

Assessing Effects of mosquito control pesticides on coral and lobster larvae in the Florida Keys National Marine Sanctuary.

Richard Pierce, P.I.,

Mote Marine Laboratory, Ecotoxicology

Project manager, field monitoring, pesticide analysis

Kimberly Ritchie, Co-PI

Mote TRL, Marine Microbiology

Coral larvae toxicity studies

Thomas Matthews, Co-PI,

FL Fish & Wildlife Research Institute

Lobster larvae toxicity studies & Field Sampling

Project Goals

1. To determine if applications of mosquito control pesticides in the FL Keys result in toxic effects to NMS organisms.
 - Monitor FKMCD applications to determine the EEC in the NMS
 - Conduct Toxicity tests on select non-target organisms
2. Work with stakeholders to assess the risk and develop appropriate response strategies as needed to maintain mosquito control while reducing the risk to the NMS.



- **Permethrin:** Applied as Permanone 30-30 (30% Permethrin, 30% Piperonyl butoxide); PM Ground ULV
- **Malathion:** Applied as Fyfanon ULV Mosquito, 96.5% Malathion; PM ground ULV



Coral larvae:
Porites astreoides,



- **Naled:** Applied as Dibrom Concentrate, 87.4% naled; AM Aerial ULV



Spiny Lobster Puerulus
(pre-juvenile larval stage)
Panulirus argus

Unique Public-Private Partnership

Including Stakeholders from Federal, State & Local Agencies
and Mote, an Independent Non-profit Research Institution





Background

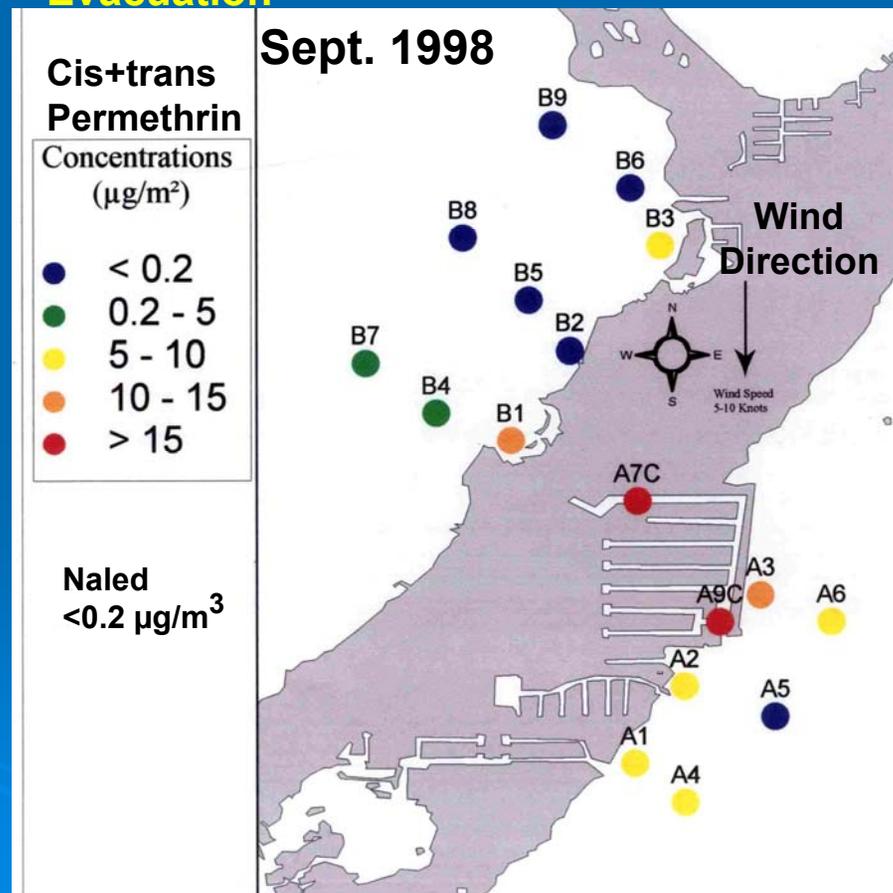
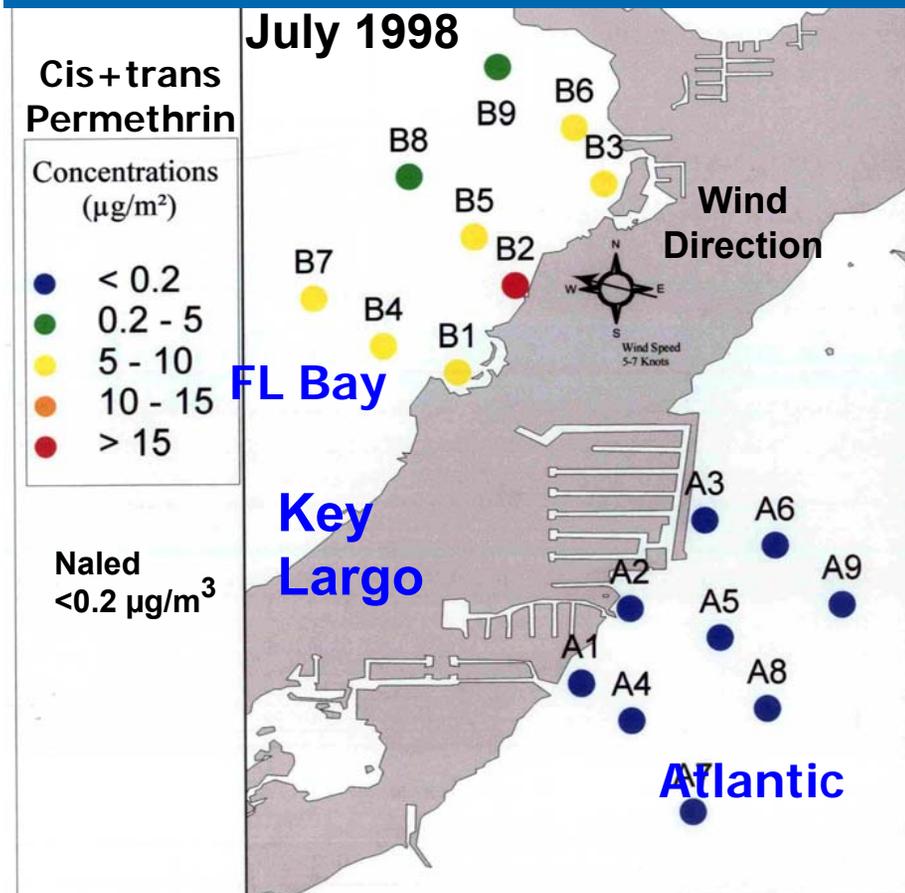
1998 Study at Key Largo Permethrin & Naled Applications:

