

An underwater photograph of a coral reef. The scene is dominated by a large, branching red coral structure in the center. To the left, there is a large, textured brown and orange coral formation. The water is clear and blue, with numerous small, bright orange and yellow fish swimming around. The lighting is bright, suggesting a shallow depth.

Assessing Effects of Mosquito Control Pesticides on Non-targeted Organisms in the Florida Keys National Marine Sanctuary

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FL Fish & Wildlife Research Institute

Unique Public-Private Partnership

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



Project Goals

Goal 1: Determine if applications of mosquito control pesticides in the FL Keys cause harmful effects to non-targeted NMS organisms.

Goal 2: Work with stakeholders to assess the potential risk and develop appropriate response strategies as needed to maintain mosquito control while reducing the risk to the NMS.

Goal 1 Objectives

- **Objective-1. How much, Where and How Long?**

Monitor distribution, concentration and persistence of mosquito control pesticides in NMS, following ground and aerial applications.



- **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

(contains both Naled and DDVP; Naled degrades to DDVP over time)

Goal 1 Objectives

Pesticide Impacts

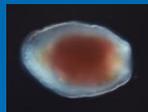
Objective-2: So What?

Determine toxicity to sentinel organisms to assess acute lethal and sublethal toxicity from exposure to Expected Environmental Concentrations (EEC) of Pesticides:

- Initial Study: Early life (larval) stages of coral and lobster
- Extension: 1st stage juvenile lobster and coral polyps



Coral Spawn



Coral planula
larvae



Lobster Pueruli

Field Sampling protocol

Cooperative with FWRI-Marathon

Naled: Morning Aerial Application

Sample Collection:

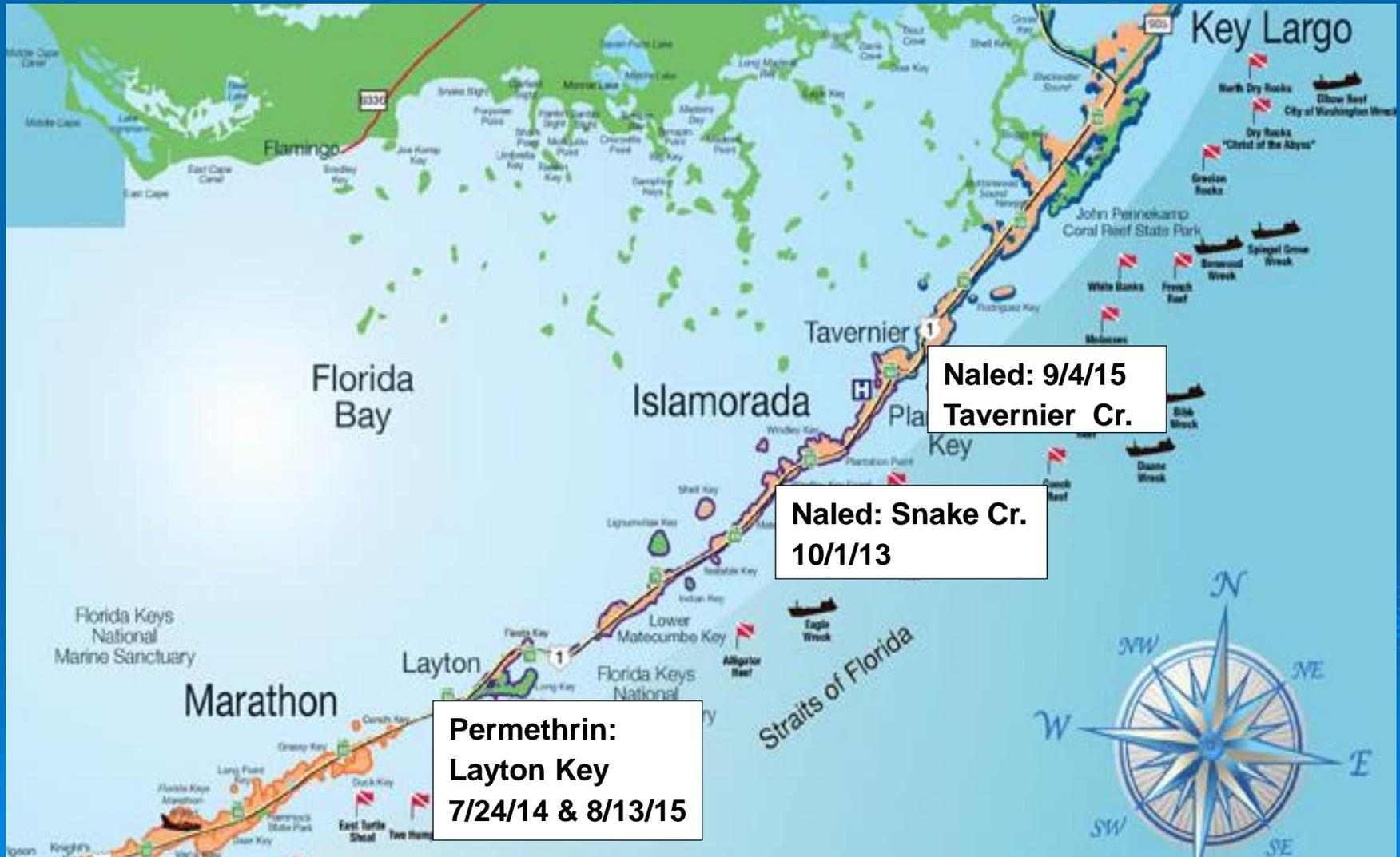
- **Pre-application;** Previous afternoon
- **Application:** early morning
- **Post-** application; 2-hrs & 5 to 6hrs

