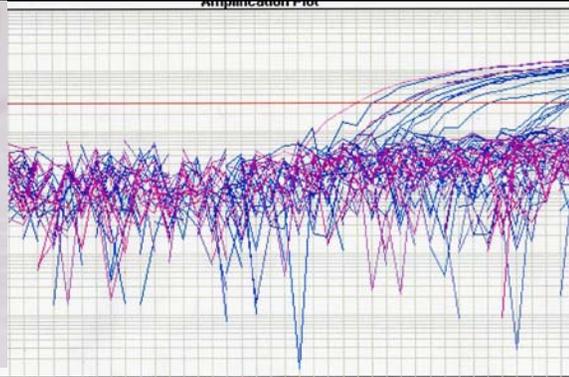




Culex pipiens



Michigan Arbovirus Mosquito Surveillance: Review of 2003 and New Ideas for 2004

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Purpose of This Presentation

- Discuss mosquito surveillance results for 2003
 - What do they tell us?
 - Lots of graphs and maps attempting to bring simplicity to a complex system
- Attempts to change the current system for more efficient processing
 - The old way: slow, laborious, costly
 - The new way: faster, less laborious, still costly
 - Electronic submission and automated processing; how we hope to change things for the better
- Why conduct mosquito surveillance?
 - It tells us who has the virus and might be transmitting it.
 - It can guide mosquito control decisions locally and regionally.
 - It can help us to define outbreak thresholds, i.e., infection rates in mosquitoes that portend human cases.
 - It really shouldn't be done as a community's primary "face" to the public for a West Nile virus program, nor as the primary surveillance tool. That is what dead crows and VecTest are for. It can supplement other activities, especially with regard to the above list.

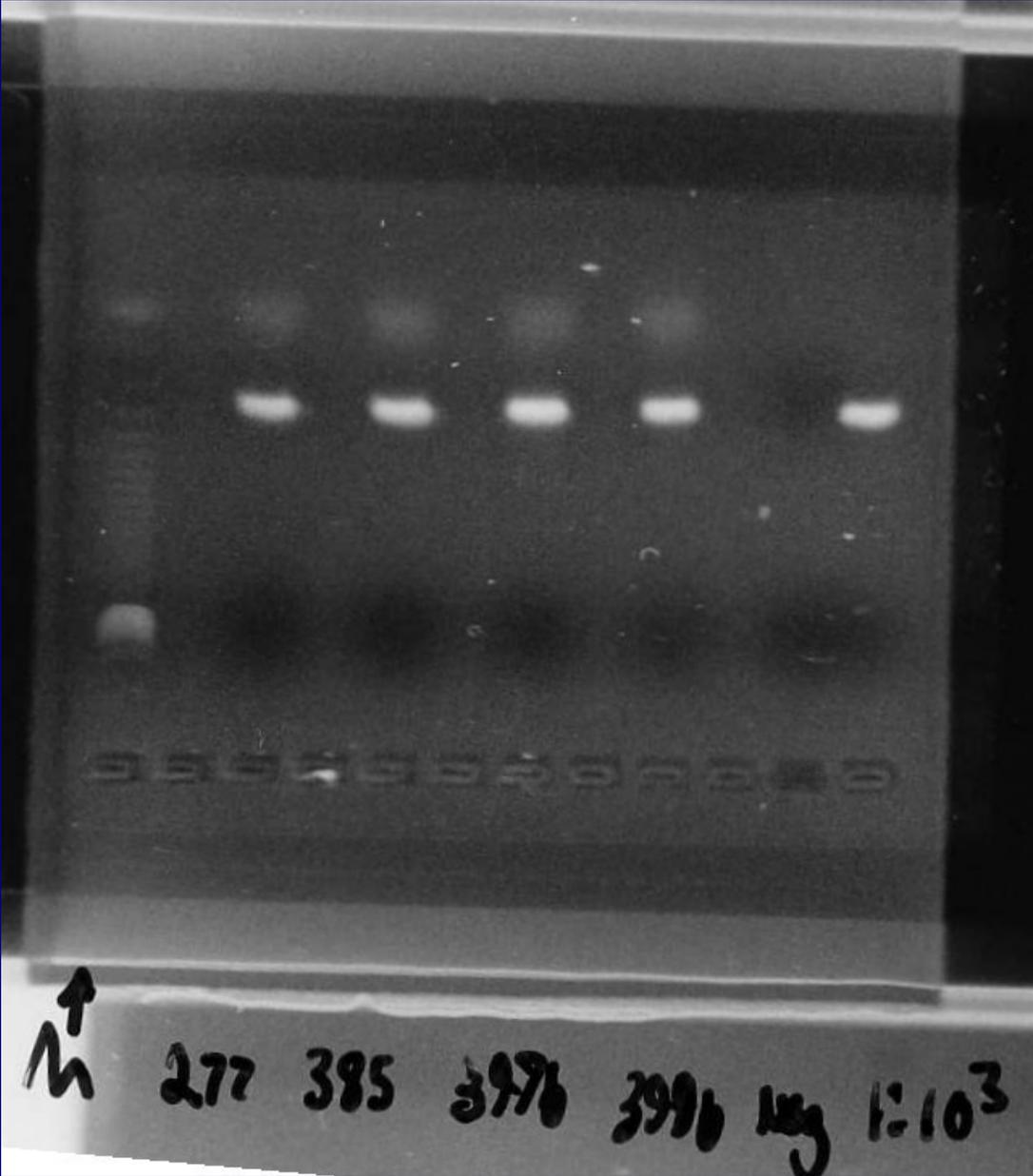
How do we do it?

