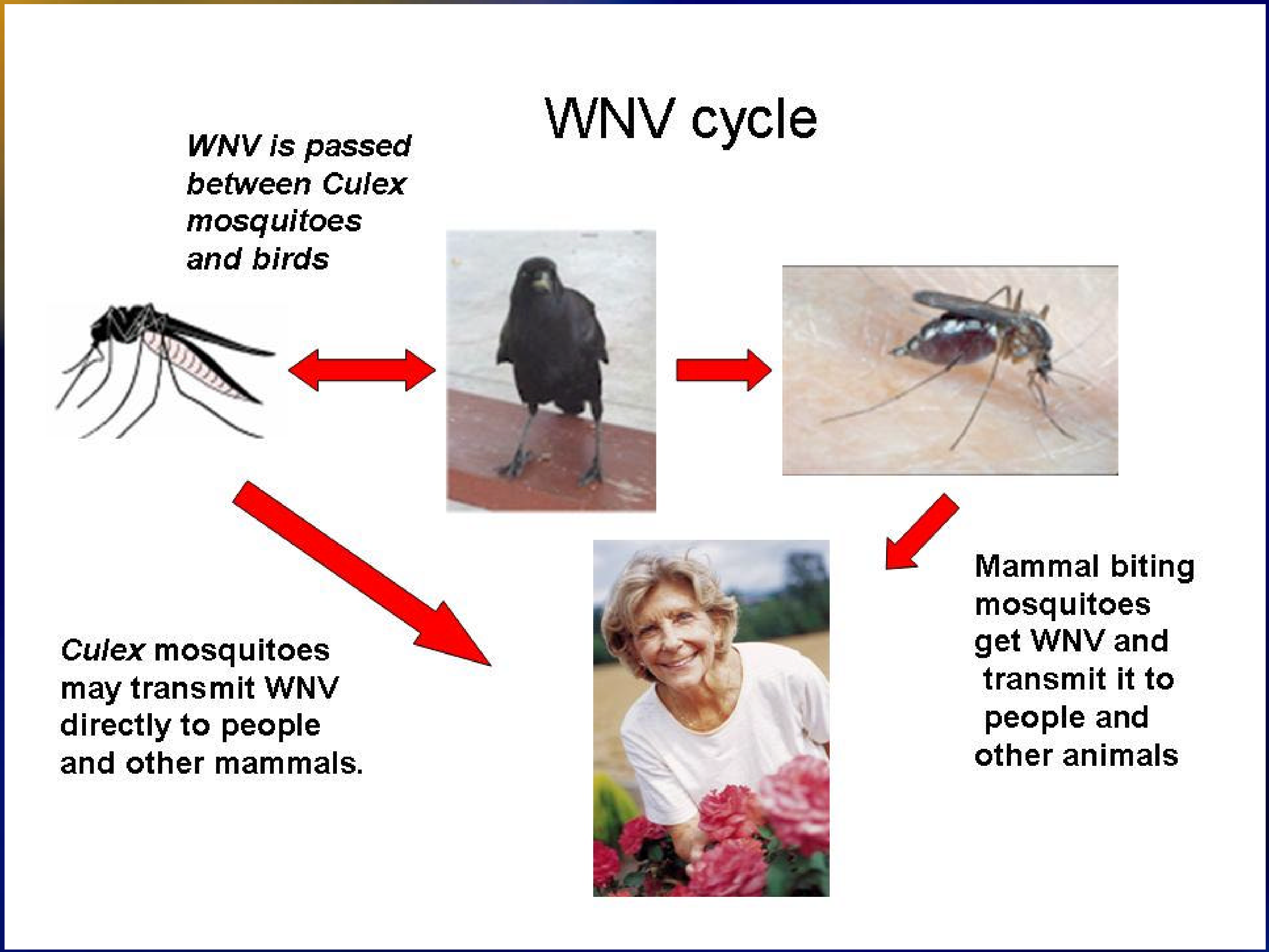
Pesticides Used to Control West Nile Virus: Toxicity to the Estuarine Grass Shrimp, *Palaemonetes pugio*

Robin K. Barnes

Outline

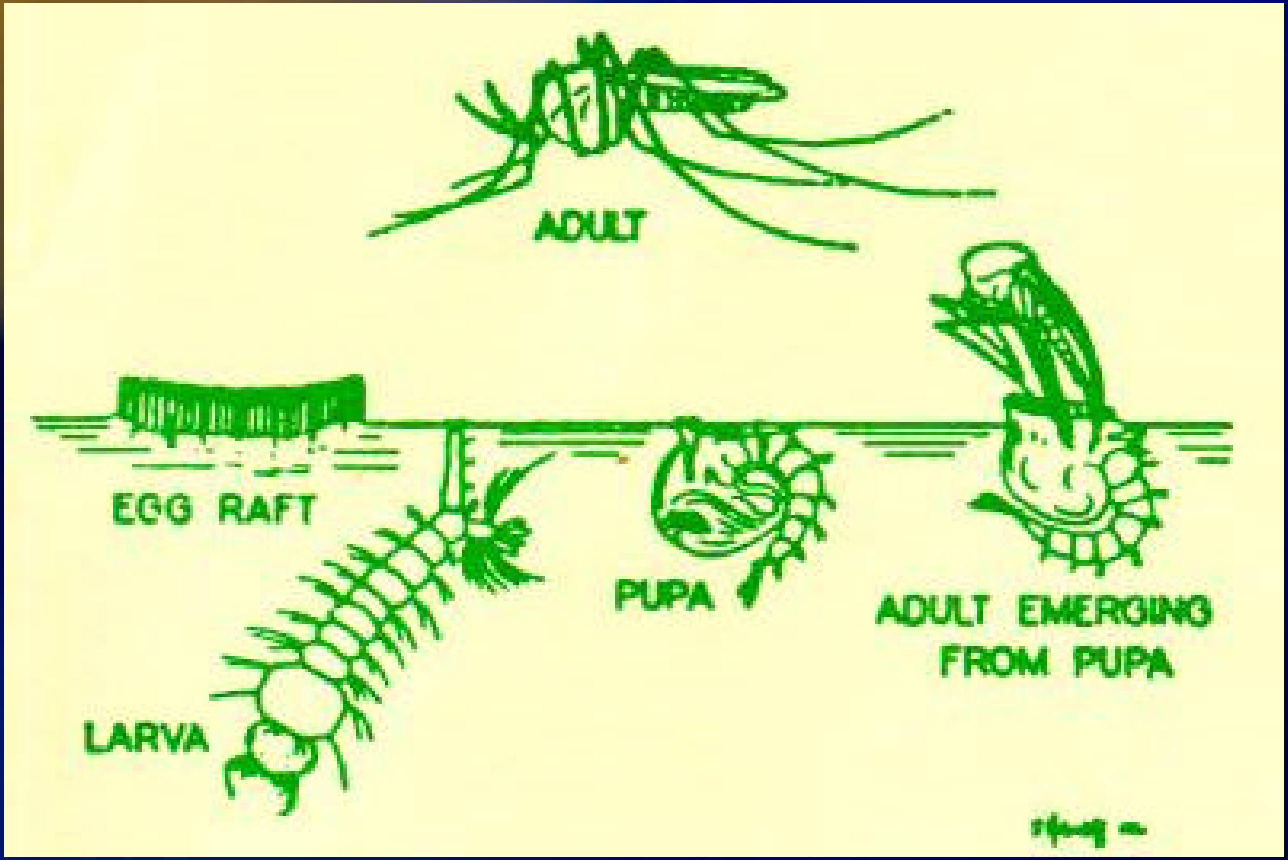
- Background
- Experimental Approach
- Field & Laboratory Results
- Conclusions
- Significance

Why is mosquito control important?



http://www.plymouthmosquito.com/wnv.htm

Life Stages of the Mosquito



- Need standing water to complete breeding cycle
- Salt marshes provide the perfect habitat

History of mosquito control on Long Island

- Began after the Civil War (post 1865), continued through Great Depression
  - Returning soldiers brought malaria
- Drainage ditches dug—remove standing water
  - Eliminated malaria; continued ditching to remove nuisance

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http://camel2.conncoll.edu/ccrec/greennet/arbo/publications/34/FRAME.HTM

Mosquito Spraying on Long Island

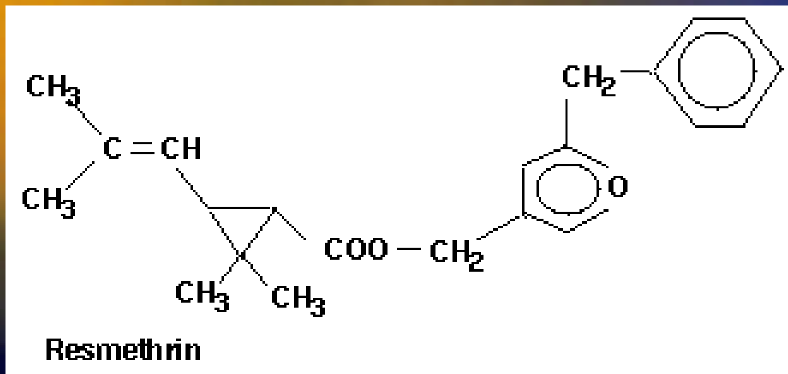
- Spraying has occurred on LI since the 1930s

- 1999---West Nile Virus first detected in U.S. (spray increases)

- Current practices:
  - larvicide with BT or Altosid® (methoprene) often
  - adulticide with Scourge® (resmethrin) when needed

http://camel2.conncoll.edu/ccrec/greennet/arbo/publications/34/FRAME.HTM

Pesticides Used on Long Island

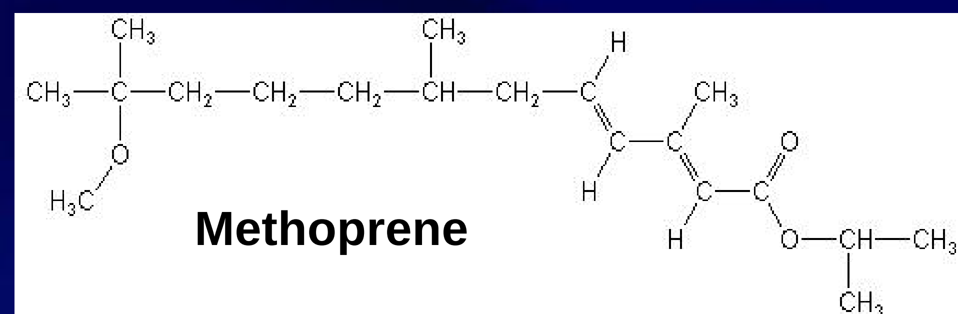


Resmethrin:

- Pyrethroid (Scourge®) -- acts as a neurotoxicant
- [1]LC50s (fish species) ~ generally < 10 µg/L
- [1]LC50s (shrimp species) ~ typically < 1µg/L

Methoprene:

- Insect growth regulator (Altosid®)
- [2]LC50s (fish species) ~ > 100 mg/L
- [3, 4]Studies with shrimp variable (LC50s range from 14 µg/L - 1 mg/L)



[1] Bradbury & Coats, 1989
[2] http://pesticideinfo.org
[3] Wirth et al., 2001
[4] Brown et al., 1996

Why study this?