Permethrin & Malathion: Evening Ground Application

Sample Collection:

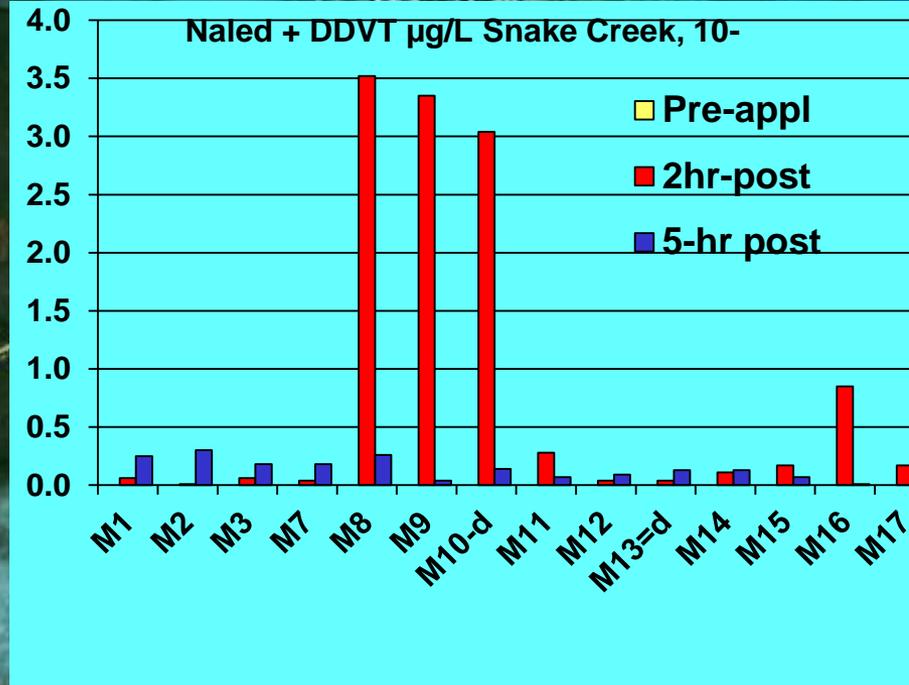
- **Pre-application;** 2 to 4 hours
- **Application:** evening, 7 to 9 PM
- **Post-application;** 2- hrs & 12 hrs