- Mosquitoes are collected from field populations in ways that preserve the quality of them and get them, and the records of them, into our hands.
 - Mosquito identification, sorting, and “pooling” (i.e., grouping) into marked tubes that are associated with a well maintained database.
 - Keep everything cold or frozen after collection, if possible.
 - Tubes are shipped by a commercial carrier. [Boxes must be returned to sender.]
 - The database contains information on location and date of collection, method of collection, a unique identity number for each collection and each subsample from each collection. It should be stored in a computer database, regularly updated and backed up, and regularly summarized.
 - Mosquitoes are received. Package opened, tubes sorted, checked against the database, and a new database created and continually updated as new shipments arrive. BOTTLENECK 1.
 - Mosquitoes are transferred to new tubes with bb’s in them and tubes relabeled. BOTTLENECK 2.
 - Reagents are added to tubes, tubes placed into blocks and a mixer mill, and they are crushed (old way was a few tubes at a time. In mid-summer 2003 we shifted to a mixer mill).

How do we do it?

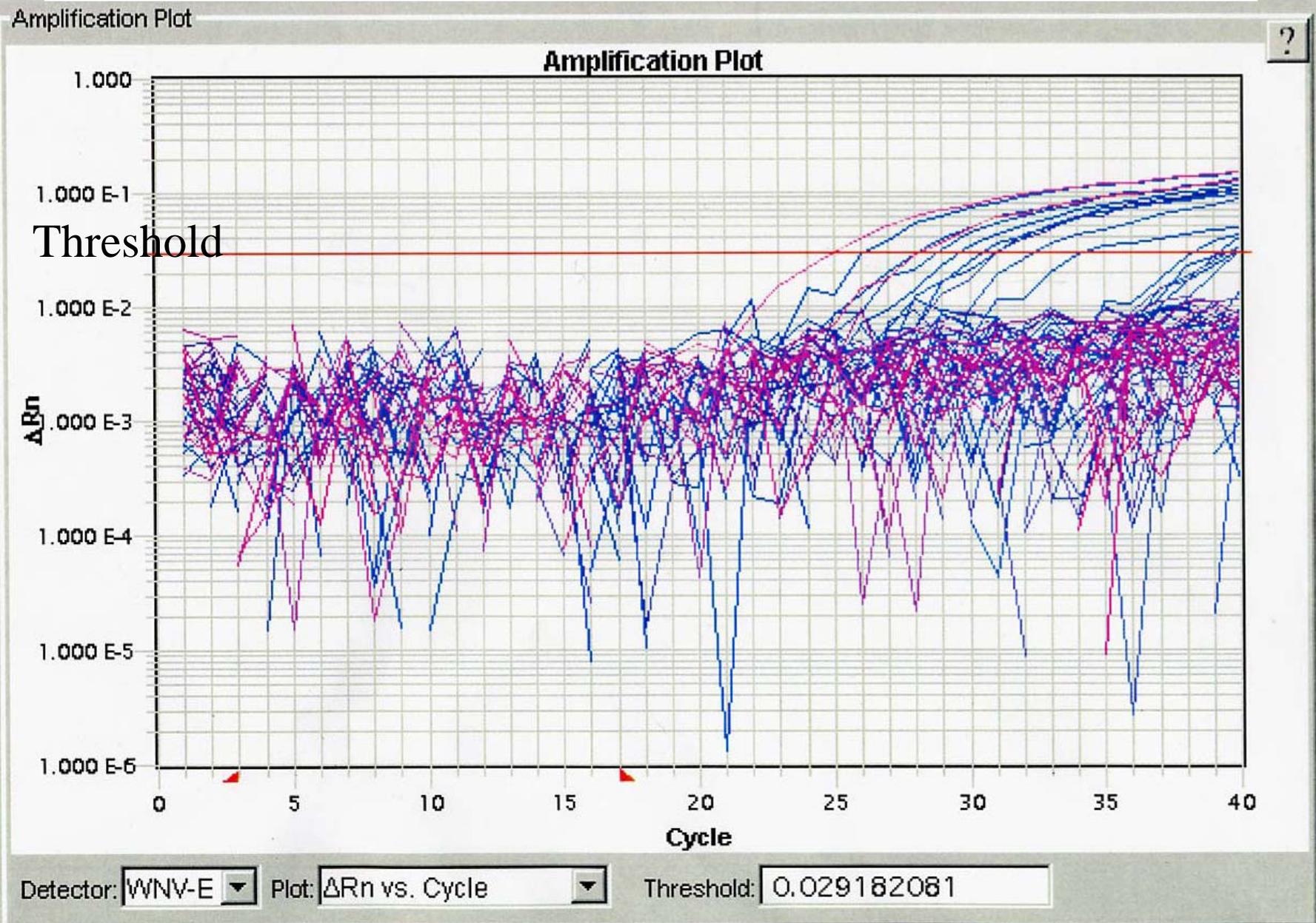
- Continuing
 - RNA is extracted from the crushed pools. Formerly, by hand. Very slow, costly, consumes reagents and tubes. Five tube changes per sample in a safety hood. SERIOUS BOTTLENECK 3.
 - Post season 2003, we have obtained an automated RNA extraction system courtesy of the MDCH laboratories. REDRESSES BOTTLENECK 3.
 - Conduct the PCR. Through 2003, we did this by standard PCR and gel electrophoresis to reveal positive pools. SERIOUS BOTTLENECK 4.
 - We have now adopted quantitative PCR at the MSU genomics facility, and no gel electrophoresis. REDRESSES BOTTLENECK 4.
 - Gels were read and positives and negatives recorded into the computer database. SERIOUS BOTTLENECK 5.
 - With quantitative PCR, we have the opportunity to retrieve the results automatically and have them dumped into the database without re-entering the data. THIS POTENTIALLY REDRESSES BOTTLENECK 5.
 - Notify submitters of results.