Filter deposition and water samples



June 16-18, and July 28-29, 1998
 Evening, ground ULV Permethrin;
 Morninr, Aerial ULV Dibrom;

September 22-23, 1998
 Evening, ground ULV Permethrin
 Morning, no Dibrom, Hurricane Georges
 Evacuation



Results of 1998 Field Applications

- **Drift into National Marine Sanctuary:**
 - **Filters:**
 - **Permethrin:** detected on filters = drift, driven by wind speed & direction
 - **Naled & DDVP,** none detected on filters
 - **Water Surface Microlayer:**
 - **Permethrin,** none detected in NMS surface water (<0.01 ug/L)
 - canal surface microlayer: 5.1 to 9.4 ug/L
 - **Naled,** none detected (<0.01 ug/L)
 - **DDVP** in one sample, windward side (0.19ug/L)
- **Tidal Transport, Subsurface Water (~ 20cm depth):**
 - **Permethrin:** one site windward side Atlantic (0.07ug/L)
 - canals, none detected, 2 hrs post appl.
 - **Naled:** no naled detected (<0.01ug/L)
 - **DDVP:** June; 5 of 9 windward sites, .08 to .56ug/L

Acute Toxicity (96 hr LC-50) of Permethrin, Dibrom, DDVP and Malathion to *Mysidopsis bahia*, and *Penaeus duorarum*. Persistence (1/2 life) and solubility in seawater.

<u>Pesticide</u>	<u>96 hr LC-50</u>	<u>ug/L (ppb)</u>	<u>Half Life</u>	<u>Solubility</u>
<u>M. Bahia</u>	<u>P. duorarum</u>	<u>days</u>	<u>mg/L (ppm)</u>	
Permethrin	.02-0.1	0.2	1-3	.006
<i>(Note: tech. ~ 1/3 cis, 2/3 trans; toxicity cis >>> trans) + PBO</i>				
Naled	4.7-8.8	1.8	< 1	2,000
DDVP	19	NA	< 1	NA
Malathion	2.2	280	2-4	130

References: Schimmel et al., 1983; Cripe, 1994; Mason and Wendel, 2010; Faria et al., 2010

Current Project

Mote EPA-WQPP FL Keys NMS Study Objectives 2012 to 2014

1: Monitor ground and aerial applications of mosquito adulticides (naled, permethrin and malathion), to assess transport, distribution, concentration and persistence in NMS.



