented in ArcGIS for district use in early 2014

Smart Trap – BSVE integration

- Proposed effort is mostly development of novel hardware/assay
- Field test data comes online in year 3
- Will work with BSVE partners & SDK for best fit into Harbinger system

Answers to questions – 1/4

- **GENERAL**
- *What need to we address? What decisions do we support?*
  - Predict, before conventional public health/vector control does, whether WNV levels are increasing in mosquitoes
  - Decision: When, and where, to undertake vector-control measures; when to use personal protective measures (repellants, mosquito nets etc)
- *Type of user targeted*: knowledgeable epidemiologist
- *How to initiate the app*: Go to a website; envision healthmap.org
- *User-input parameters*: Date range; zoom level
- *Easy to use?*: Haven't implemented yet
- *Clearly understandable?*: See above
- *Results accurately represented?*: Both kriging and point-process will come with an explicit measure of modeling error in the WNV incidence estimate
- *Are limitations/caveats mentioned in output*: Will be, when done

Answers to questions – 2/4

- **ANALYTIC METHOD**
- *Language and platform*: Javascript, Java (grails), Python, R and AWS
- *What technology used in visualization*: Google maps for risk maps; graphs: DOJO for time-series plots
- *How is new data used to update things?*: nightly data updates all maps; nightly batch process
- *Designed as a SaaS*: Yes
- *Designed with big data approaches*: No, not needed
- *Proprietary licenses*: No. *COTS*: everything
- *Core analytic method mathematically sound?*
  - Methods are well established; their application to Coachella valley & our data is new
  - Chose these methods because of previous use with WNV (logistic regression) and their use in epidemiology (inhomogeneous point process, Kelsall & Diggle, 1995)
  - Have we modified these methods – Too early to tell
- *Pros*: Accuracy, expense & scalability; *Cons*: Flexibility; customized to WNV in Coachella.

Answers to questions – 3/4

- *How flexible / general is the method*: General (used in WNV modeling elsewhere); implementation is for Coachella
- *How is the output visualized, manipulated and stored?*
  - Visualized: translucent incidence map overlaid on relevant met. & land-use covariates
  - Manipulated: time-range, zoom function; plot with CDPH reports
  - Stored: incidence maps computed nightly and databased
- *Current implementation status and improvements planned*: N/A
- DATA
- *Source data*:
  - Public, free; determined usefulness of data sources from literature; data rights - *During 3-year DTRA funded effort – data will be freely available. Subsequently traps/assays will be licensed to 3rd party to manufacture; traps & data will be controlled by end users (state/local public health/vector control).*
- *Examples of input data, data model, XML scheme and outputs*: under development

Answers to questions – 4/4

- How is the data stream archived? XML files in SimpleDB
- Public or private cloud?: public
- Does data stream require pre-processing?
  - Python scripts: SMS -> XML conversion; R scripts for incidence map generation; incidence maps archived in SimpleDB
- How is input data fed into analytic app: uploaded by relay computer into AWS as a XML file.