FL Keys Map showing pesticide monitoring areas



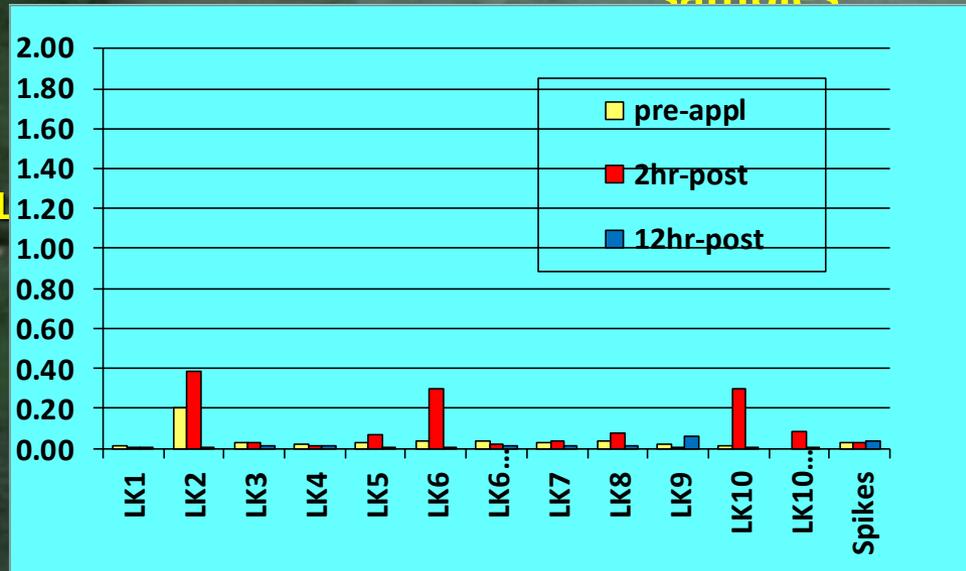
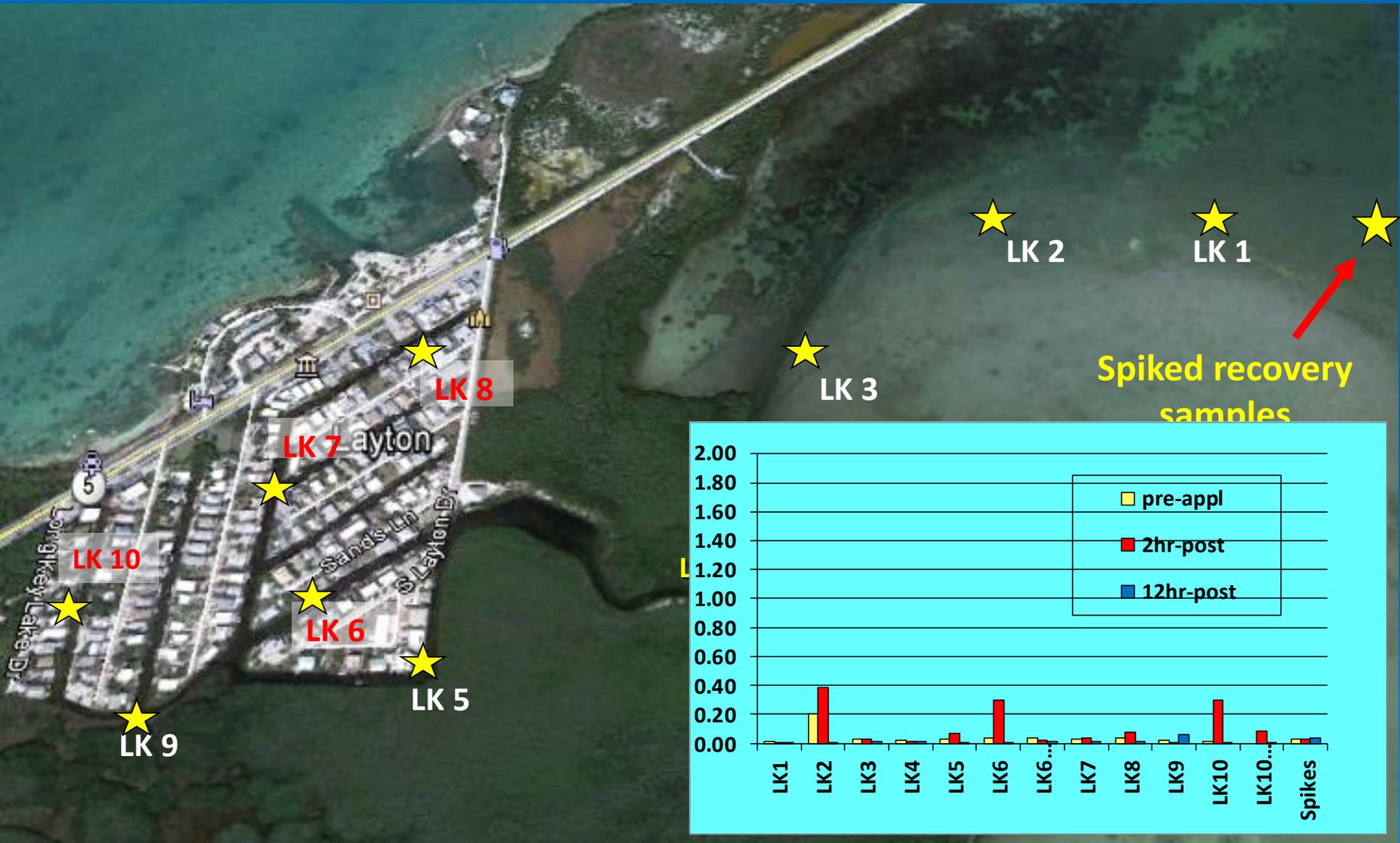
Naled $\mu\text{g/L}$ Application Monitoring Sites Snake Creek October 1, 2013