THE OLD WAY



Reverse transcription PCR
detection of WNV in
field caught mosquitoes
from Michigan

Quantitative PCR amplification of WNV naturally infected mosquitoes in Michigan: The new way



2003 Mosquito Surveillance Results

County	No. pools per county	Total tested per county
Lapeer	1	5
Calhoun	4	16
Washtenaw	13	134
Arenac	27	241
Macomb	45	271
Barry	47	285
Muskegon	70	658
Kent	109	824
Wayne	110	842
Lenawee	113	892
Cass	119	927

2003 Mosquito Surveillance Results, continued

Monroe	159	1763
Livingston	156	1748
Tuscola	163	2547
Ottawa	289	3697
Genessee	293	3705
Midland	272	3594
Ingham	229	3166
Oakland	346	4082
Isabella	364	3292
Bay	713	8432
Saginaw	874	8875

2003 Mosquito Surveillance Results, continued

	POOLS	Mosquitoes	Positives
TOTALS	15630	133559	203

2003 Mosquito Surveillance Results, "Aedes"

County	Species	No. pools	Total tested	No. pos	MIR
Arenac	Aedes	3	32	0	0
Barry	Aedes	4	28	0	0
Calhoun	Aedes	6	29	0	0
Cass	Aedes	3	34	0	0
Genessee	Aedes	4	9	0	0
Isabella	Aedes	79	79	0	0
Lenawee	Aedes	5	35	0	0
Livingston	Aedes	81	1425	4	2.807018
Macomb	Aedes	4	4	0	0
Monroe	Aedes	1	4	0	0
Muskegon	Aedes	8	165	0	0
Oakland	Aedes	3	3	0	0
Ottawa	Aedes	44	374	0	0
Tuscola	Aedes	7	130	0	0

2003 Mosquito Surveillance Results, Anopheles

Macomb	Anopheles	1	1	0	0
Ottawa	Anopheles	4	5	0	0
Washtenaw	Anopheles	4	58	0	0
Wayne	Anopheles	3	26	0	0
Arenac	Anopheles	2	5	0	0
Barry	Anopheles	3	13	0	0
Bay	Anopheles	25	306	0	0
Genessee	Anopheles	138	1516	1	0.659631
Lapeer	Anopheles	1	4	0	0
Lenawee	Anopheles	4	15	0	0
Livingston	Anopheles	72	897	3	3.344482
Midland	Anopheles	10	78	0	0
Monroe	Anopheles	73	1638	0	0
Oakland	Anopheles	82	909	0	0

2003 Mosquito Surveillance Results, *Oc. canadensis*

Arenac	Canad	1	1	0	0
Barry	Canad	3	20	0	0
Bay	Canad	113	2243	9	4.012483
Cass	Canad	2	3	0	0
Genessee	Canad	1	1	0	0
Isabella	Canad	700	700	0	0
Macomb	Canad	2	2	0	0
Midland	Canad	7	111	0	0
Muskegon	Canad	2	3	0	0
Saginaw	Canad	90	695	0	0
Wayne	Canad	3	26	0	0