- **Permethrin:** Applied as Permanone 30-30 (30% Permethrin, 30% Piperonyl butoxide); PM Ground ULV

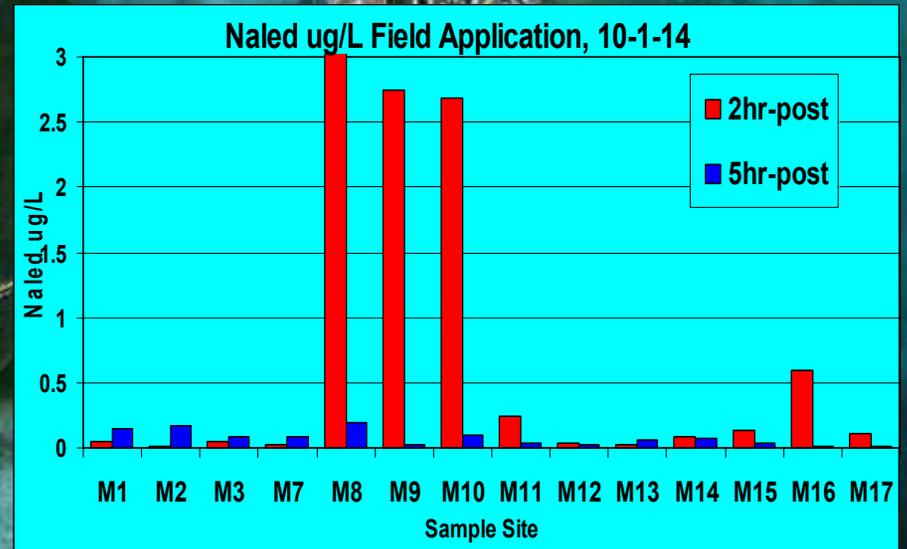
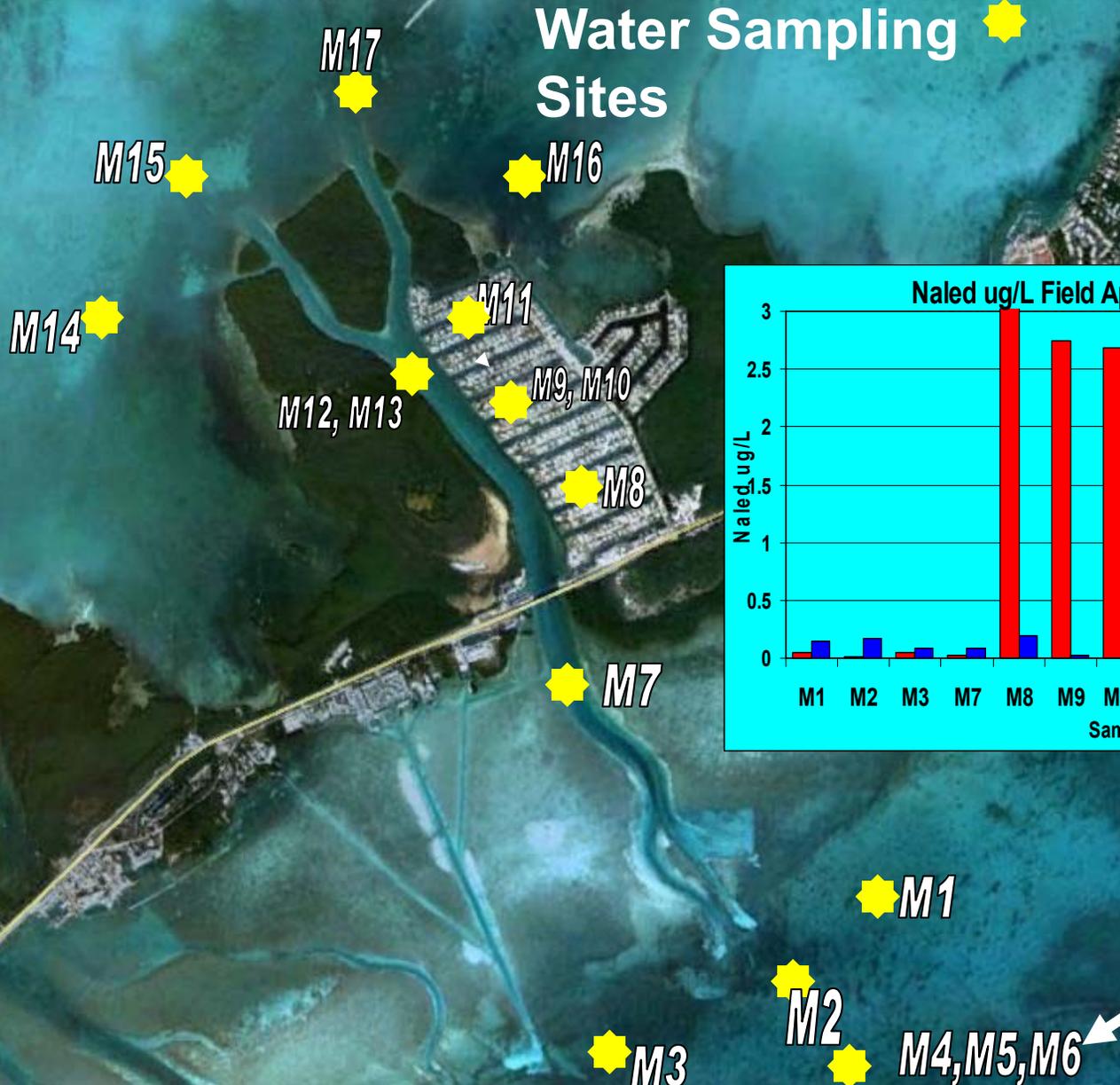
- **Malathion:** Applied as Fyfanon ULV Mosquito, 96.5% Malathion; PM ground ULV