Water Sampling Sites 

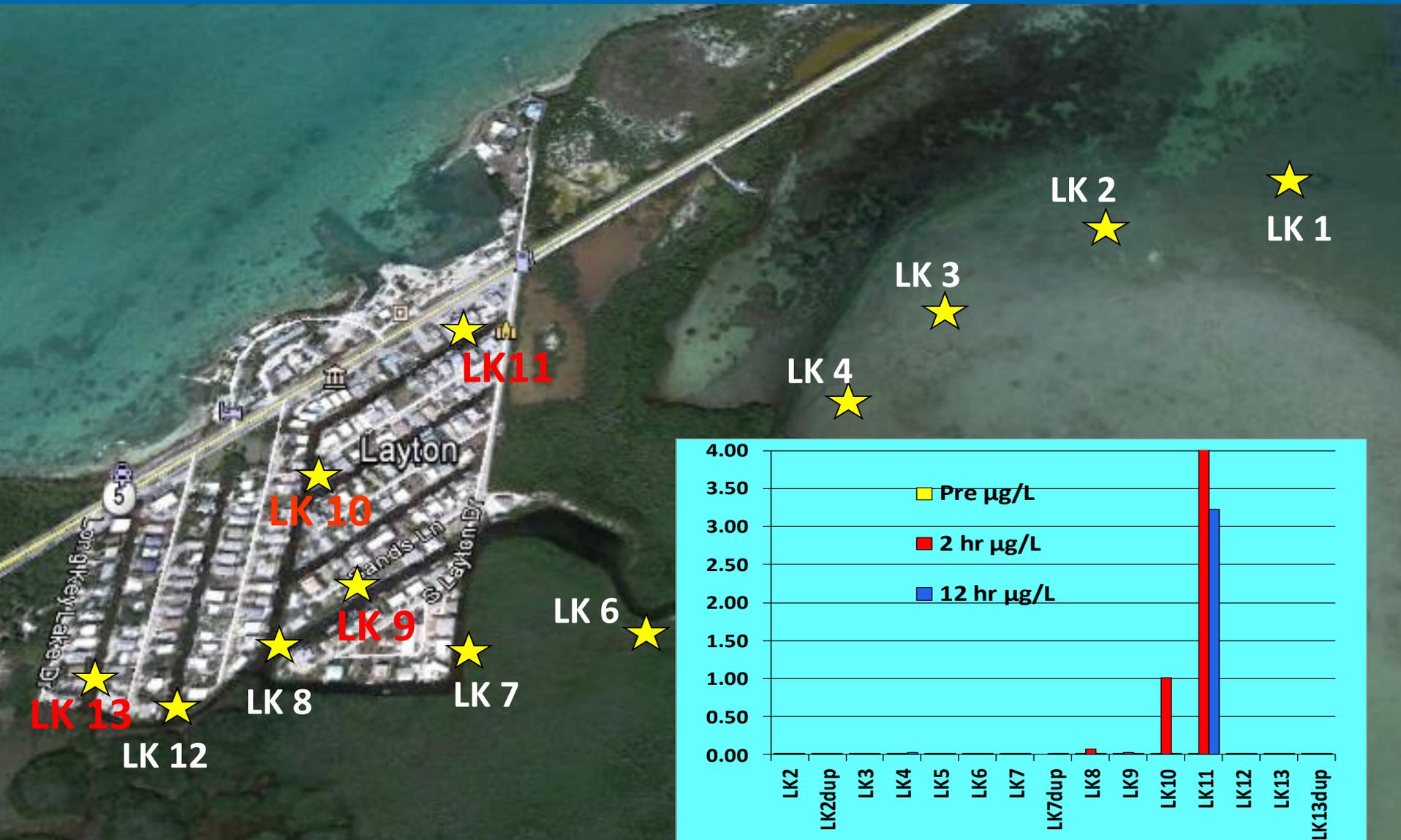


 M-1
 M-2
 M-3
 M-4,5,6 ← Spiked stds.