Arenac	Culex	3	5	0	0
Barry	Culex	5	44	0	0
Bay	Culex	521	5907	4	0.677163
Cass	Culex	2	2	0	0
Genessee	Culex	88	568	0	0
Ingham	Culex	274	1236	1	0.809061
Isabella	Culex	411	411	0	0
Lapeer	Culex	2	5	0	0
Lenawee	Culex	18	86	0	0
Livingston	Culex	58	467	2	4.282655
Midland	Culex	283	4035	1	0.247831
Monroe	Culex	12	187	0	0
Muskegon	Culex	2	3	0	0
Oakland	Culex	427	6234	19	3.047802
Ottawa	Culex	28	75	0	0
Tuscola	Culex	143	2575	0	0
Washtenaw	Culex	2	14	0	0
Wayne	Culex	11	168	0	0

2003 Mosquito Surveillance Results, Culex “Pip/Rest”

Arenac	Pip/Res	2	5	0	0
Barry	Pip/Res	5	39	0	0
Calhoun	Pip/Res	6	23	0	0
Cass	Pip/Res	4	5	0	0
Genessee	Pip/Res	30	163	1	6.134969
Lenawee	Pip/Res	7	32	0	0
Macomb	Pip/Res	9	124	0	0
Monroe	Pip/Res	5	15	0	0
Muskegon	Pip/Res	6	19	0	0
Oakland	Pip/Res	108	1105	2	1.809955
Washtenaw	Pip/Res	4	29	0	0
Wayne	Pip/Res	1	2	0	0

2003 Mosquito Surveillance Results, Culex pipiens

Arenac	Pipiens	1	1	0	0
Barry	Pipiens	1	2	0	0
Bay	Pipiens	9	19	0	0
Genessee	Pipiens	2	5	0	0
Ingham	Pipiens	9	9	0	0
Isabella	Pipiens	1	1	0	0
Kent	Pipiens	14	23	0	0
Lenawee	Pipiens	1	1	0	0
Macomb	Pipiens	13	56	0	0
Muskegon	Pipiens	4	20	0	0
Oakland	Pipiens	50	271	3	11.07011
Ottawa	Pipiens	115	885	1	1.129944
Saginaw	Pipiens	1077	7254	15	2.067825
Wayne	Pipiens	16	41	2	48.78049

2003 Mosquito Surveillance Results, Culex restuans

Arenac	Restuans	1	1	0	0
Bay	Restuans	79	931	0	0
Genessee	Restuans	15	44	0	0
Ingham	Restuans	14	22	0	0
Kent	Restuans	21	31	0	0
Macomb	Restuans	11	24	0	0
Muskegon	Restuans	3	6	0	0
Oakland	Restuans	102	704	2	2.840909
Ottawa	Restuans	2	2	0	0
Saginaw	Restuans	603	3562	17	4.7726
Tuscola	Restuans	1	2	0	0
Wayne	Restuans	19	76	3	39.47368

2003 Mosquito Surveillance Results, *Aedes vexans*

Lapeer	Vexans	1	5	0	0
Washtenaw	Vexans	9	118	0	0
Arenac	Vexans	14	107	0	0
Macomb	Vexans	18	30	0	0
Muskegon	Vexans	23	373	0	0
Kent	Vexans	39	166	1	6.024096
Cass	Vexans	6	35	0	0
Tuscola	Vexans	73	1441	2	1.387925
Ottawa	Vexans	56	492	0	0
Ingham	Vexans	39	155	0	0
Isabella	Vexans	137	137	0	0
Barry	Vexans	7	39	0	0
Bay	Vexans	744	13994	6	0.428755
Calhoun	Vexans	5	17	0	0
Genessee	Vexans	98	773	0	0

2003 Mosquito Surveillance Results, *Aedes vexans*, continued

Lenawee	Vexans	12	98	0	0
Livingston	Vexans	2	2	0	0
Midland	Vexans	177	2398	0	0
Monroe	Vexans	20	318	1	3.144654
Oakland	Vexans	112	777	1	1.287001
Saginaw	Vexans	1289	12592	49	3.89136
Wayne	Vexans	28	230	1	4.347826

2003 Mosquito Surveillance Results, Coq. perturbans

Arenac	Perturbans	4	18	0	0
Genessee	Perturbans	27	134	0	0
Livingston	Perturbans	22	248	0	0
Monroe	Perturbans	2	8	0	0
Oakland	Perturbans	41	425	0	0
Washtenaw	Perturbans	1	17	0	0
Arenac	Perturbans	3	24	0	0
Barry	Perturbans	11	118	0	0
Bay	Perturbans	247	3800	4	1.052632
Calhoun	Perturbans	6	56	0	0
Cass	Perturbans	14	82	0	0
Genessee	Perturbans	41	320	0	0
Isabella	Perturbans	254	254	0	0