- **Naled:** Applied as Dibrom Concentrate, 87.4% naled; AM Aerial ULV

Snake Creek Naled Monitoring Site Pre & post application Oct. 1-2, 2013

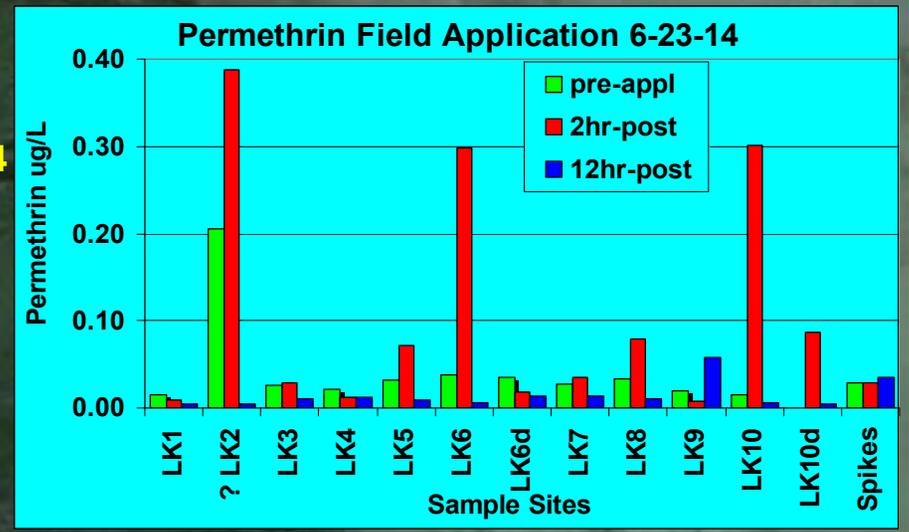
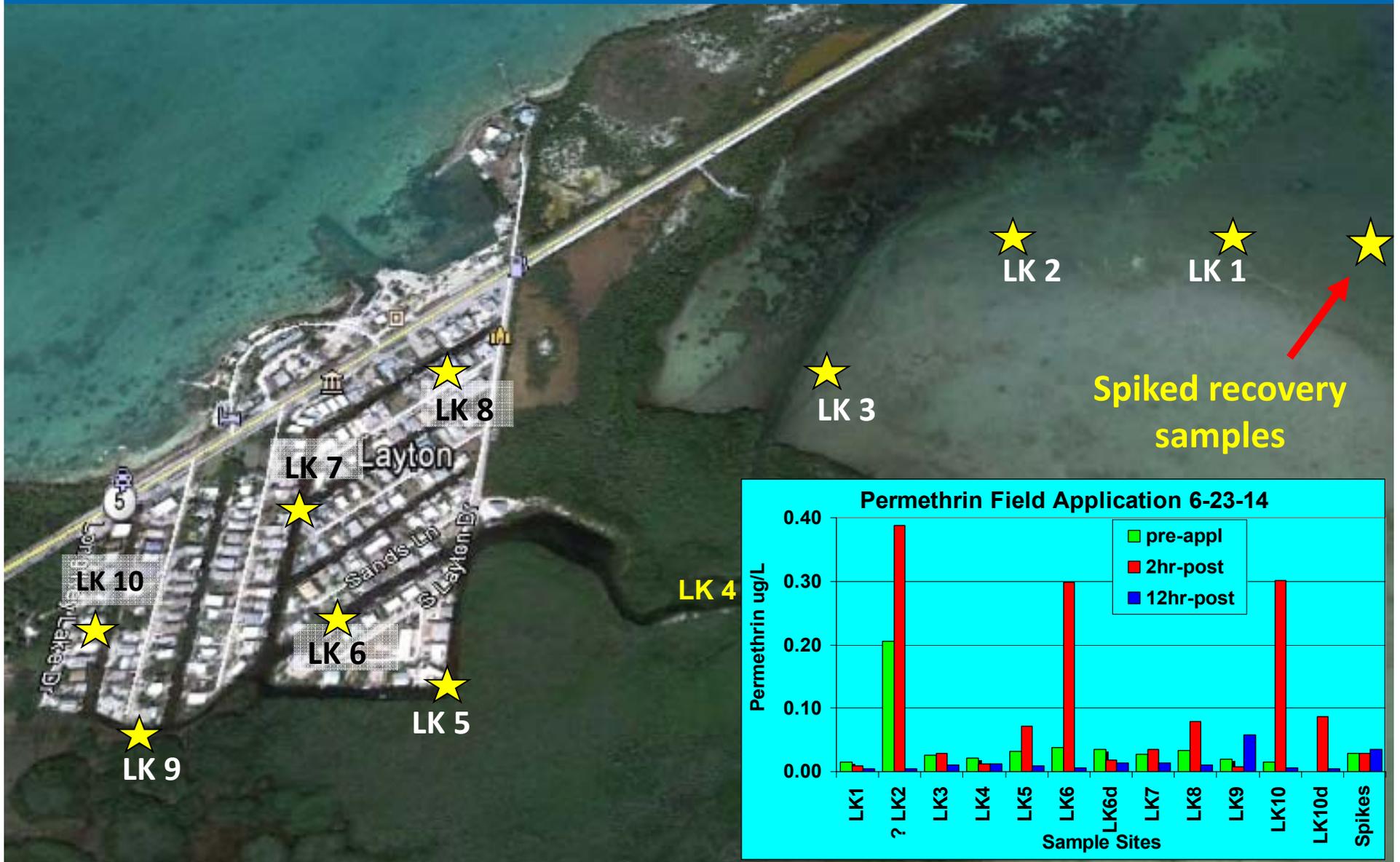
Water Sampling Sites