- Spraying is an important component of disease/nuisance control

- Both chemicals can be very toxic to organisms at low concentrations

    - Potential for non-target organisms to be affected

- No local EIS performed by vector control…

As part of the long-term assessment proposed by the NYSDEC, Cashin Associates coordinated a caging study that included many parties…

Cashin Associates (*coordination*)

Suffolk County Department of Health Services Division of Environment Quality (*sampling*)

Suffolk County Department of Public Works – Vector Control (*spraying*)

U.S. Geological Survey (*sampling & analysis*)

Brownawell's Laboratory Stony Brook University (*sampling & analysis*)

RTP Environmental Corp. (*modeling*)

Integral Consulting Inc. (*risk assessment*)

Long Island University (*caged fish assessment*)

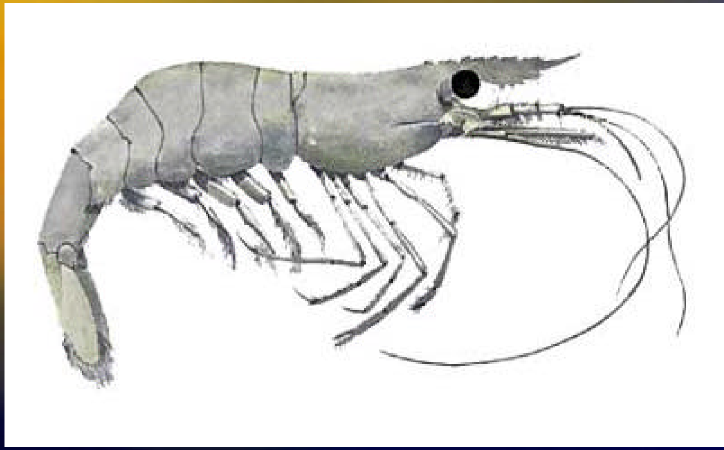
Caging study design—original plan

- Original plan
  - Multiple study sites
  - Multiple reference sites
  - Multiple sprays
- Investigate both larvicide and adulticide use
- Limited approval by the NYSDEC
  - No planned sprays allowed, only operational sprays

Caging study design—what happened

- Three larvicides
  - First two: 8/3 and 8/10
  - Both had the same two spray & reference sites
  - The third: 9/1—only one spray site
- Two adulticides performed
  - On 8/18 and 8/25
  - Same sites as spray & reference

Why Shrimp??



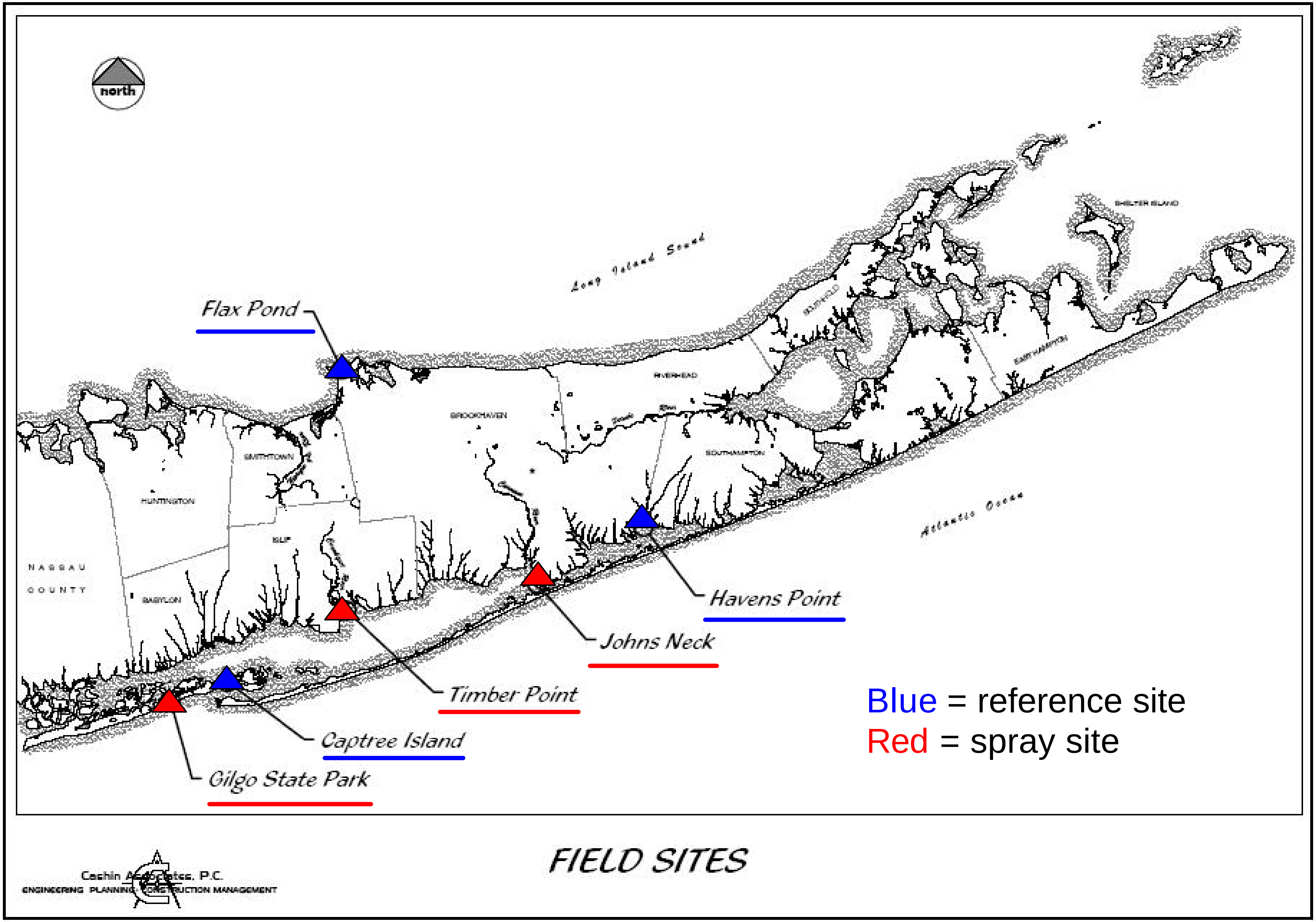
❖ *Palaemonetes pugio*

- Crustaceans sensitive to pesticides
- Used as an estuarine health indicator by US EPA
- Important component in food web (prey species)
- Aid in breakdown of detritus

Experimental Approach

Three part approach

Part I: Caging study with shrimp



north
Long Island Sound
Atlantic Ocean
NASSAU COUNTY
HUNTINGTON
SMITHTOWN
ISLIP
BABYLON
BROOKHAVEN
RIVERHEAD
SOUTHAMPTON
SOUTHOLD
EAST HAMPTON
SHELTER ISLAND
Flax Pond
Havens Point
Johns Neck
Timber Point
Captree Island
Gilgo State Park
Blue = reference site
Red = spray site
FIELD SITES
Cashin Associates, P.C.
ENGINEERING PLANNING CONSTRUCTION MANAGEMENT

Flax Pond field site

Black circles indicate final cage positions.

Havens Point field site

Black circles indicate final cage positions.

Johns Neck field site

Black and white circles indicate final and preliminary cage positions, respectively.

Captree Island field site

Black circles indicate final cage positions.

Timber Point field site

Black and white circles indicate final and preliminary cage positions, respectively.

Details of caging study

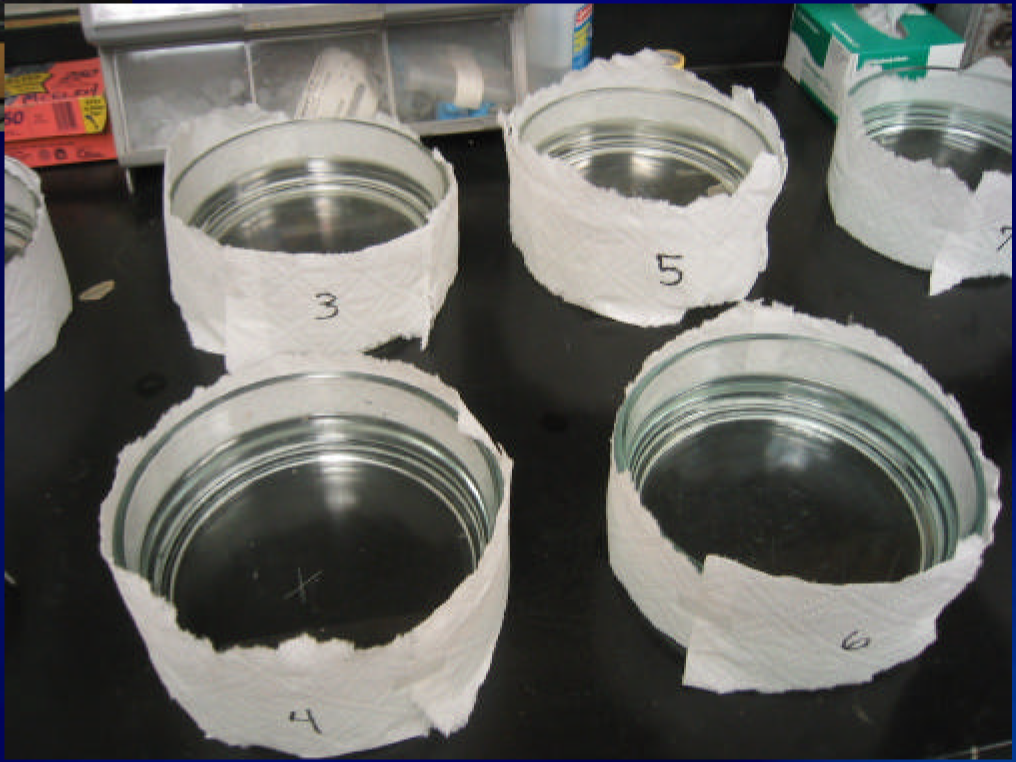
- Fish/shrimp placed 24 h pre-spray
- Survival checked daily for 4 days
- DO & temperature monitored every 30 min for duration of all experiments
- Chemistry—for both larvicide & adulticide
  - Water samples taken 30 min pre-spray; 1-96 h post-spray
  - Sediment samples taken pre-spray; 1 & 4 days post-spray
  - Analysis of sediment & water by GC-MS & LC-MS
  - Brownawell and Terracciano performed analyses on water and sediment samples

Part II: Laboratory Studies

Test Type:	Method Summary:	Why Studied?
Static Tests:	-water taken after spray -replenished daily in lab	-independent measure of toxicity without environmental variables
Prey Capture Tests:	-1 or 2 h in duration -5 brine shrimp prey -replenished every 15 m	-assessment of non-lethal effects of pesticide exposure on shrimp
Dosing Experiments:	-3 or 4 replicates -dosed 1 time/day for 4 d -water & acetone controls	-measure acute toxicity of LI pesticides on shrimp