2003 Mosquito Surveillance Results, Coq. Perturbans, continued

Lenawee	Perturbans	6	49	0	0
Macomb	Perturbans	1	1	0	0
Midland	Perturbans	61	763	0	0
Monroe	Perturbans	7	43	0	0
Muskegon	Perturbans	8	21	0	0
Oakland	Perturbans	43	274	0	0
Ottawa	Perturbans	5	7	0	0
Saginaw	Perturbans	117	620	3	4.83871
Tuscola	Perturbans	28	476	0	0
Washtenaw	Perturbans	1	6	0	0
Wayne	Perturbans	5	65	0	0

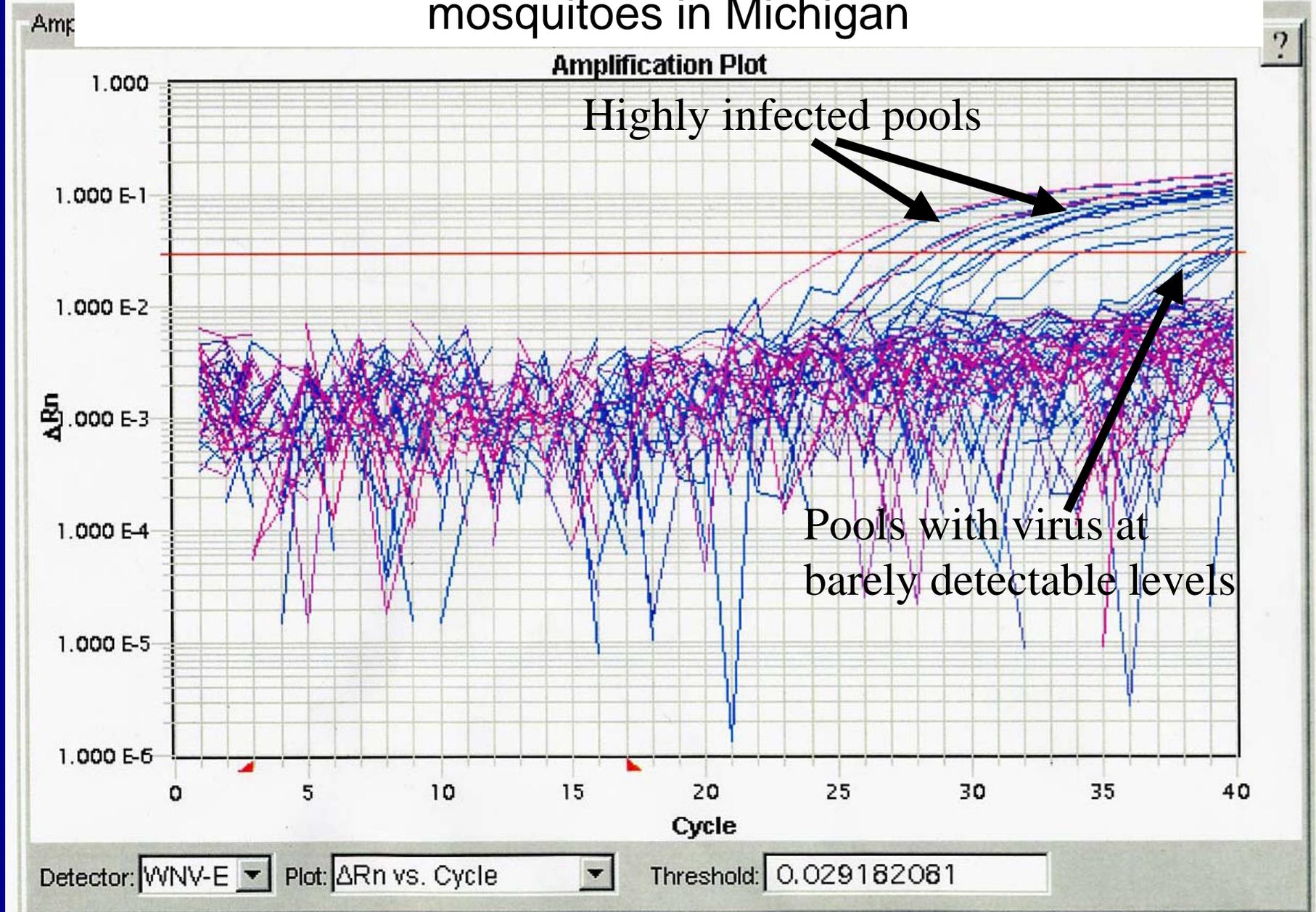
2003 Mosquito Surveillance Results, Oddities of concern