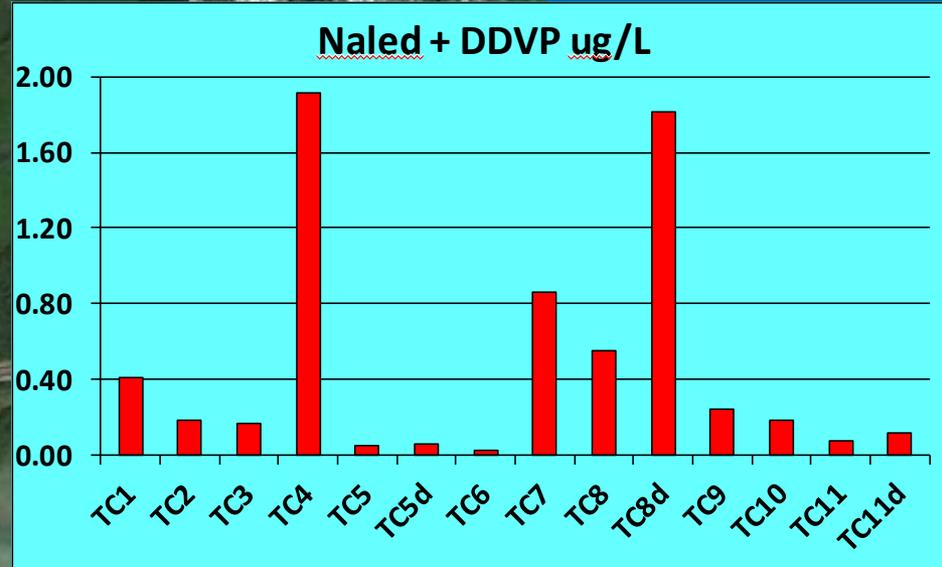
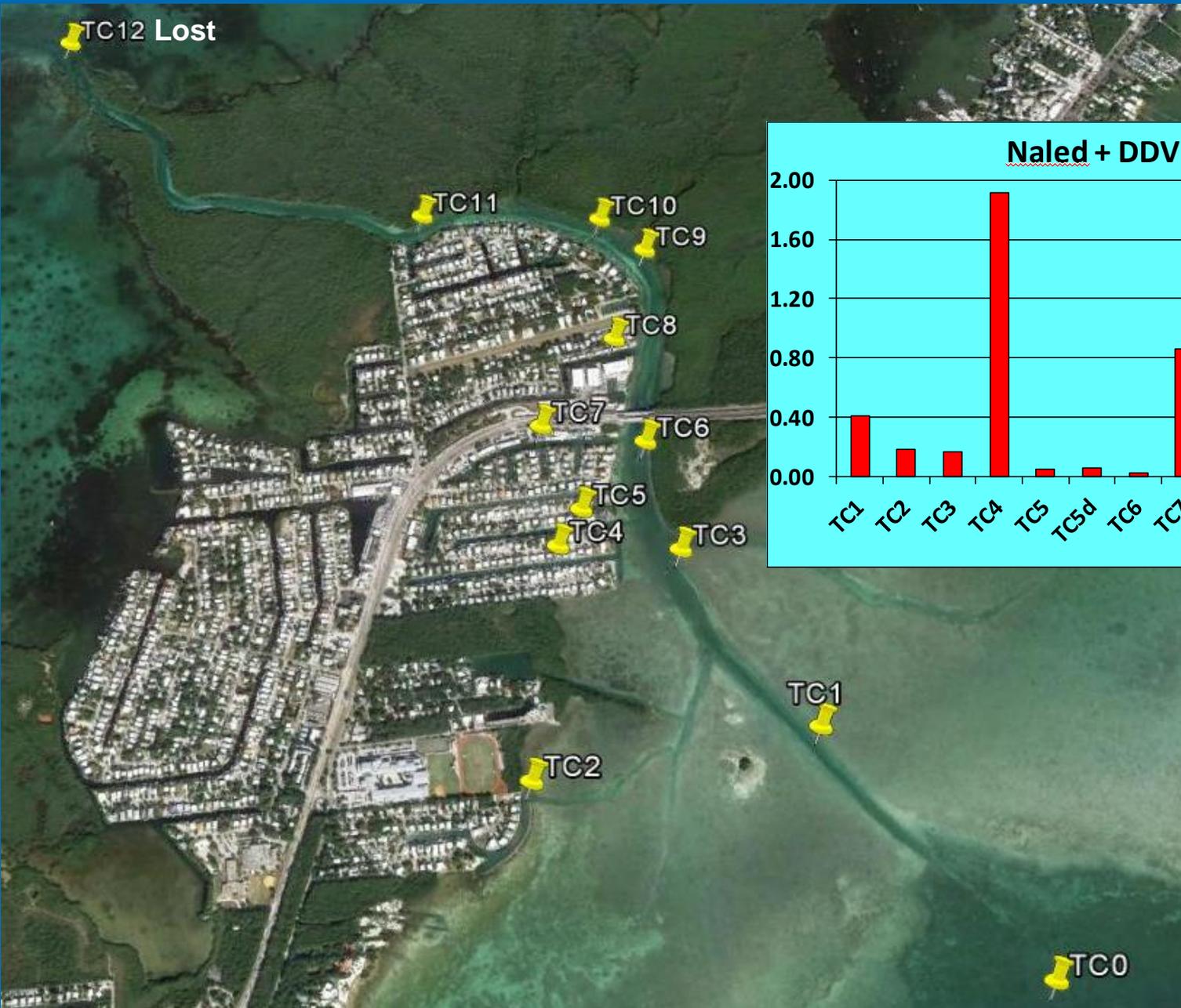
Permethrin $\mu\text{g/L}$ Application Monitoring Sites Layton Key, July 23-24, 2014