Static & Dosing Experiments…

Prey Capture Experiments…

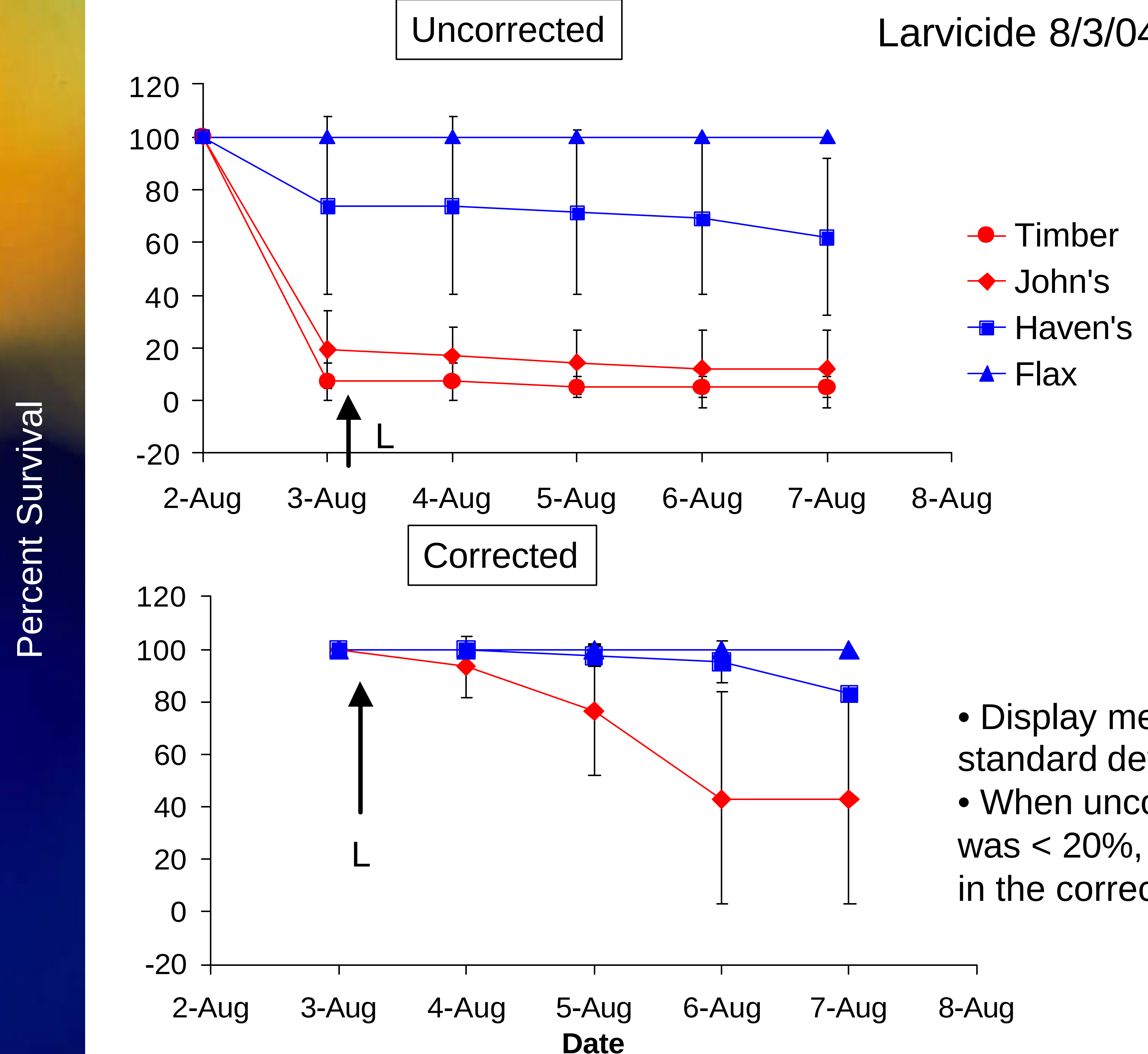


Part III: Benthic community structure analysis

- Cores taken from all field sites after spraying done for the year
- Abundance and identification of species determined
- Compared reference sites (no known recent spraying) with sprayed sites
- Analysis performed to assess chronic effects associated with pesticides

Caging Study Results

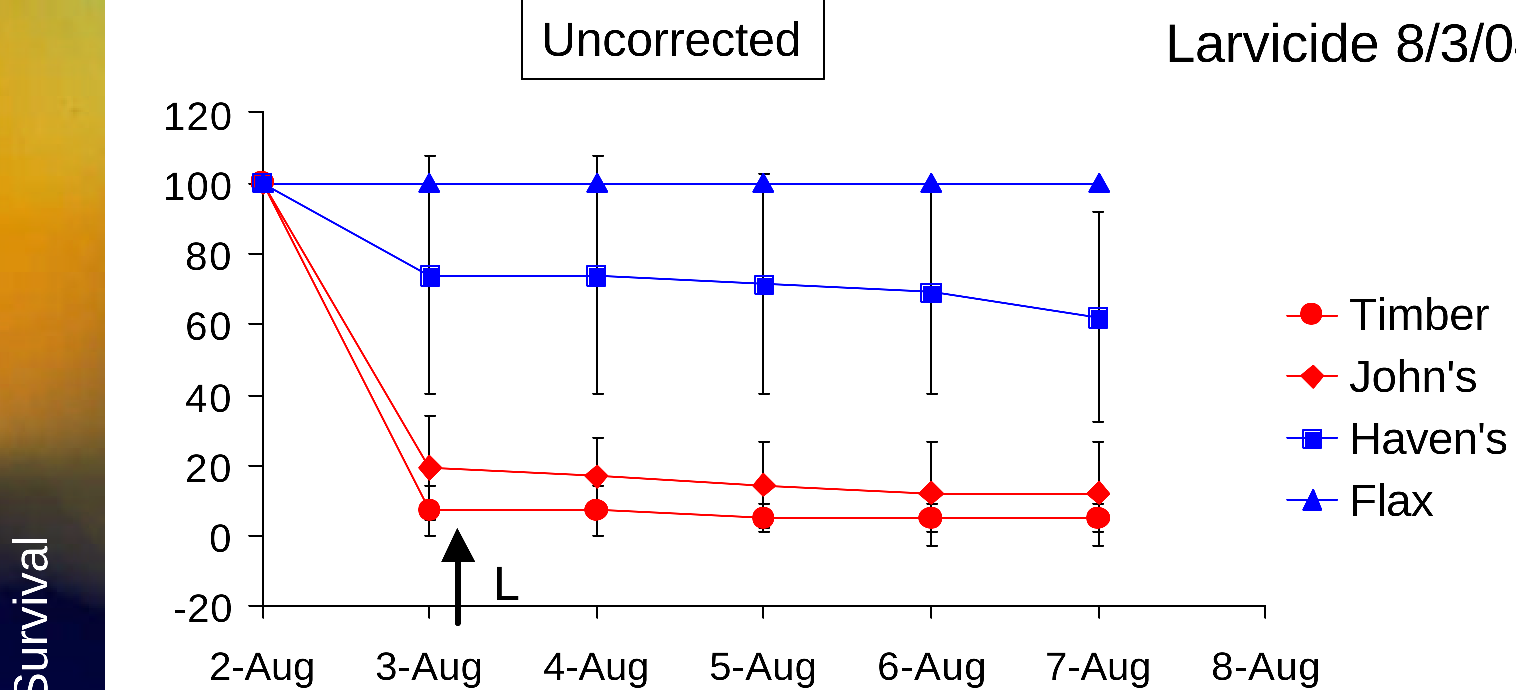
Larvicide 8/3/04

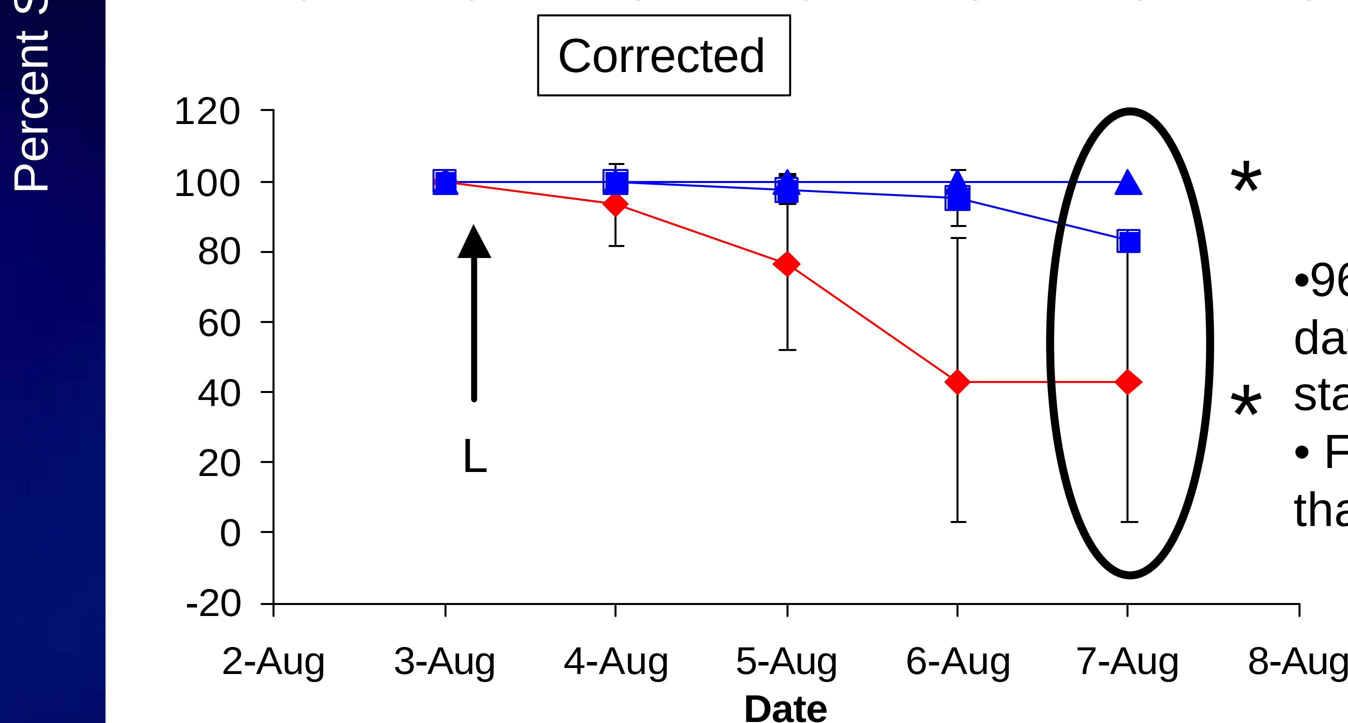


RED = Spray sites
BLUE = Control sites

- Display means of 3 reps. & standard deviations
- When uncorrected survival was < 20%, it was not used in the corrected data

Larvicide 8/3/04



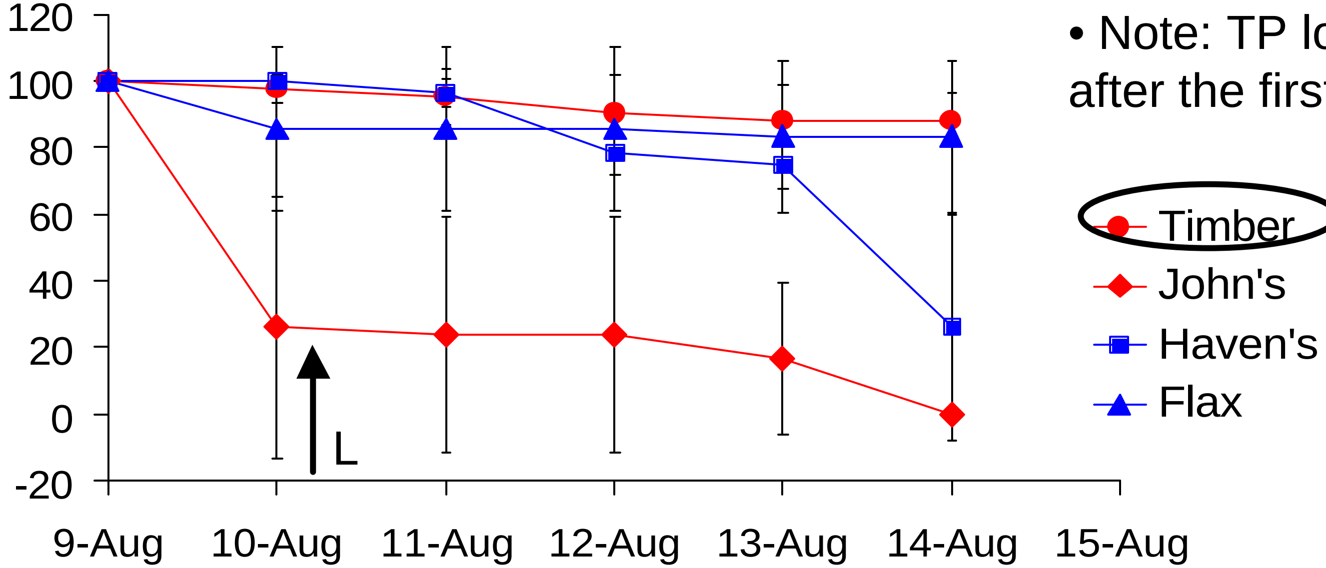


RED = Spray sites
BLUE = Control sites

- 96 h corrected survival data was used to perform statistics
- Flax significantly higher than Johns