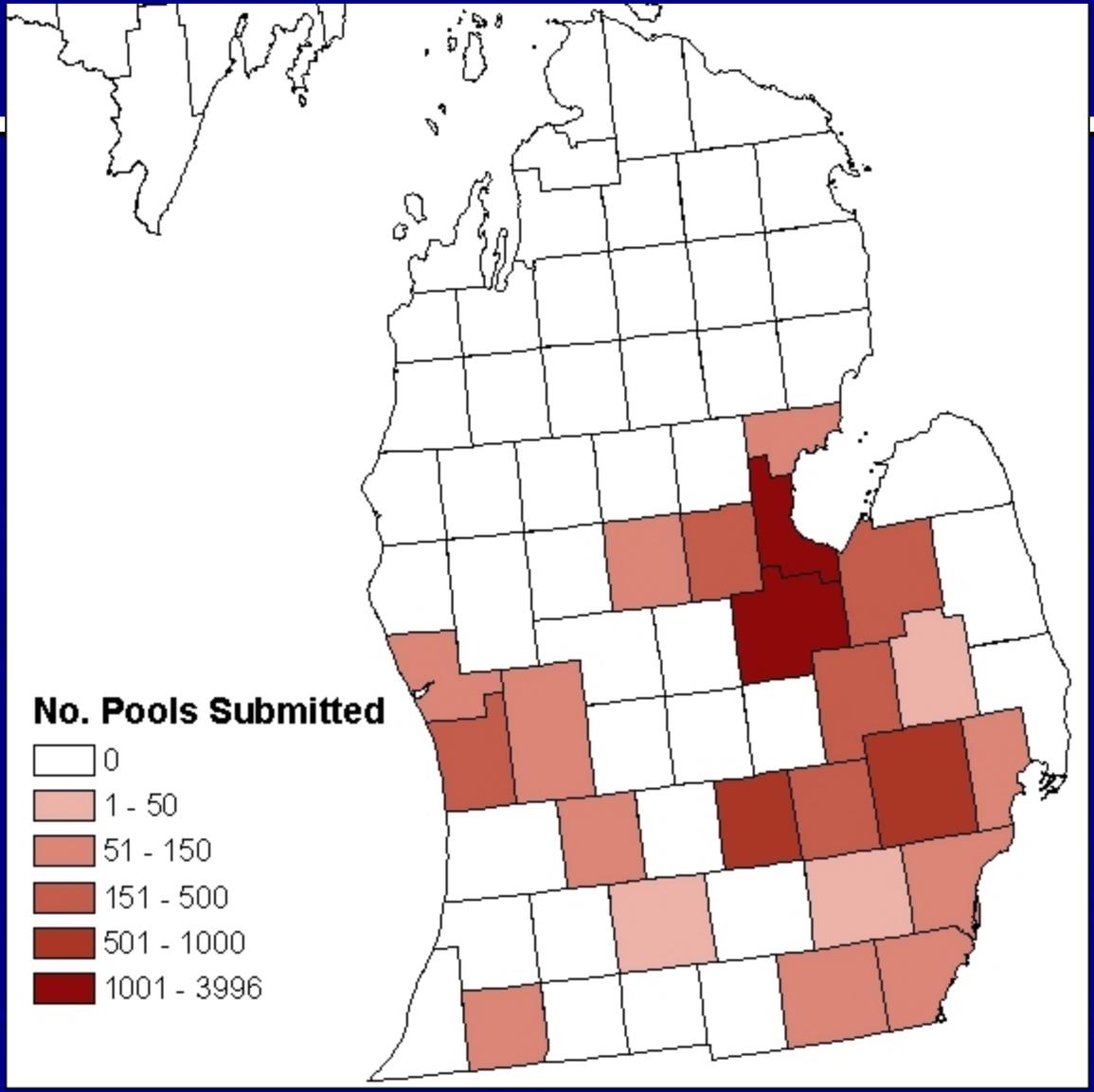
County	Species	No. pools	Total tested	No. pos	MIR
Monroe	Stim/Fitch	2	7	1	142.8571
Saginaw	Provocans	10	15	1	66.66667
Bay	Inornata	15	23	1	43.47826
Oakland	Trivittatus	11	59	1	16.94915
Saginaw	Stim	91	358	5	13.96648
Saginaw	Tris	148	307	4	13.02932
Saginaw	Punct	213	337	4	11.86944
Saginaw	Stim/Fitch	121	359	4	11.14206
Saginaw	Sticticus	207	1180	9	7.627119
Saginaw	Walkereri	161	443	2	4.514673
Monroe	Walkereri	40	836	2	2.392344