Permethrin, $\mu\text{g/L}$, Monitoring Sites Layton Key, August 13, 2015



Naled & DDVP $\mu\text{g/L}$ Application Monitoring Sites Tavernier Creek 9/14/2015 (pre-appl. = $<0.1 \mu\text{g/L}$)



Summary of Field Monitoring Results permethrin, naled/DDVP in $\mu\text{g/L}$

Naled + DDVP (Dibrom Concentrate):

Snake Creek, 10-1-13 & Tavernier Key, 9/14/15:

inside canals **2hr** = <0.05 to 3.5; **5hr** = <0.05 to 0.3

outside canal **2hr** = < 0.05 to 0.4; **5hr** = <0.05 to 0.3

Permethrin (Permanone 30-30):

Layton Key, 7-14-14 & 8/13/15

inside canals **2hr** = < 0.05 to 4.0; **12hr** = <0.05 to 3.2

Note: (*high 4.0 & 3.2 $\mu\text{g/L}$ in only one canal*)

outside canal **2hr** = < 0.05 to 0.9; **12hr** = <0.05 to 0.1

Objective 2: Pesticide toxicity to coral and lobster larvae

Lobster Larvae Toxicity Tests:

With: Tom Matthews, Gabby Renchen & Casey Butler, FWRI

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

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



Phyllosome Larvae

Puerulus Larvae
& juvenile

Adult Spiny Lobster

Lobster Pueruli Toxicity Tests

FWRI & Mote



Lobster larvae dosing, FWRI



Pesticide extraction, FWRI



Lobster larvae dosing, Mote



Monitoring water quality, Mote



Pesticide extraction Mote



Pesticide Analysis
HPLC-MS/MS Mote

Coral Larvae Toxicity Tests

Kim Ritchie, Emily Hall, Erich Bartels;
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 larvae