Spiked samples to verify recovery

Permethrin Monitoring Site

Long Key/Layton July 23-24, 2014; pre & post application



Lobster Larvae Toxicity Tests:

With: Tom Matthews, Gabrielle Renchen & Casey Butler, at FWRI

Spiny lobster (*Panulirus argus*) Exposed to environmentally relevant concentrations of pesticide technical formulations .
Toxicity end points will include:

- **Acute toxicity, % survival and LC-50, 96 hr.**



Phyllosome Larvae



Puerulus Post Larva



Adult Spiny Lobster

National Geographic photos

Lobster Juvenile, Pesticide Exposure Effects Tests

Pesticide	Start Date	End Date	Trial #	# lobsters
Permethrin	10/19/12	10/31/12	1	151
Permethrin	2/19/13	3/1/13	2	175
Permethrin	1/7/14	1/16/14	3	175
Permethrin*	2/6/14	2/20/14	4	50
Malathion	11/17/12	12/14/12	1	150
Malathion	3/19/13	3/27/13	2	172
Malathion	3/7/14	3/18/14	3	84
Naled	1/16/13	2/1/13	1	150
Naled	5/16/13	5/24/13	2	61
Naled	12/7/13	12/20/13	3	175

* Sublethal effects juveniles

Lobster Pueruli Toxicity Tests FWRI, Marathon



Pesticide extraction



Lobster
Puerulus Larvae



Lobster larvae dosing

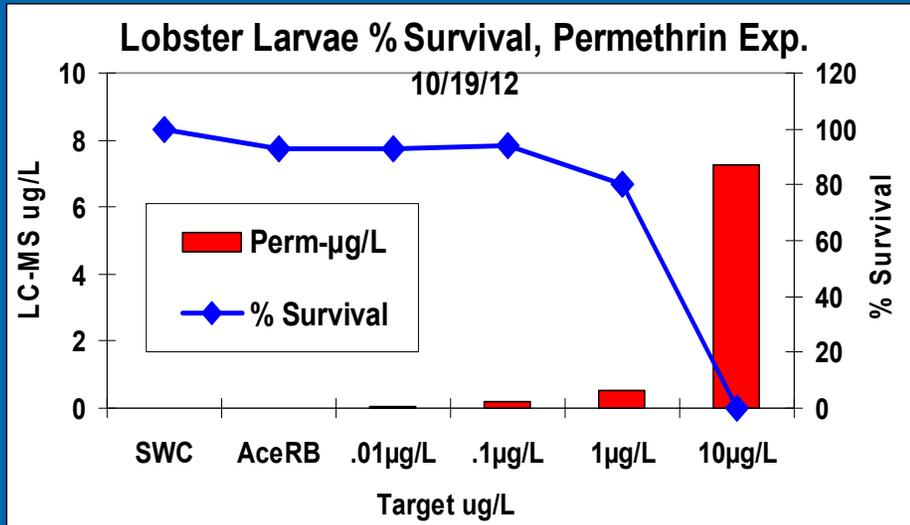


Pesticide analysis
LC-MS/MS

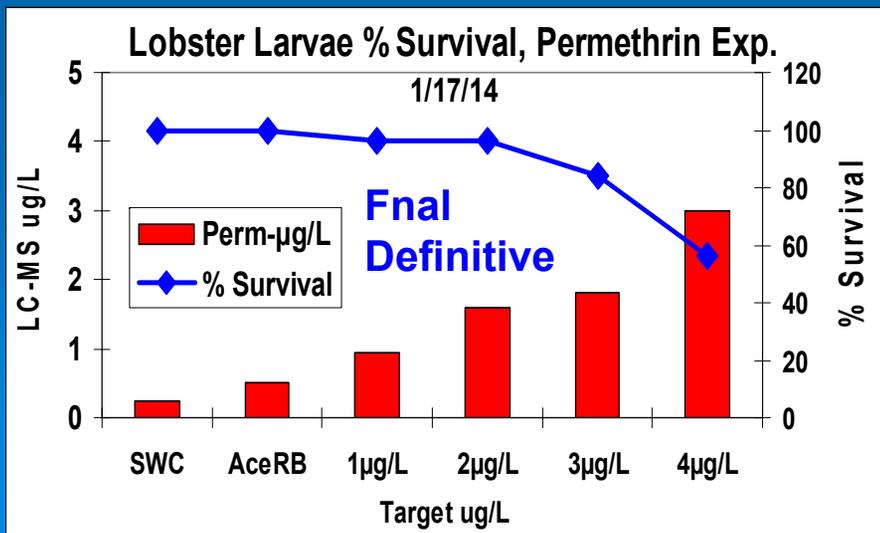
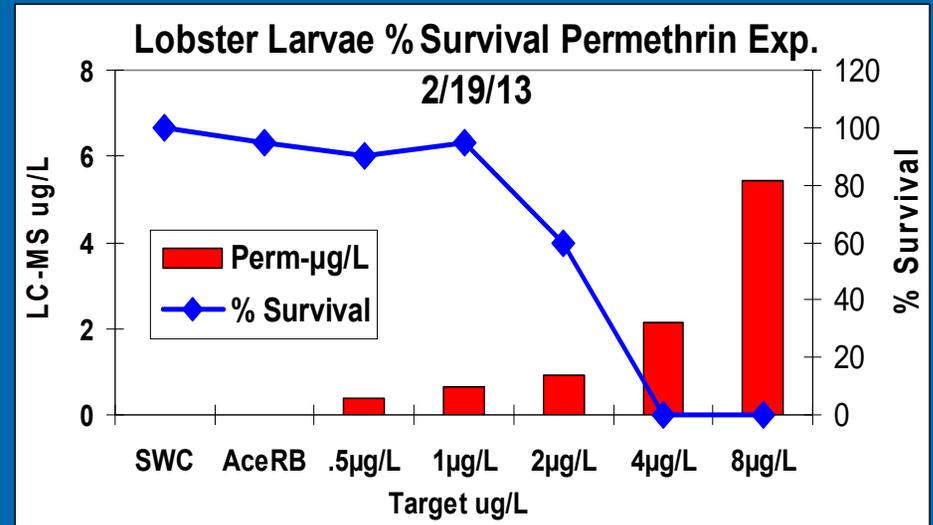
Lobster Larvae Exposure Results