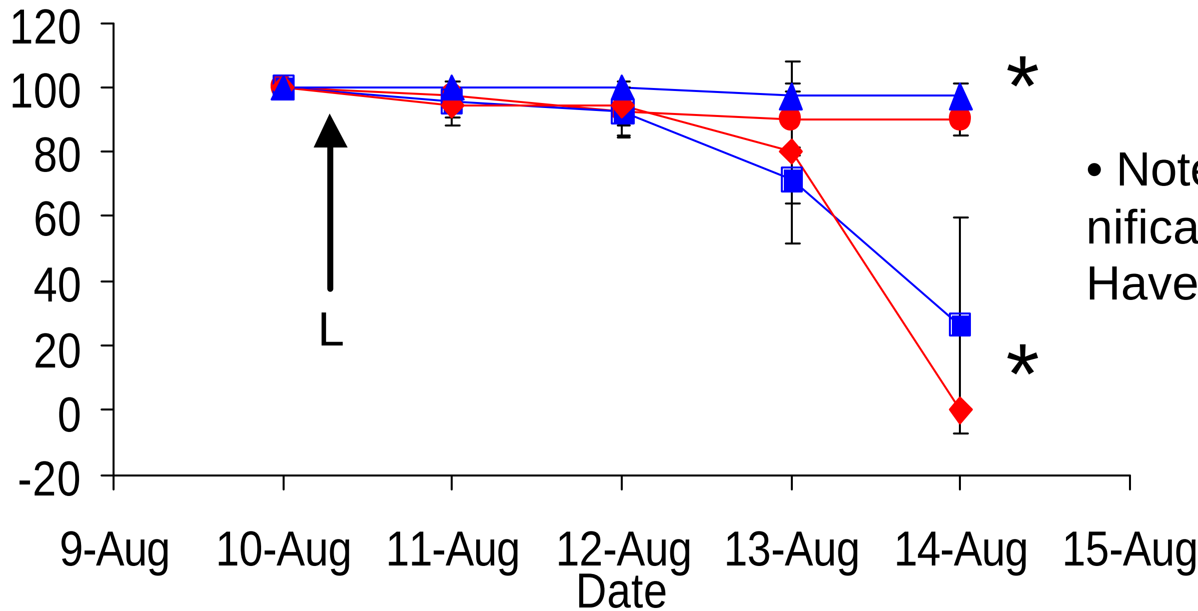
Larvicide 8/10/04

Uncorrected



- Note: TP location changed after the first larvicide

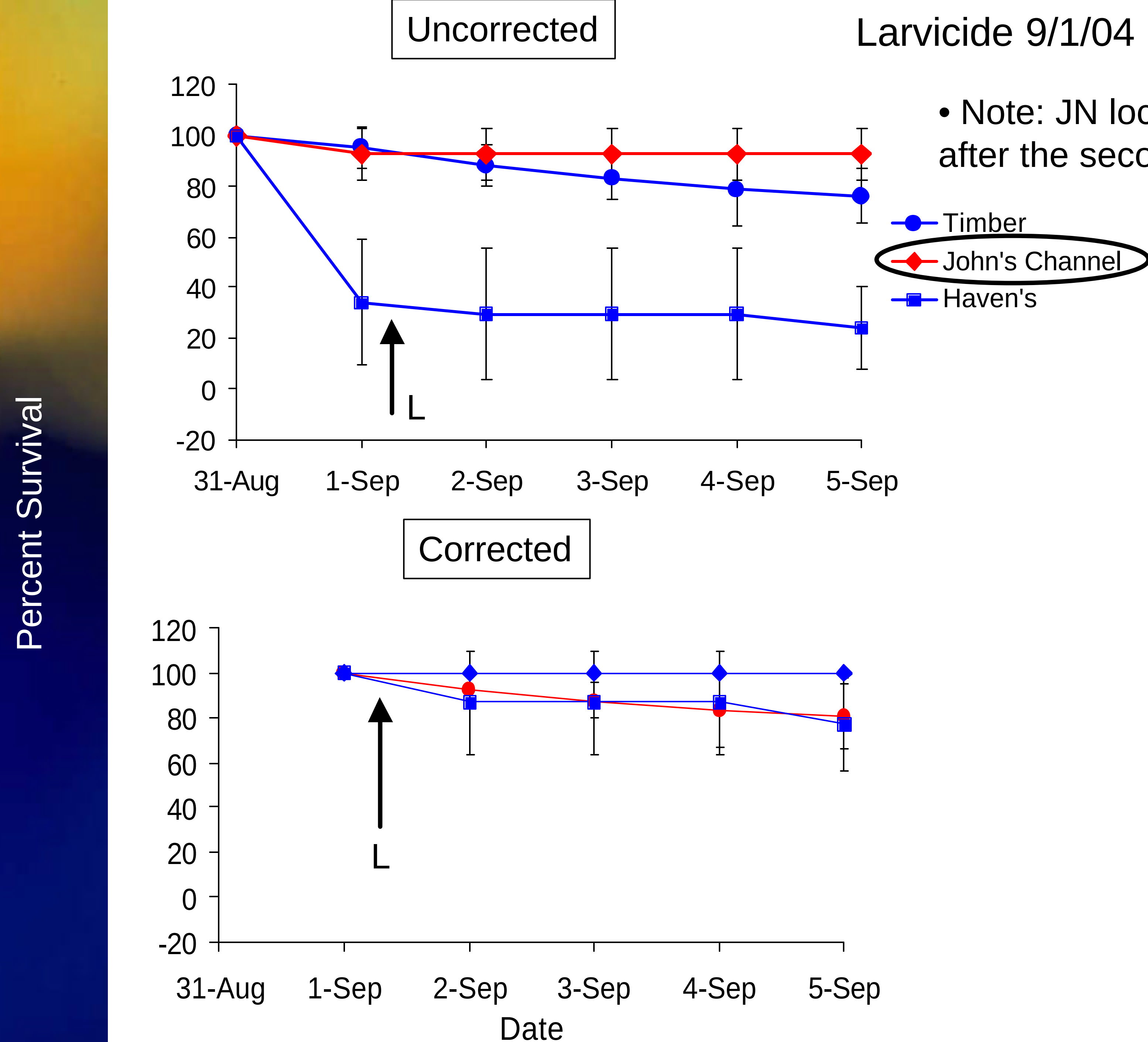
Corrected



RED = Spray sites
BLUE = Control sites

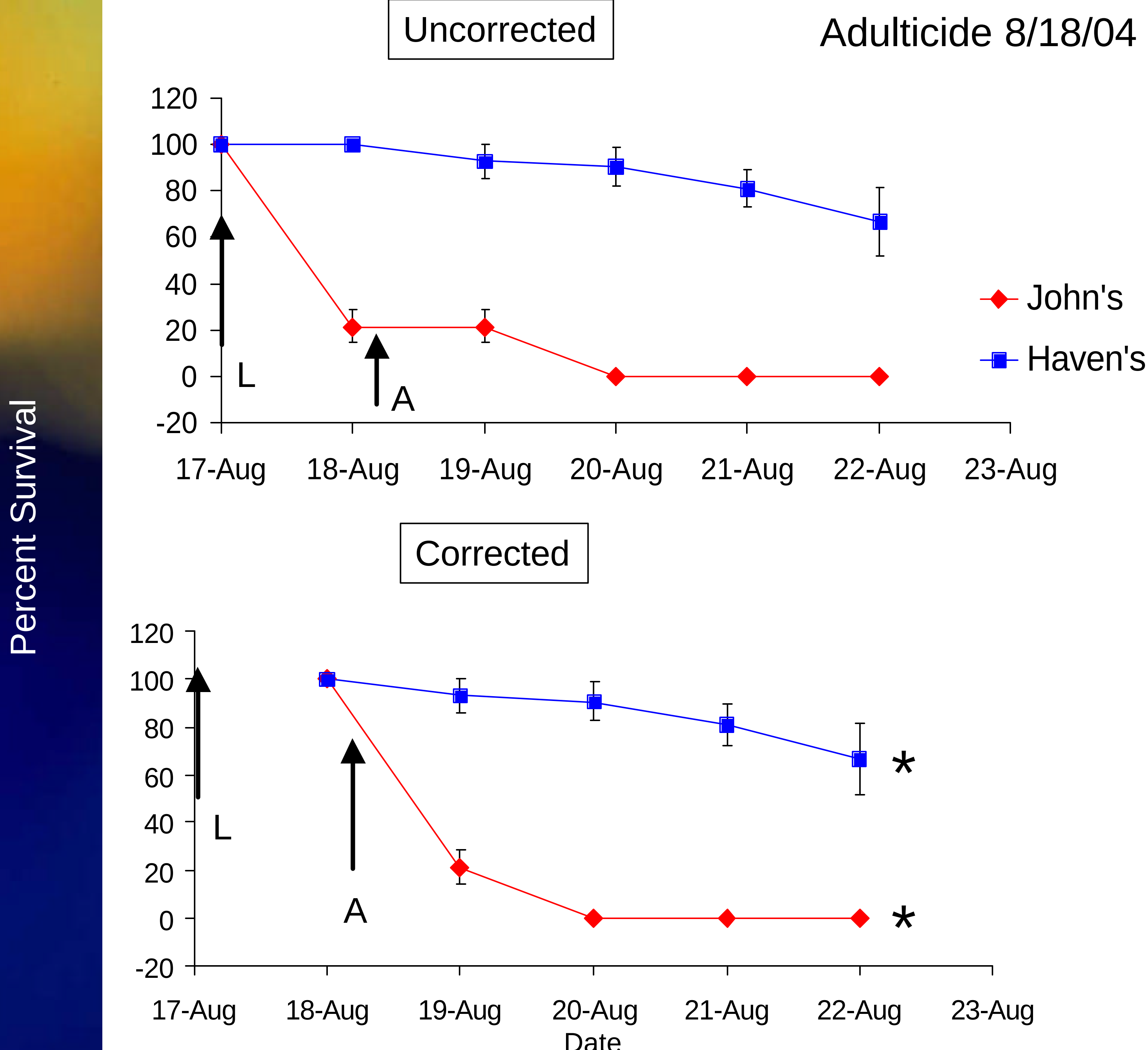
- Note: Timber & Flax significantly different from Havens & Johns