Primary Polyp



Adult Polyp

Collecting Dosing & Monitoring Coral Larvae



Live Coral Spawning



Collecting larvae



Coral larvae in
petri dish



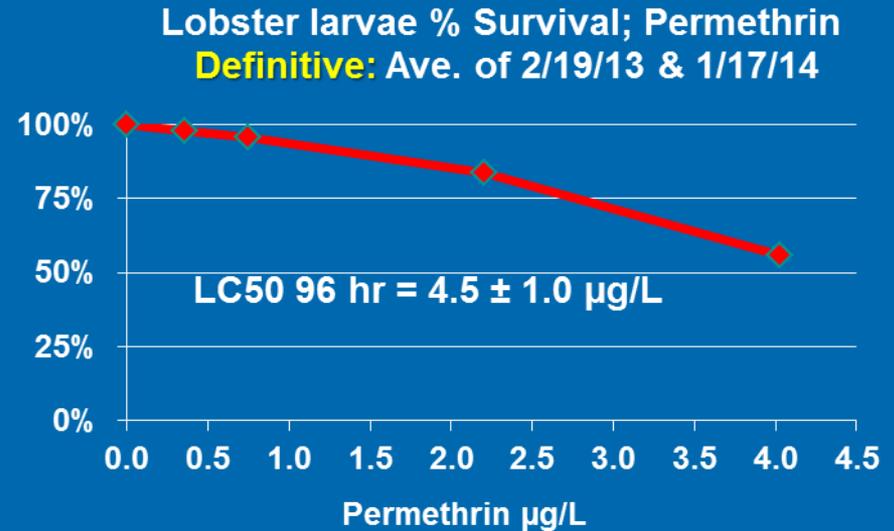
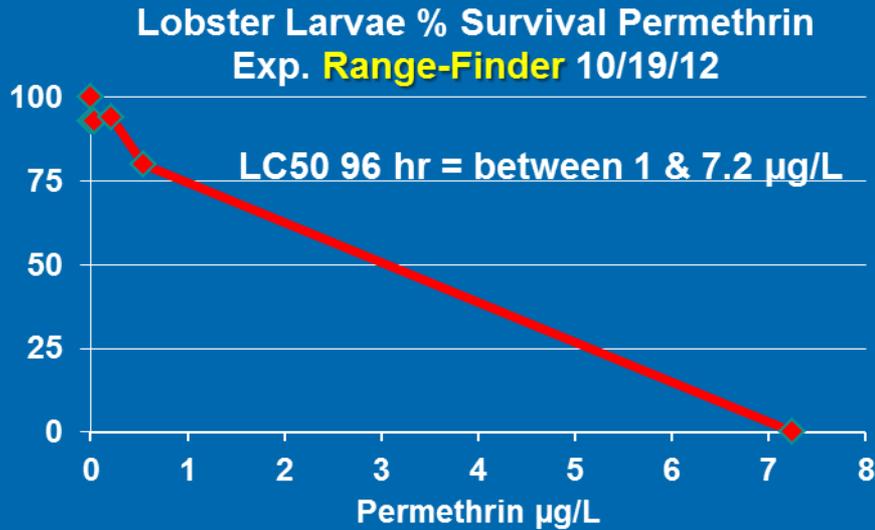
Coral dosing



Monitoring Coral

Lobster Larvae Exposure

Permethrin 96 hr exposure



Permethrin acute toxicity:

Range-finder: LC50 , 96 hr = between 1 and 10µg/L

Definitive LC50, 96 hr = 4.5 ± 1.0 µg/L

Results from Naled Lobster pueruli Exposure LC50 96 hr Acute Toxicity,

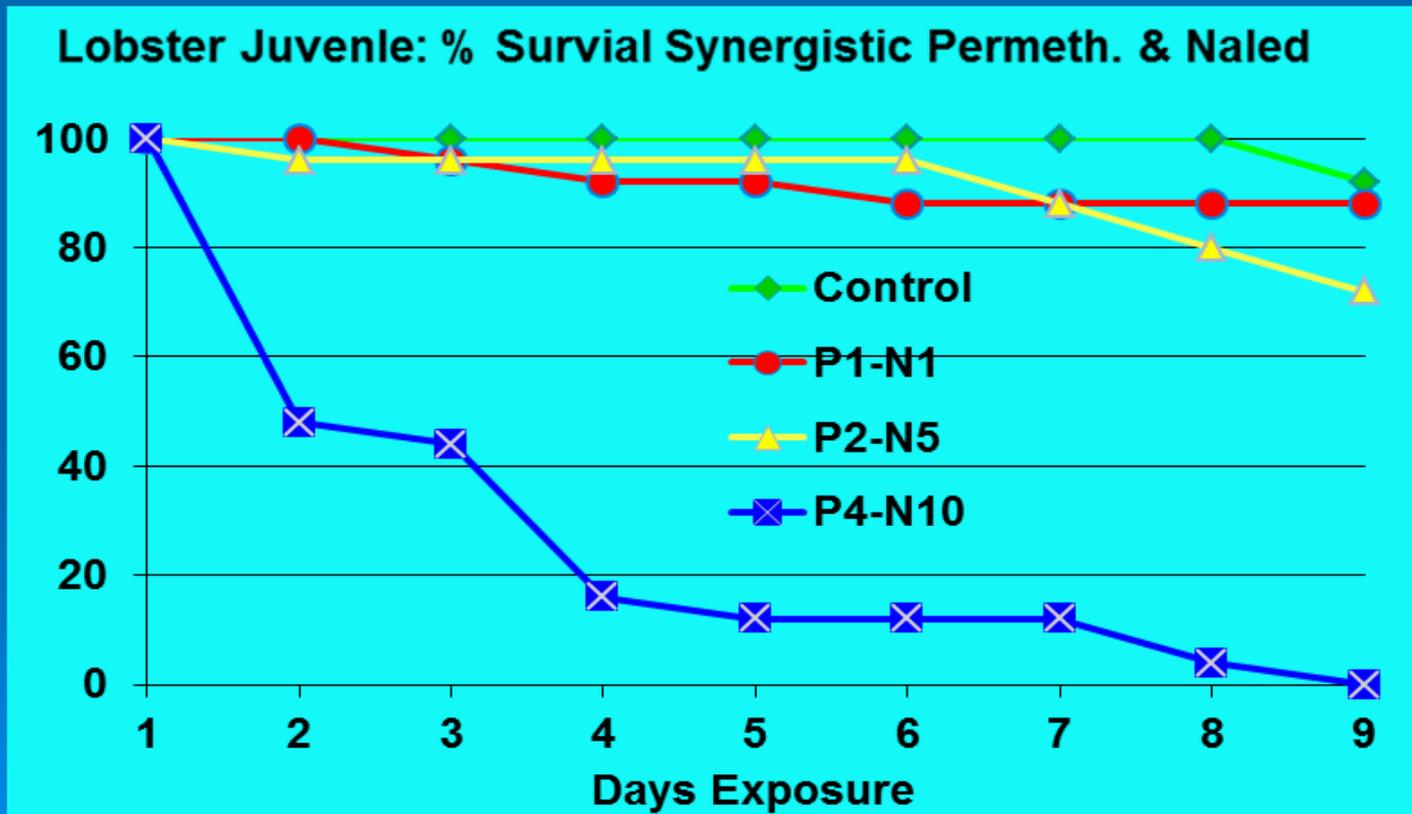
Naled LC-50 96hr:

- No significant difference between control and exposures through 9.5 $\mu\text{g}/\text{L}$ Naled
- 100% mortality at 21 $\mu\text{g}/\text{L}$ Naled

1st Stage Lobster Juveniles

Synergistic Effects From Exposure to both Permethrin and Naled

% Survival through 9 days



Summary of Coral Larvae Exposures

% Survival at 96 hours Exposure

96 hour % survival Naled + DDVP µg/L

N+D µg/L	stdev	% survival	stdev
0.19	0.07	84%	7.4
0.40	0.45	85%	8.1
0.60	0.26	87%	4.5
4.00	1.27	87%	4.5
7.00	0.54	83%	7.9
10.40	2.04	82%	8.4

Mean & standard deviation of multiple exposure studies

Naled: 96-hr LC-50 > 10 µg/L

96 hour % survival Permethrin µg/L

Permethrin µg/L		% survival at	96 hrs
Mean	stdev	mean	stdev
0.1	0.8	80.8	94%
0.7	0.2	93.6	94%
1.9	0.7	93.6	94%
5.7	1.8	91.2	93%
9.5	0.0	92.8	95%

Permethrin 96-hr LC-50 > 9.5 µg/L

SUMMARY

1. Expected Environmental Concentrations (EEC):

- **Naled:**
 - 2 hours post appl.= <0.05 to 3.5 µg/L (in canals)
 - 5 hours post appl.= 0.01 to 0.4 µg/L
- **Permethrin**
 - 2 hrs post = < 0.5 µg/L to 4.0 µg/L (in canals)
 - 12 hrs post = <0.5 to 0.9 µg/L

2. Acute Toxicity; LC-50, 96 hr

- **Lobster larvae**
 - Naled; $>9.5 \pm 1$ µg/L ; - Permethrin; 4.5 ± 1.0 µg/L
- **Coral larvae;**
 - Naled; > 10 µg/L;
 - permethrin; > 9.5 µg/L

Conclusions

- **Naled: No Acute Toxicity (LC50 96 hr) for Coral larvae, *P. acropora*:**

EEC \ll LC50 96 hr toxic concentration from field applications of **Naled** or **Permethrin** in the FL Keys NMS.

- **Permethrin: EEC at toxic level for lobster larvae in restricted flow canal.**

No Acute toxicity for coral or lobster larvae outside canals.

- **Possible synergistic effect from exposure to multiple pesticides.**

Need To Know

1. Chronic, Sublethal effects at EEC

- development, reproduction
- Biomarkers of effect
- Cell ultrastructure

2. Synergistic effects with:

- Other pesticides: bifenthrin,
- Pharmaceuticals: (endocrine disruptors): progesterone, estradiol,
- Climate change: Changing Temperature & pH

3. Toxicity to additional FL Keys Organisms:

- Sea urchins, sponges, fish

Residential Pesticide applications

Monitor Drift and runoff from:

- **Pesticide misting systems:
(This Study)**



- **Lawn maintenance:
(Future Study)**



Common Goal for All Stakeholders:

- 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.