Permethrin Exposures

Range-finder



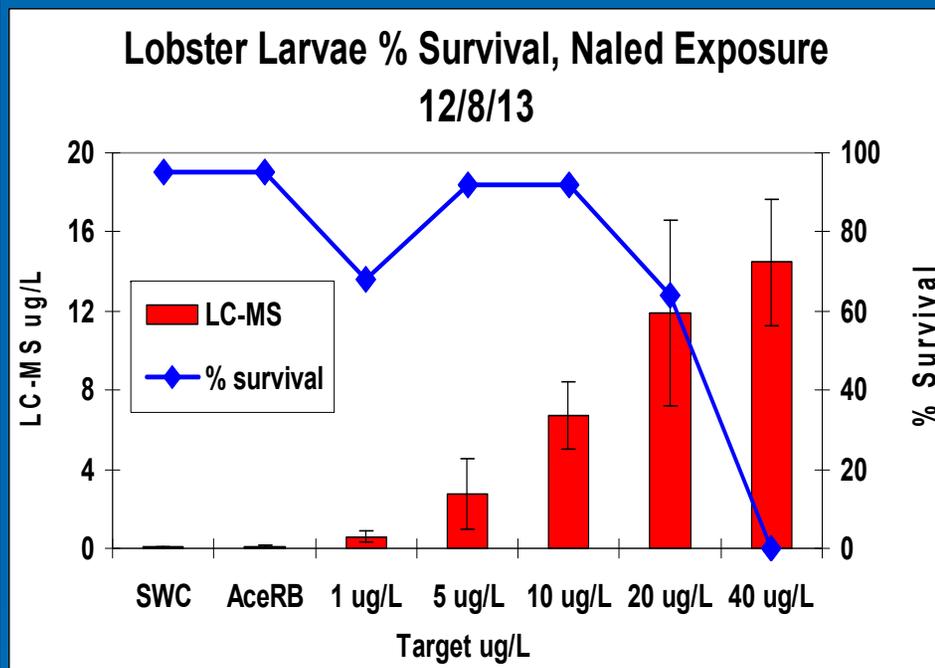
1st definitive



Results for Permethrin acute toxicity:

- **LC-50** = $3.0 \pm 0.5 \mu\text{g/L}$
- **NOEL No Observable Effect Level** = (no difference from control) = $1.8 \pm 0.3 \mu\text{g/L}$

Summary of Naled & Malathion Lobster Exposures



Naled: 96-hr LC-50 = $12 \pm 4 \mu\text{g/L}$
- 96hr NOEL = $7 \pm 2 \mu\text{g/L}$

Malathion: 96hr NOEL $> 20 \mu\text{g/L}$

Malathion Exposure

Lobster larvae, 3/7/14 96-hr % Survival

LC-MS $\mu\text{g/L}$	stdev	% survival
2.20	0.27	100
5.08	0.69	100
10.20	1.78	100
15.33	0.66	100
20.59	3.55	100

Summary; Lobster 96 hour Acute Toxicity Tests

- **Permethrin:**
 - LC-50 = $3.0 \pm 0.5 \mu\text{g/L}$
 - NOEL (No Observable Effect Level) = $1.8 \pm 0.3 \mu\text{g/L}$
- **Naled:**
 - LC-50 96hr = $12 \pm 4 \mu\text{g/L}$
 - NOEL = $7 \pm 2 \mu\text{g/L}$
- **Malathion:** 96-hr LC-50 > $20 \mu\text{g/L}$
 - LC-50 96hr > $20 \mu\text{g/L}$
 - NOEL > $20 \mu\text{g/L}$

Coral Larvae Toxicity Tests

Dr. Kim Ritchie & Dr. Emily Hall;
Mote Tropical Research Lab, Summerland Key, FL

Larvae of the scleractinian coral (*P. astreoides*) exposed to environmentally relevant concentrations of the technical formulations of each pesticide. Toxicity end points include:

- Acute toxicity; 96 hour % Survival and LC-50.