Larvicide 9/1/04



- Note: JN location changed after the second larvicide

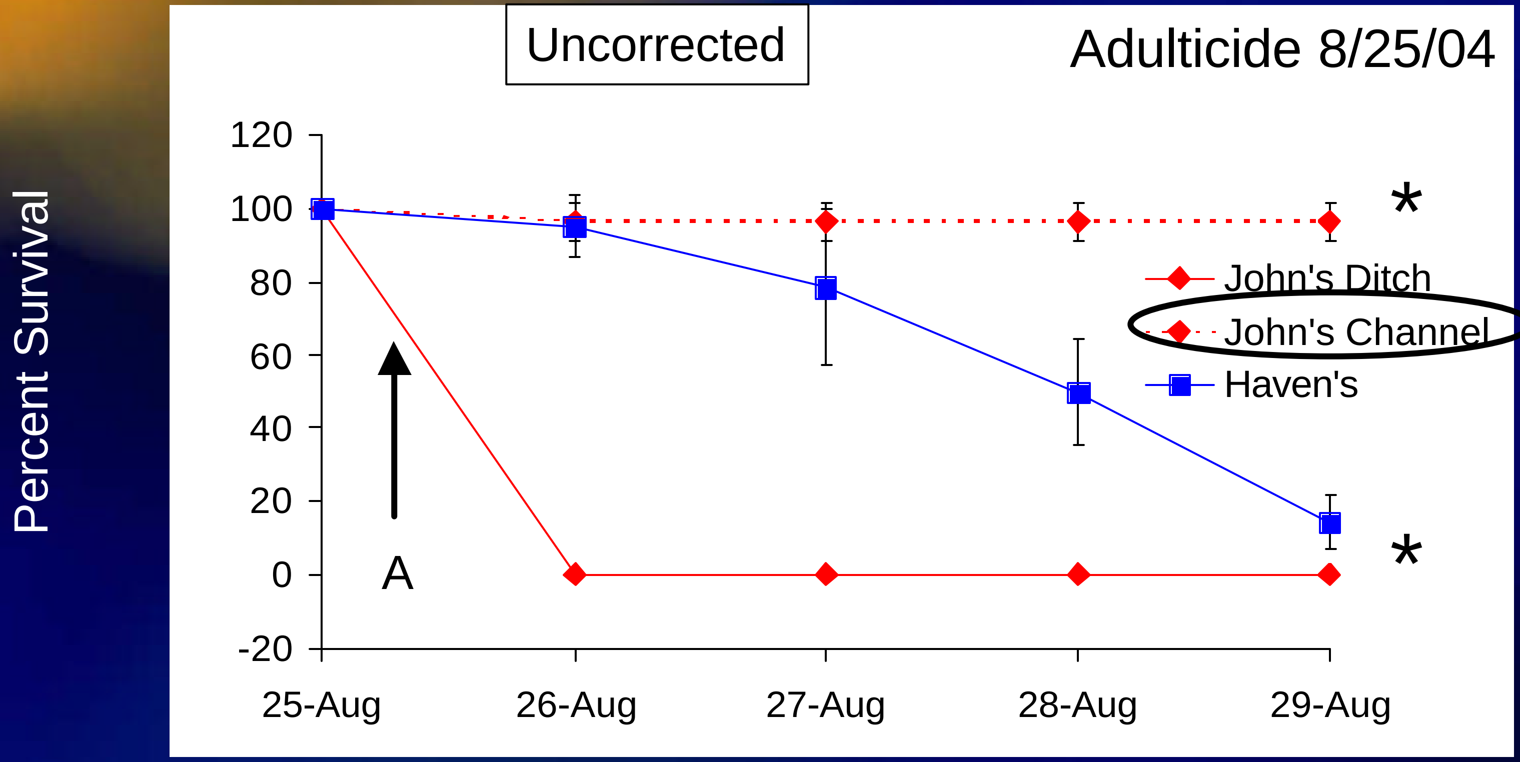
RED = Spray sites
BLUE = Control sites



Adulticide 8/18/04

RED = Spray sites
BLUE = Control sites

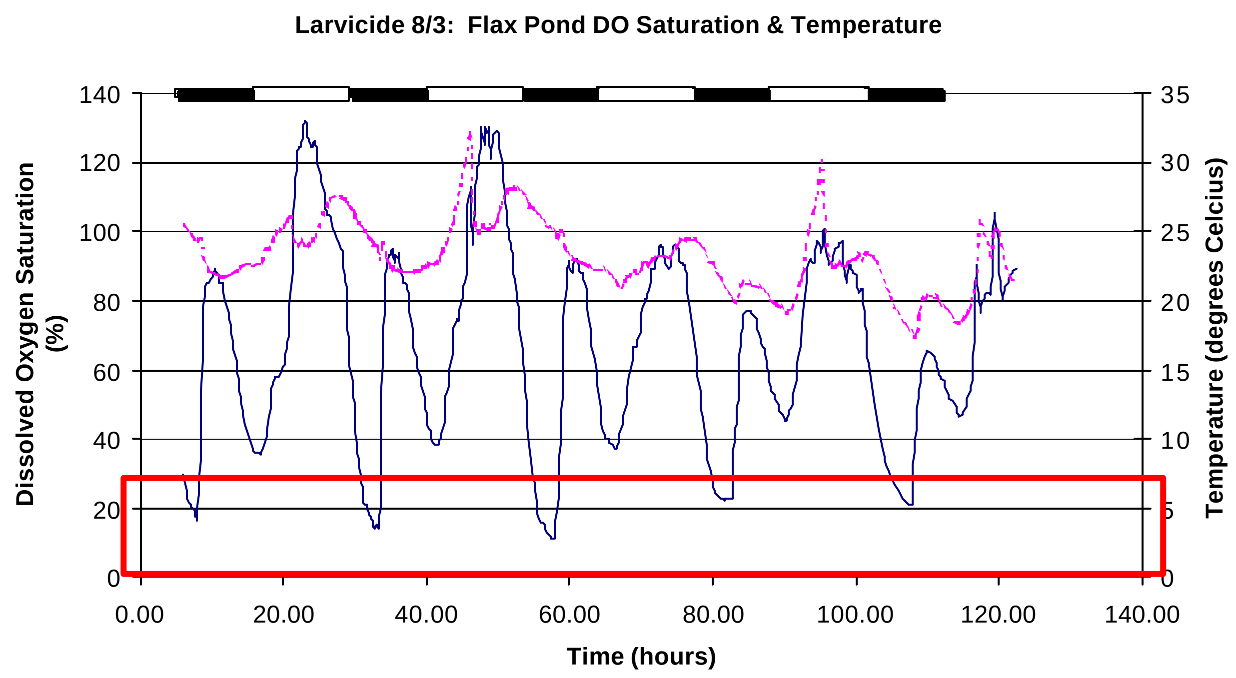
• Note: Johns channel significantly different from Johns ditch & Havens