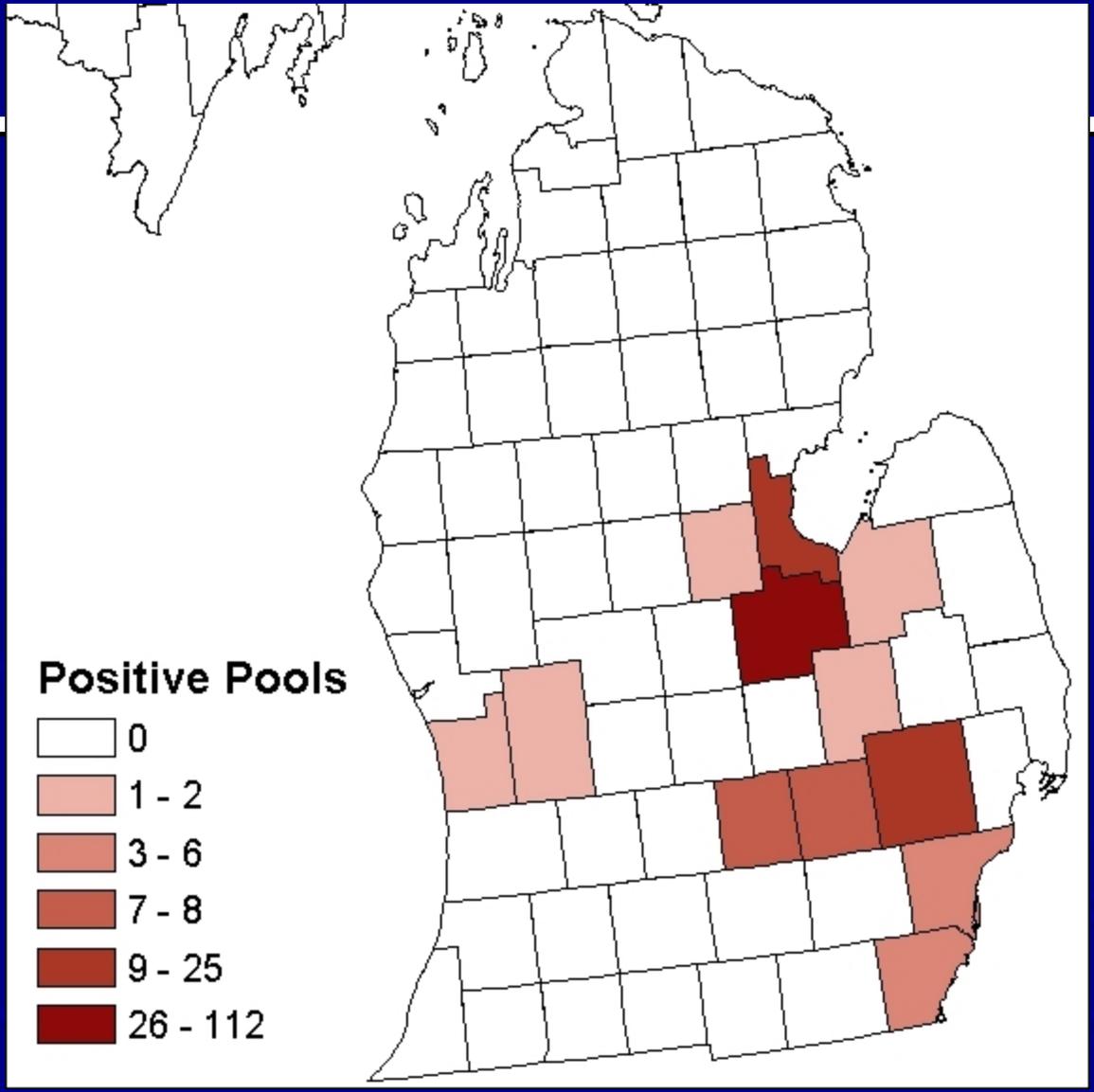
What are we learning?