Planula



Primary Polyp



Adult Polyp

Collecting & Dosing Coral Larvae



Live Coral Spawning



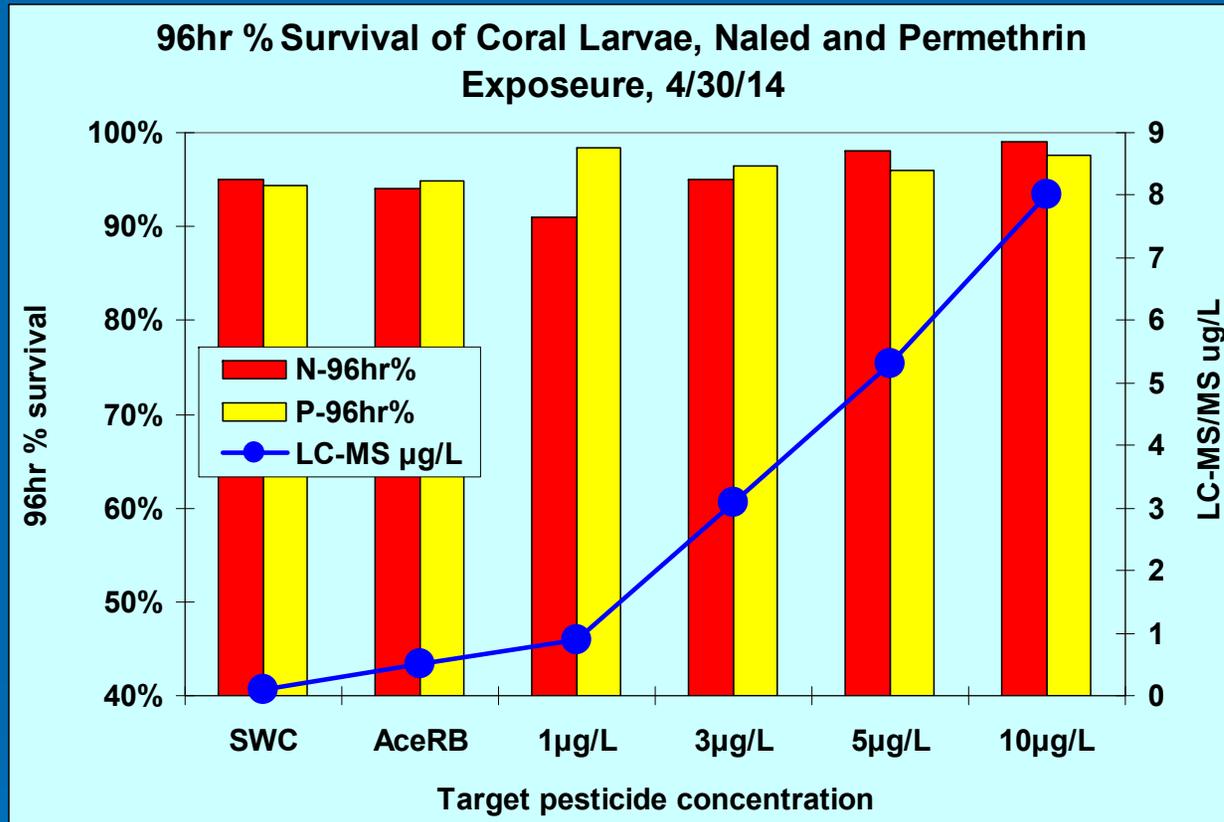
Collecting larvae



Coral larvae dosing & Monitoring



Coral Larvae Exposure to Naled and Permethrin



Summary of Coral Larvae 96hr Acute Toxicity Tests: Naled & Permethrin Results:

- NOEL Naled > 8 µg/L of Naled;
- NOEL Permethrin > 8 µg/L permethrin.

Results & Conclusions

1. Expected Environmental Concentrations (EEC):

- **Naled:** ● 2 hours post appl.= 0.2 to 3.14 µg/L (in canals); 0.02-0.6 µg/L outside
 - 5 hours post appl.= 0.01 to 0.17 µg/L
- **Permethrin:** < 0.5 µg/L 2 hr & 12 hr post appl.

2. Acute Toxicity; NOEL: 96 hr % survival vs Controls

- **Coral larvae;**
 - Naled; > 8 µg/L;
 - permethrin; > 8 µg/L
 - Malathion; ? No mortality, LC-MS verification malfunction
- **Lobster larvae**
 - Naled; 7 ± 2 µg/L
 - Permethrin; 1.8 ± 0.3 µg/L
 - Malathion > 20 µg/L

3. Conclusions

- **Coral larvae, *P. astreoides*:** **EEC** << toxic concentration = No Acute toxicity to coral larvae for field applications of Naled, Permethrin or Malathion in the Atlantic or FL Bay adjacent to the Snake Creek study area.

- **Lobster larvae, *P. argus*:** **EEC** << Toxic concentration = No Acute toxicity to lobster larvae for field applications of Naled or Permethrin in the Atlantic adjacent to the Layton Key Canals.

Recommendations

- Conduct monitoring of additional pesticide field applications and residential misting systems.