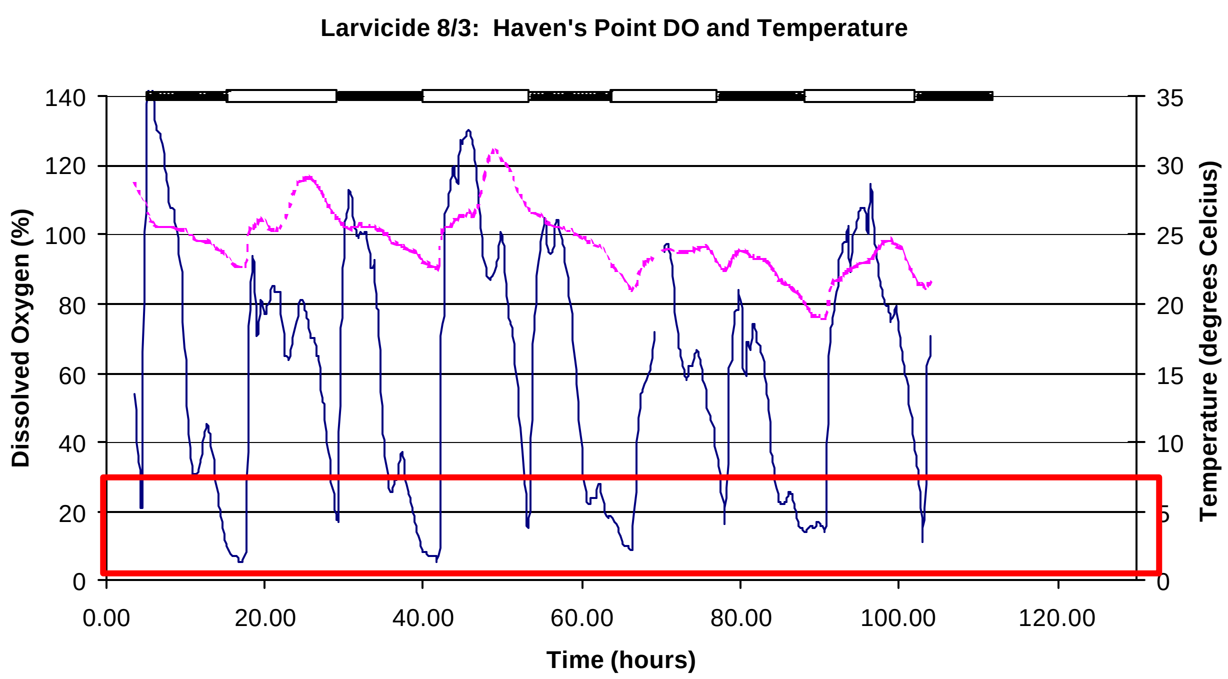


RED = Spray sites
BLUE = Control sites

• Note: JN ditch was the original site; channel was the location change

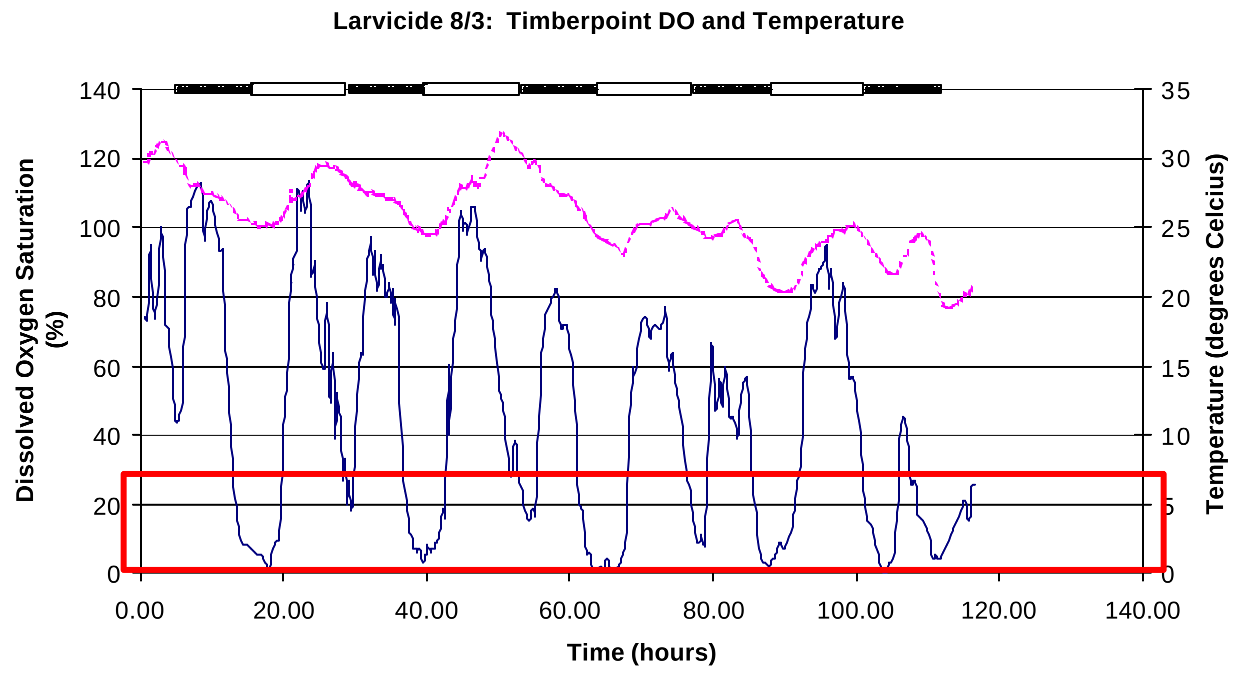
DO Graph Comparison Reference Sites

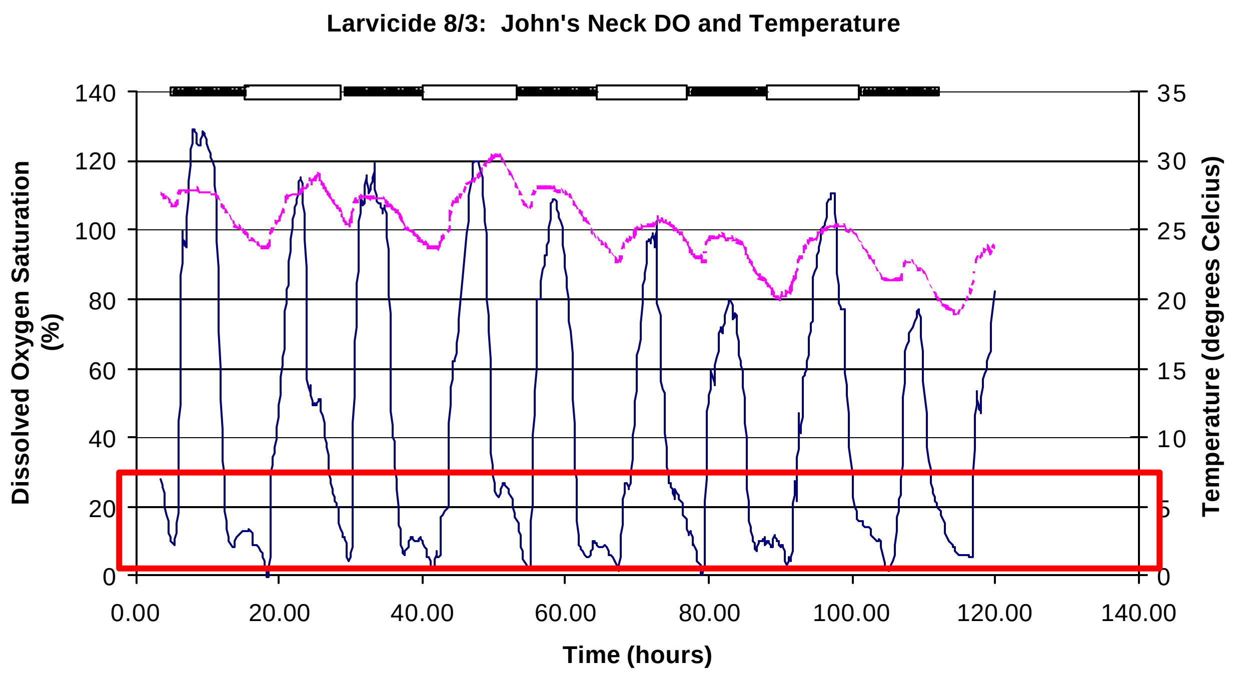




-blue line = DO
-pink line = temperature
-black/white strip = day & night sequence

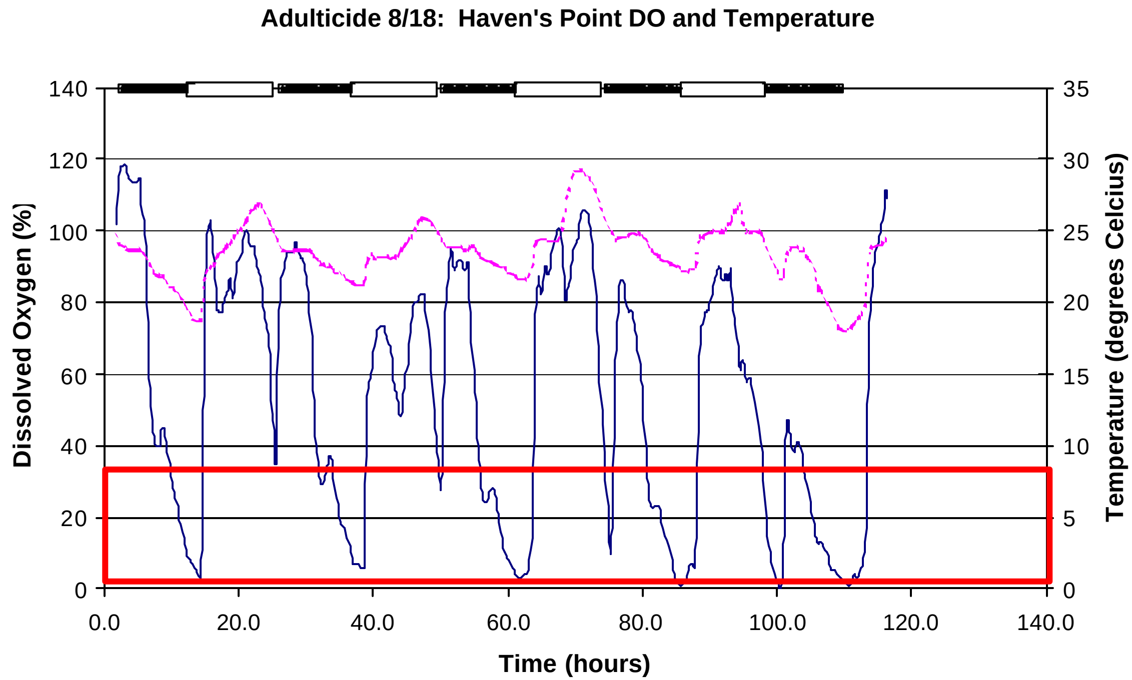
DO Graph Comparison Spray Sites

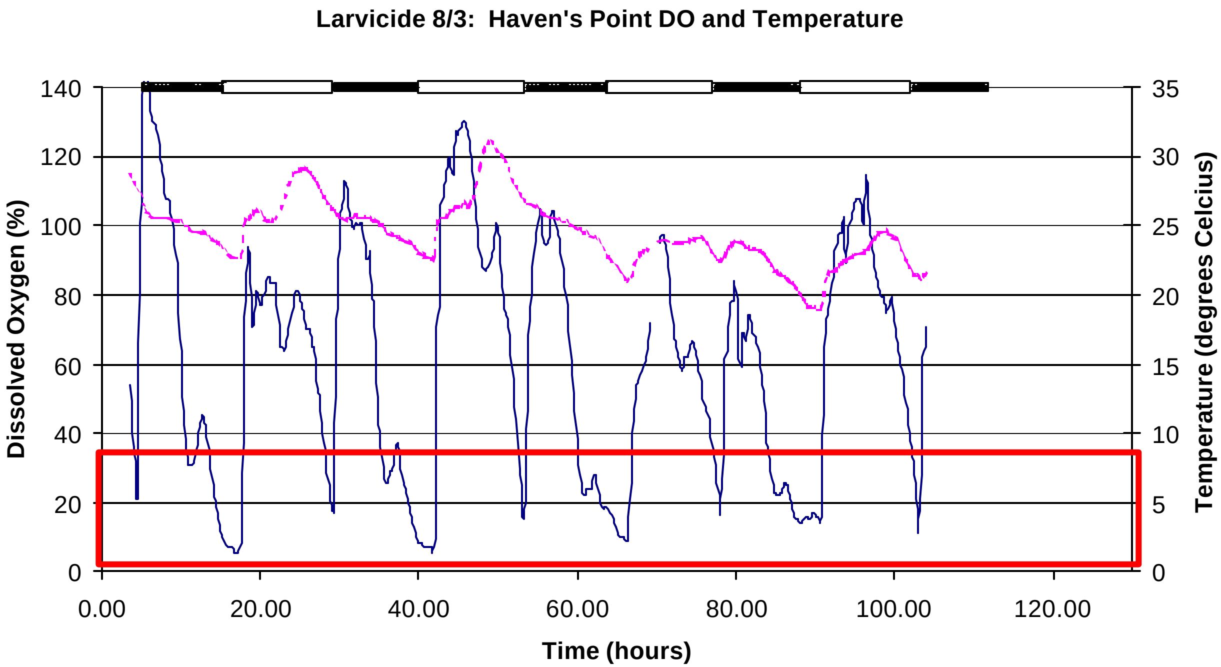




-blue line = DO
-pink line = temperature
-black/white strip = day & night sequence

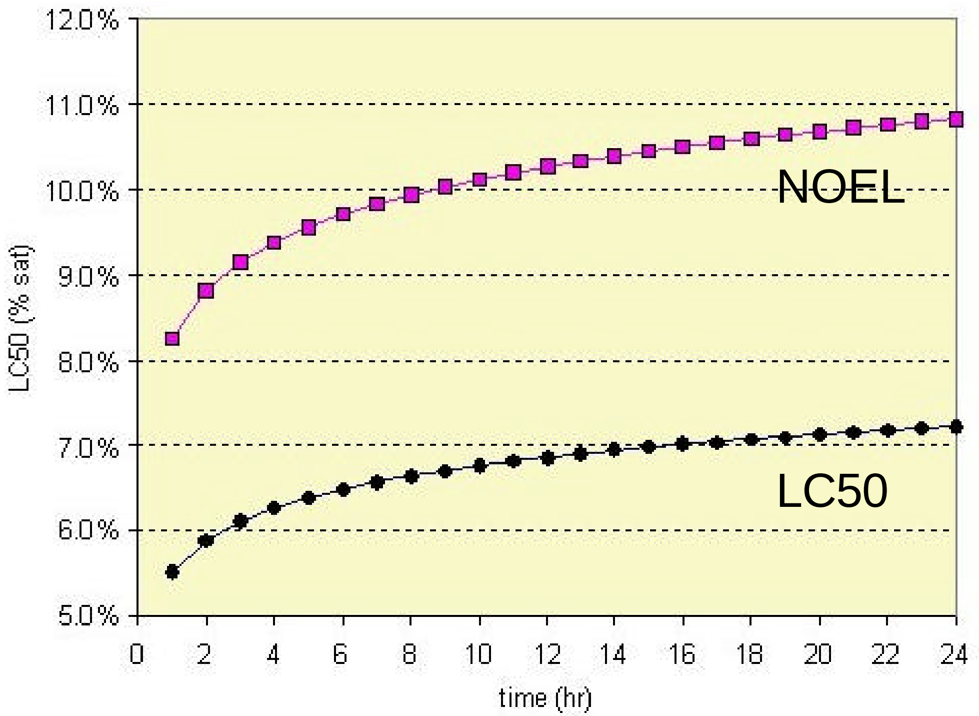
DO Graph Comparison Havens Point





-blue line = DO
-pink line = temperature
-black/white strip = day & night sequence

EPA DO Method: Time to death approach using daily cumulative low DO excursions



Adult *P. pugio* data

Two Criteria:

- LC50
  - <5% for > 1 h
  - 5-7 % for > 4 h
- NOEL
  - <9 % for >2 h
- Determined time below thresholds for both criteria

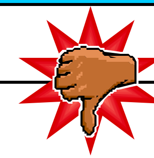
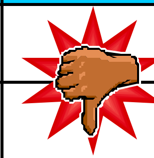
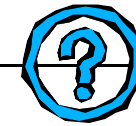
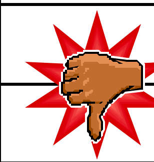
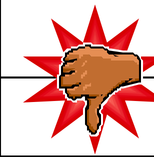
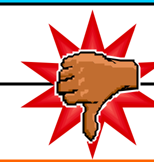
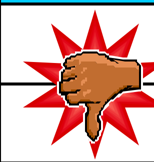
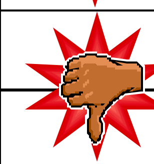
EPA-822-R-00-012 & G. Thursby

Caging Study: Summary of DO

Denotes when low DO could have caused mortality

Denotes that the DO is for some reason unknown…

Summary of Mortaltiy Observed in Caged Organisms - Oxygen Effect

			Spray Sites:		Control Sites:	
Spray Type:	**Dates:**	**Species**	**TP**	**JN**	**FP**	**HP**
Larvicide	**8/3/04**	**Fish**				
		Shrimp				
	8/10/04	**Fish**				
		Shrimp				
	9/1/04	**Fish**				
		Shrimp				
Adulticide	**8/18/04**	**Fish**				
		Shrimp				
	8/25/04	**Fish**				
		Shrimp				

denotes when cages were moved to deeper water

Caging Study: Summary of Survival

Summary of Mortaltiy Observed in Caged Organisms

			Spray Sites:		Control Sites:	
Spray Type:	**Dates:**	**Species**	**TP**	**JN**	**FP**	**HP**
Larvicide	8/3/04	Fish	☠	☠		
		Shrimp	☠	☠		
	8/10/04	Fish				☠
		Shrimp		☠		☠
	9/1/04	Fish				☠
		Shrimp				
Adulticide	8/18/04	Fish				☠
		Shrimp		☠		
	8/25/04	Fish				☠
		Shrimp				☠

denotes when cages were moved to deeper water

☠ Statistically significant mortality relative to another site at one or more days