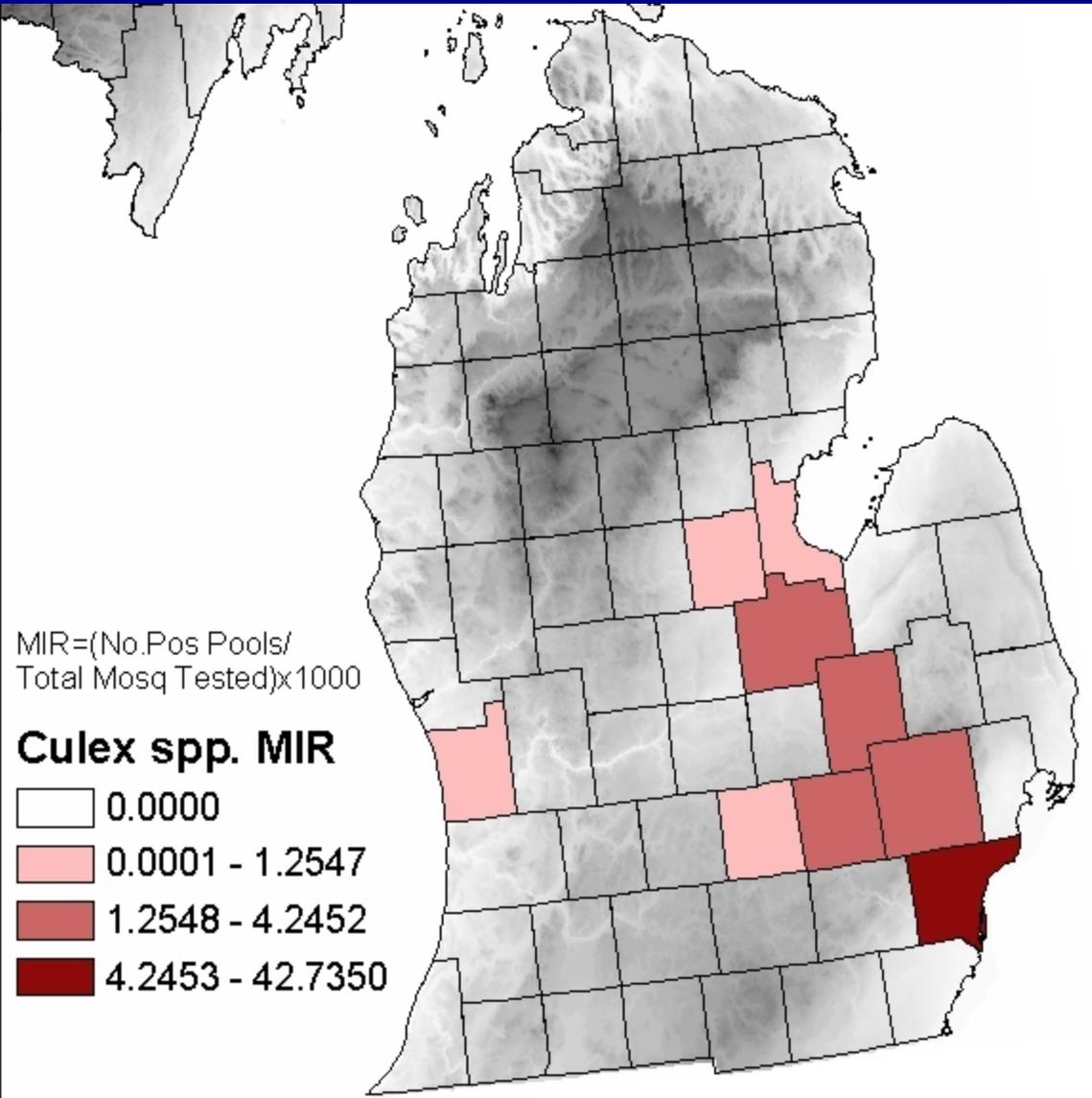
- WNV is widespread in Michigan mosquitoes, in many different areas, at low to high infection rates
- The “right” species are infected, i.e., the Culex species, and in some places at alarmingly high infection rates
- Other species are also infected. What does it mean?
 - Bridge vectors?
 - Infected but not infectious?
 - Transovarial transmission?

Quantitative PCR amplification of WNV naturally infected mosquitoes in Michigan









Quantitative PCR

- Rapid, reasonably priced, potential for automated data reporting, low error rate, high sensitivity and specificity
- Can provide a quantitative estimate of the amount of virus in a “positive pool;” this could help with interpretation of the results. Pools with “low virus” may not be meaningful to vector status.

Automated RNA extraction

- Rapid, reasonably priced, low contamination rate, moves to quantitative PCR quickly; technician time intensive
- You can help! How?
 - Send in pools in pre-labeled extraction tubes with mosquitoes and bb's already in them
 - Send by email attachment an Excel database of the submitted pools with standardized variable fields (columns) and standardized

Implementing New Standards in Mosquito Submission and Processing

- Historically
 - No universal guidelines governing the submission of mosquito pools for screening
- Limited capacity
- Reasons
 - Increased surveillance and submissions
 - Time consuming molecular and serologic methods
 - Lack of uniform submission guidelines
 - Bottlenecks in results reporting

Increasing Submissions

- Historically, MDCH, MSU, and MDA have supported targeted mosquito surveillance activities for endemic diseases such as St. Louis Encephalitis and Eastern Equine Encephalomyelitis.
- With the emergence of WNV in 1999 (New York) and the subsequent 2002 outbreak in Michigan, public health and mosquito abatement have become increasingly involved in surveillance, control, and public education.
- The demand has risen for mosquito screening from ~2000 mosquito pools in 2001, to over 15,000 by 2003.

Submission of Specimens

- Tubes are pre-labeled and have bb's
- Boxes have a return FedEx slip
- Specimen numbers are standardized and unique
- Database is uniform among all sample submitters
- The database is submitted by email attachment

THANK YOU!

- This is a collaborative interagency program
- It is worthwhile because we learn what is going on out there
- Please try to help us make the system better
- We would like a list of 2004 submitters; the season is coming up