 - Test toxicity to coral polyps and lobster 1st stage juvenile = includes ingestion of contaminated prey
 - Initiate studies of sublethal effects using cellular biomarkers & physiological impact: Biomarkers of effects, including;
 - Catalase and Superoxide Dismutase activity;
 - Phenoloxidase (PO) activity;
 - Lipid peroxidation
 - Investigate synergistic effects from simultaneous exposure to two or more chemical contaminants.
 - Study synergistic effects of climate change with pesticide exposure.
 - Temperature; pH
- 

Application of Results

Provide FL Keys NMS Resource Managers and
FL Keys Mosquito Control District Managers
with empirical data to:

- preserve and enhance the living resources of the National Marine Sanctuary
- while maintaining adequate mosquito control to protect the public health and economic well being of the FL Keys.





Shared Project Support

<u>Funding/Collaborators:</u>	<u>Year-1</u>	<u>Year-2</u>
US EPA WQPP, FL Keys NMS;	\$70,000	\$30,000 (\$100,000 max)
FL Keys Mosquito Control Dist.;	\$25,000	\$25,000
<u>Levi Research Fund (Mote);</u>	<u>\$25,000</u>	<u>\$25,000</u>
Project Budget	\$120,000	\$80,000 (\$200,000/ 2 yrs)

In-Kind Support

NOAA-National Marine Sanctuary; In-Kind Support (advice; interpretation)

FL FWRI field & lobster toxicity; In-Kind Support (\$33,670)
(FWRI- in kind staff time for collecting and monitoring lobster larvae)

Mote, Field monitoring & Coral toxicity; In-kind Support (\$33,000)
(POR coral and Ocean Acidification-in Kind staff time for collecting & monitoring coral larvae)