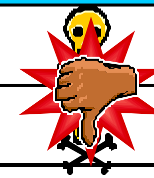
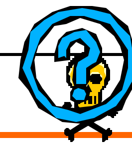
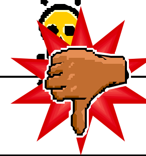
Summary of DO & mortality

• Mortality that can be attributed to low DO

Denotes when low DO could have caused mortality

Denotes that the DO is for some reason unknown…

Summary of Mortaltiy Observed in Caged Organisms - Oxygen Effect

			Spray Sites:		Control Sites:	
Spray Type:	**Dates:**	**Species**	**TP**	**JN**	**FP**	**HP**
Larvicide	**8/3/04**	**Fish**				
		Shrimp				
	8/10/04	**Fish**				
		Shrimp				
	9/1/04	**Fish**				
		Shrimp				
Adulticide	**8/18/04**	**Fish**				
		Shrimp				
	8/25/04	**Fish**				
		Shrimp				

denotes when cages were moved to deeper water

Statistically significant mortality relative to another site at one or more days

Conclusions from caging study survival & DO

- The field caging study did not indicate toxicity due to pesticide spraying

- All of mortality could have been caused by low DO alone using an EPA time-to-death approach

Laboratory Study Results

Static Testing Results

(with water collected 30-min post-spray and taken back to the lab)

Shrimp Static Exposure Experiments Concurrent with Spray Events

Start Date:	Site:	Mean % Survival: (after 96 hours)	Std. Dev.:
8/3/2004	Flax Pond	100.0	0.0
	Timber Point	100.0	0.0
	Johns Neck	83.3	40.8
8/10/2004	Flax Pond	100.0	0.0
	Timber Point	100.0	0.0
	Johns Neck	100.0	0.0
8/18/2004	Johns Neck	100.0	0.0
	Havens Point	100.0	0.0
8/25/2004	Johns Neck	100.0	0.0
	Havens Point	100.0	0.0
9/1/2004	Timber Point	83.3	40.8
	Havens Point	66.7	51.6

Conclusions from static tests

- Concentrations of chemicals applied during sprays was not directly toxic to grass shrimp under controlled conditions in the laboratory

Prey Capture Results

Caged & Static Tested Shrimp Only!

Date:	Site ID:	Number of Shrimp Tested:	Avg. eaten after 60 min.:	Std. Dev.:
8/3/04 Larvicide	Flax	7	9.14	7.01
	Havens	7	10.29	5.44
	Johns Neck	5	18.60	1.67
	Timber	2	9.00	9.90
	Flax static	5	8.40	5.98
	J.N. static	5	10.00	4.64
	T.P. static	5	8.60	2.19
8/9/04 Larvicide	Flax	10	12.10	4.72
	Havens	10	12.90	5.07
	J.N. static	6	12.50	6.69
	Timber	10	10.20	5.03
8/18/04 Adulticide	Havens	10	11.80	4.85
	J.N. static	5	10.20	3.27
	H.P. static	5	9.40	4.72
8/25/04 Adulticide	Havens	6	12.67	2.73
	H.P. static	5	11.00	1.22
	J.N. channel	14	11.00	3.26
	J.N. static	5	9.00	1.41
9/1/04 Larvicide	Havens	9	12.78	2.95
	Johns Neck	10	5.80	3.55
	Timber	11	9.82	4.60

Conclusions from prey capture tests

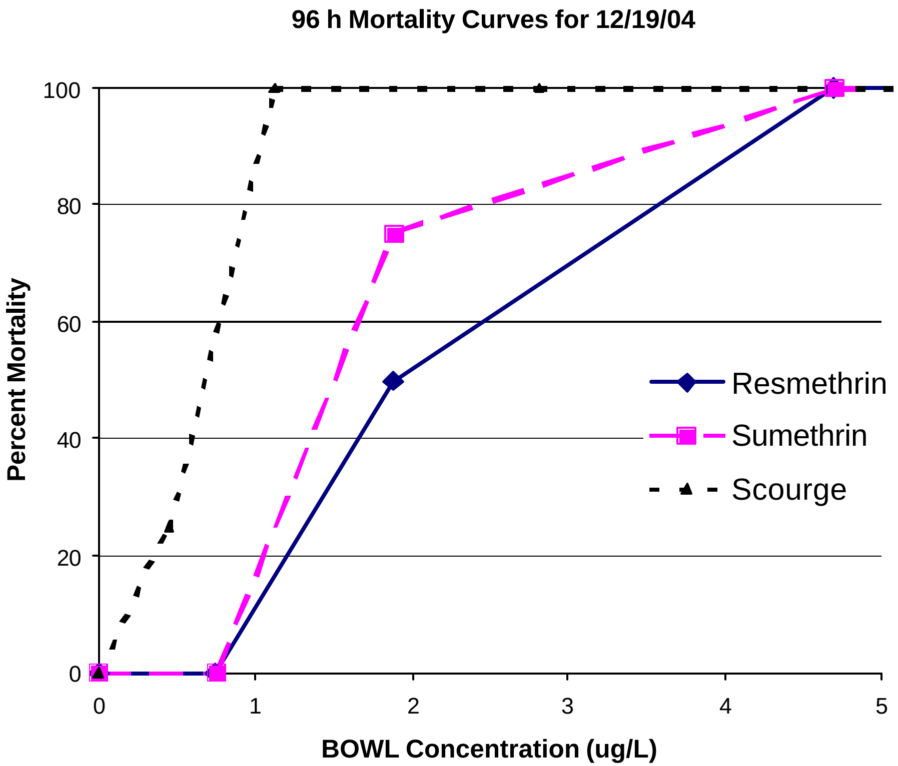
- Concentrations of chemicals applied during sprays did not affect the prey capture ability of grass shrimp under controlled conditions in the laboratory

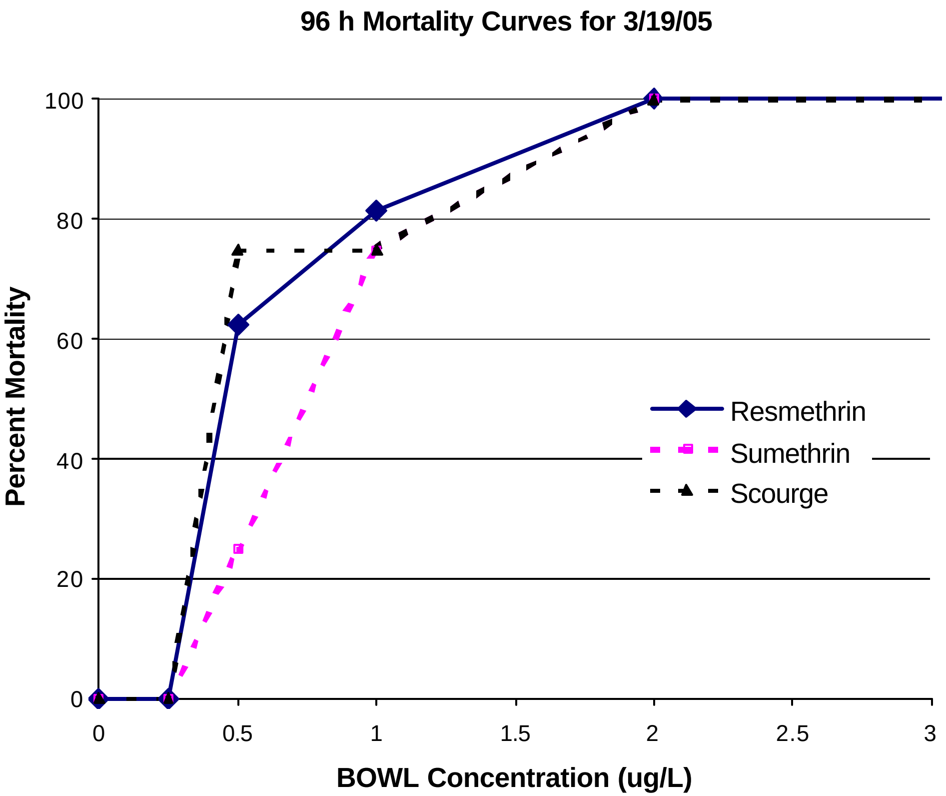
Chemicals involved in dosing experiments

- Pyrethroids
  - Resmethrin (active ingredient in Scourge®)
  - Sumethrin (active ingredient in Anvil®)
  - Scourge ® (received from county—has piperonyl butoxide, PBO, as synergist)

- Methoprene-based
  - R,S mixture of methoprene
  - S-methoprene (active isomer used in Altosid®)
  - Altosid® (received from county)

Dosing Experiment Results





Average LC50s:

Scourge® ~ 0.58 µg/L
Sumethrin: ~ 1.1 µg/L
Resmethrin ~ 1.2 µg/L

TOXICITY:
Scourge® > Sumethrin ~ Resmethrin

NOTE: no methoprene-based chemical was toxic up to 1 mg/L concentrations

Conclusions from dosing experiments

- Resmethrin & sumethrin are toxic around 1.1 ug/L, and Scourge® appears to be more toxic, which is to be expected. Scourge® is toxic at 0.58 ug/L.

- Altosid® or methorprene-based chemical concentrations of 1 mg/L were not observed to be toxic to shrimp in the laboratory

Altosid® and Scourge® Chemistry Findings (Analyses performed by Dr. Bruce Brownawell, Joe Ruggieri & Steve Terracciano)

Methoprene

- Not detected in pre-spray or reference samples
- Exceeding 1,000 ng/L were observed 30 min post- spray after 3 of 4 sprays
- 2 hrs post-spray, < 25 ng/L--detectable for 1 to 2 days post-spray
- More persistent in sediment, but no evidence of build-up

Resmethrin

- Not found in pre-spray of water or sediment
- Much higher at water interface (320 ng/L) than inches below surface (60 ng/L), but often non-detectable
- Rapidly became undetectable >2 hrs after spray
- Not found in sediments after spraying

Mortality hazard assessment

- Low concentrations of pesticides observed in field samples after spraying are much lower levels needed to cause mortality in the laboratory, further supporting the absence of toxicity in the field

Benthic Community Analysis Results

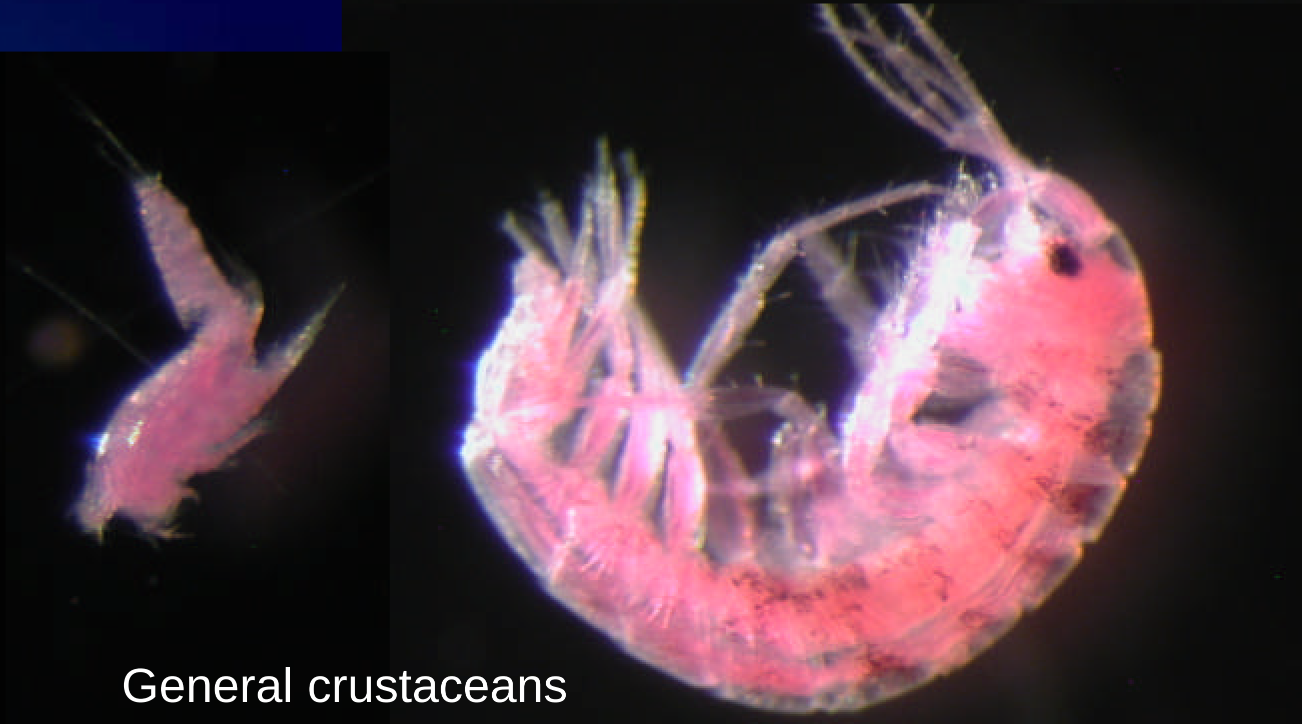
Nereis succinea

Crustaceans

Our original hypothesis was that the crustacean community would be most affected by sprays due to phylogenic similarities…

Cumacean

Amphipod

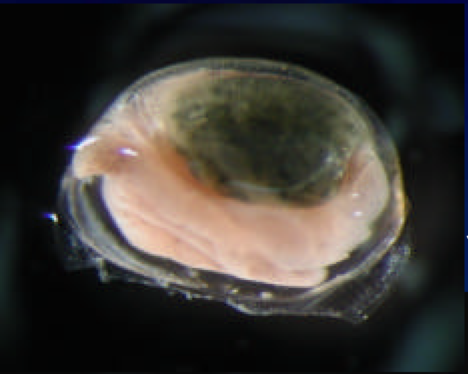


General crustaceans

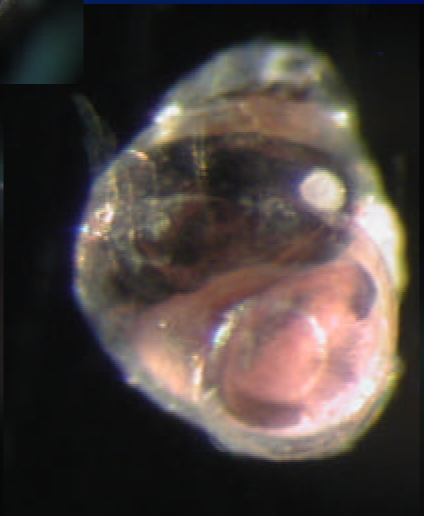
Polychaetes…

Mollusks & others…

Snout beetle



Snails



Mites

Midge larvae

Clams

26 Species identified in samples

Number	Organism ID	Phylum
1	oligochaete	Annelida
2	*Paranaitis speciosa*	Annelida
3	Spionidae	Annelida
4	*Nereis succinea*	Annelida
5	Streblospio	Annelida
6	trumpet-butt worm	Annelida
7	Cirratulidae	Annelida
8	fan worm	Annelida
9	Amphareidae	Annelida
10	crustacean #1	Arthropoda
11	tick	Arthropoda
12	cladocerans	Arthropoda
13	midge larvae	Arthropoda
14	isopod	Arthropoda
15	amphipod	Arthropoda
16	snout beetle	Arthropoda
17	Trombidiid mite	Arthropoda
18	crustacean #2	Arthropoda
19	Corophium crust.	Arthropoda
20	Cumacean	Arthropoda
21	Polyps	Cnidaria
22	snail #1	Mollusca
23	snail #2	Mollusca
24	clams	Mollusca
25	worms	Nematoda
26	foram	Sarcodina

Worms

Insects/Crustaceans

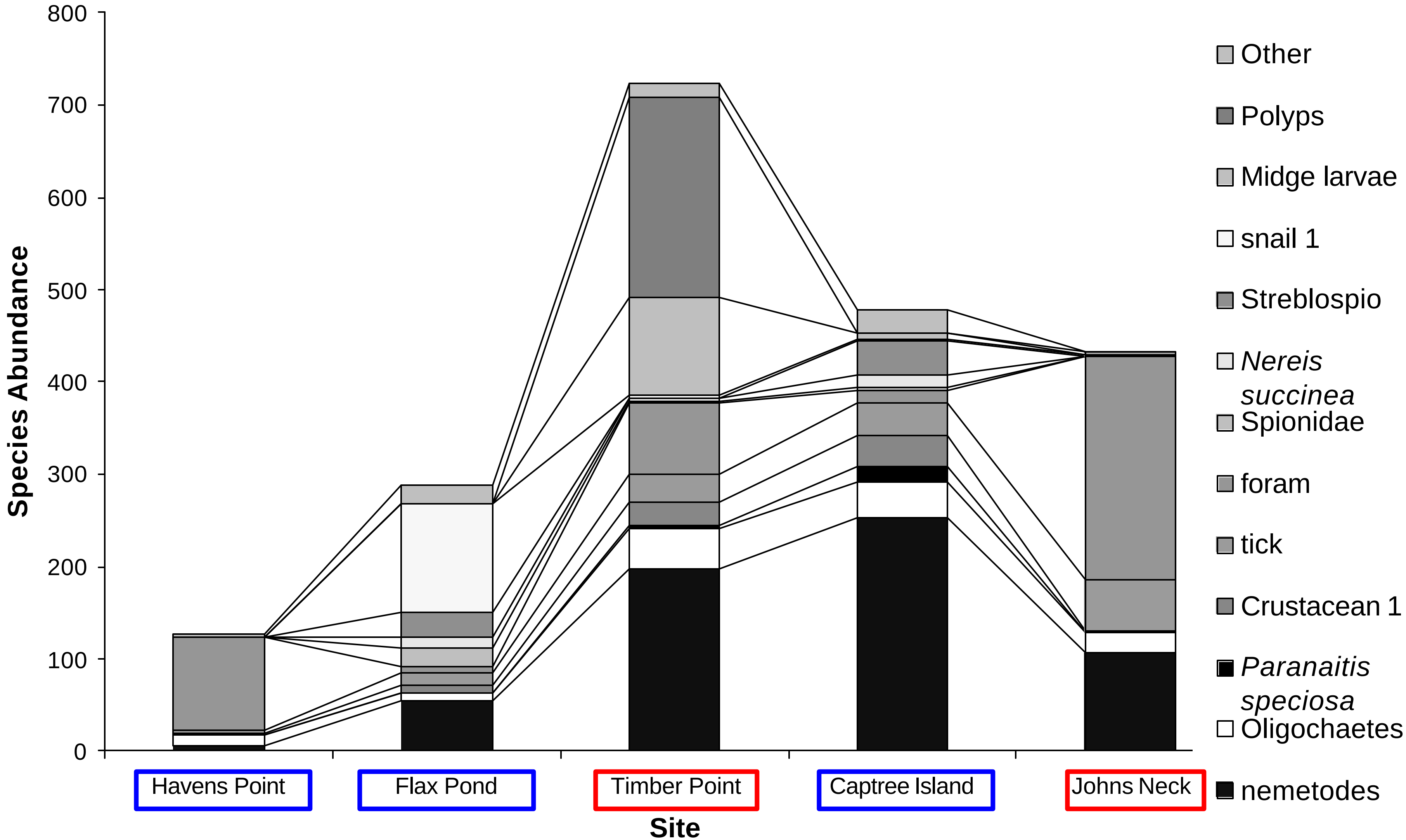
Anemones

Mollusks

Nemetodes

Foraminifera

Species abundance of 99% of species



Note: "Other" represents the remaining 1% of species

Benthic community statistical analysis

- ❖ PERMANOVA v. 1.6 was used to analyze site differences with respect to species abundance and type
- ❖ Program runs ANOVAs on multivariate data
  - Data was run using a two-way nested ANOVA
- ❖ Data was analyzed in four ways
  - all species; marine animals only; crustaceans only; all Arthropods
- ❖ No significant differences between spray and reference sites during any of the four runs

Conclusions from benthic community analysis

- Spray sites were not significantly different from reference sites in species abundance and composition
- Sites were significantly different from each other regardless of spray status
- Benthic organism composition more dependent on location rather than spray status

Conclusions

- Altosid® and Scourge® had no demonstrable effect on caged animals, which was supported in the laboratory studies and chemical analyses

- Low DO primary source of mortality in caged organisms (i.e. changes in cage location significantly increased survival)

- Pesticide exposure was not an important factor affecting benthic community structure

Significance

- The spraying of pesticides in Suffolk County for mosquito control does not seem to cause adverse effects in a non-target organism, *P. pugio*, or benthic community structure (when measured in the fall for this study)

- Bottom line: we failed to see pesticide-related toxicity with studied parameters

- WNV is now a national problem--the results of this study are pertinent for US mosquito control

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- Suffolk County Department of Public Works – Vector Control

Questions?

Methoprene water column concentrations

8/3/04--Timber Point

Time post spray (h)	Sample type	Methoprene ng/L	Lab
0.5	Interface	3,300	USB
		216	USGS
	Subsurface	490	USB
		82	USGS
2	Subsurface	6.3	USB
		< 5	USGS
24	Subsurface	17	USB
		< 5	USGS
48	Subsurface	< 5	USGS
96	Subsurface	< 5	USGS

8/3/04--Johns Neck

Time post spray (h)	Sample type	Methoprene ng/L	Lab
0.5	Interface	23	USB
		< 5	USGS
	Subsurface	> 2,500	USB
		10	USGS
2	Subsurface	< 5	USGS
24	Subsurface	< 0.5	USB
		< 5	USGS
48	Subsurface	< 5	USGS
96	Subsurface	< 5	USGS

Methoprene continued

8/10/2004				
Site	Time post spray (h)	Sample type	Methoprene ng/L	Lab
Johns Neck	0.5	Subsurface	1,100	USB
	24	Subsurface	24	USB
Timber Point	0.5	Subsurface	8	USB
	24	Subsurface	22	USB

Scourge® water column concentrations at Johns Neck

8/18/2004

Time post spray (h)	Sample type	Resmethrin ng/L	PBO ng/L	Lab
0.5	Interface	320	18,000	USB
		270	59,000	USGS
	Subsurface	7.8	210	USB
		< 5	1,310	USGS
2	Subsurface	36	2,900	USB
		38	457	USGS
9	Subsurface	< 0.5	24	USB
		< 5	61	USGS
48	Subsurface	< 5	6	USGS
96	Subsurface	< 5	< 5	USGS

8/25/2004

Time post spray (h)	Sample type	Resmethrin ng/L	PBO ng/L	Lab
0.5	Interface	< 0.5	26	USB
		< 5	12	USGS
	Subsurface	0.8	11	USB
		< 5	15	USGS
2	Subsurface	< 0.5	2	USB
		< 5	28	USGS
9	Subsurface	< 0.5	88	USB
		< 5	113	USGS
48	Subsurface	< 5	< 5	USGS
96	Subsurface	< 5	< 5	USGS