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

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Mote Marine Laboratory, Ecotoxicology
Project manager, field monitoring, pesticide analysis

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

Coral larvae: *Porites astreoides*,
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 Permanone;
Morning, Aerial ULV Dibrom;

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

Cis+trans Permethrin

<table>
<thead>
<tr>
<th>Concentrations (µg/m³)</th>
<th>July 1998</th>
<th>Sept. 1998</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.2</td>
<td>B4, B7</td>
<td>A7C, B9</td>
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<tr>
<td>0.2 - 5</td>
<td>B8, B9</td>
<td>B9</td>
</tr>
<tr>
<td>5 - 10</td>
<td>B1, B3</td>
<td>B3, B6</td>
</tr>
<tr>
<td>10 - 15</td>
<td>B5</td>
<td>B5</td>
</tr>
<tr>
<td>&gt; 15</td>
<td>B2</td>
<td>B2</td>
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</table>

Naled <0.2 µg/m³

Cis+trans Permethrin

<table>
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<tr>
<th>Concentrations (µg/m³)</th>
<th>July 1998</th>
<th>Sept. 1998</th>
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<tr>
<td>&lt; 0.2</td>
<td>A2, A6</td>
<td>A2, A6</td>
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<tr>
<td>0.2 - 5</td>
<td>A1, A4</td>
<td>A1, A4</td>
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<td>5 - 10</td>
<td>A3, A5</td>
<td>A3, A5</td>
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<tr>
<td>10 - 15</td>
<td>A3, A5</td>
<td>A3, A5</td>
</tr>
<tr>
<td>&gt; 15</td>
<td>A3, A5</td>
<td>A3, A5</td>
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</table>
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 duoarum*. Persistence (1/2 life) and solubility in seawater.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>M. Bahia 96 hr LC-50 ug/L (ppb)</th>
<th>P. duoarum 96 hr LC-50 ug/L (ppb)</th>
<th>Half Life</th>
<th>Solubility mg/L (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permethrin</td>
<td>.02-0.1</td>
<td>0.2</td>
<td>1-3</td>
<td>.006</td>
</tr>
<tr>
<td></td>
<td><em>(Note: tech.~1/3 cis, 2/3 trans; toxicity cis&gt;&gt;&gt;trans)</em> + PBO</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naled</td>
<td>4.7-8.8</td>
<td>1.8</td>
<td>&lt; 1</td>
<td>2,000</td>
</tr>
<tr>
<td>DDVP</td>
<td>19</td>
<td>NA</td>
<td>&lt; 1</td>
<td>NA</td>
</tr>
<tr>
<td>Malathion</td>
<td>2.2</td>
<td>280</td>
<td>2-4</td>
<td>130</td>
</tr>
</tbody>
</table>

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

Naled ug/L Field Application, 10-1-14

Sample Site

- M1
- M2
- M3
- M4, M5, M6
- M7
- M8
- M9, M10
- M11
- M12, M13
- M14
- M15
- M16
- M17

2hr-post

5hr-post

Naled ug/L
Permethrin Monitoring Site
Long Key/Layton  July 23-24, 2014; pre & post application

Spiked recovery samples

Permethrin Field Application 6-23-14
- pre-appl
- 2hr-post
- 12hr-post

Sample Sites
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

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

* Sublethal effects on 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

Lobster Larvae % Survival, Permethrin Exp.

10/19/12

<table>
<thead>
<tr>
<th>Target ug/L</th>
<th>SWC</th>
<th>AceRB</th>
<th>.01µg/L</th>
<th>.1µg/L</th>
<th>1µg/L</th>
<th>10µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC-MS ug/L</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>

% Survival

Perm-µg/L

% Survival

Results for Permethrin acute toxicity:

- **LC-50** = 3.0 ± 0.5 µg/L
- **NOEL** No Observable Effect Level = (no difference from control) = 1.8 ± 0.3µg/L

1st definitive

Lobster Larvae % Survival Permethrin Exp.

2/19/13

<table>
<thead>
<tr>
<th>Target ug/L</th>
<th>SWC</th>
<th>AceRB</th>
<th>.5µg/L</th>
<th>1µg/L</th>
<th>2µg/L</th>
<th>4µg/L</th>
<th>8µg/L</th>
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<tbody>
<tr>
<td>LC-MS ug/L</td>
<td>0</td>
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<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>12</td>
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% Survival

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Results for Permethrin acute toxicity:

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Final Definitive

Lobster Larvae % Survival, Permethrin Exp.

1/17/14

<table>
<thead>
<tr>
<th>Target ug/L</th>
<th>SWC</th>
<th>AceRB</th>
<th>1µg/L</th>
<th>2µg/L</th>
<th>3µg/L</th>
<th>4µg/L</th>
</tr>
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<tbody>
<tr>
<td>LC-MS ug/L</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
</tr>
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% Survival

Perm-µg/L

% Survival

Results for Permethrin acute toxicity:

- **LC-50** = 3.0 ± 0.5 µg/L
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Final Definitive
Summary of Naled & Malathion Lobster Exposures

**Naled:** 96-hr LC-50 = 12 ± 4 µg/L
- 96hr NOEL = 7 ± 2 µg/L

**Malathion:** 96hr NOEL > 20 µg/L

### Malathion Exposure

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

<table>
<thead>
<tr>
<th>LC-MS µg/L</th>
<th>stdev</th>
<th>% survival</th>
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</thead>
<tbody>
<tr>
<td>2.20</td>
<td>0.27</td>
<td>100</td>
</tr>
<tr>
<td>5.08</td>
<td>0.69</td>
<td>100</td>
</tr>
<tr>
<td>10.20</td>
<td>1.78</td>
<td>100</td>
</tr>
<tr>
<td>15.33</td>
<td>0.66</td>
<td>100</td>
</tr>
<tr>
<td>20.59</td>
<td>3.55</td>
<td>100</td>
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</table>
Summary; Lobster 96 hour Acute Toxicity Tests

- **Permethrin:**
  - LC-50 = 3.0 ± 0.5 µg/L
  - NOEL (No Observable Effect Level) = 1.8 ± 0.3 µg/L

- **Naled:**
  - LC-50 96hr = 12 ± 4 µg/L
  - NOEL = 7 ± 2 µg/L

- **Malathion:** LC-50 96hr > 20 µg/L
  - LC-50 96hr > 20 µg/L
  - NOEL > 20 µg/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.
Collecting & Dosing Coral Larvae

Live Coral Spawning

Collecting larvae

Coral larvae dosing & Monitoring
Coral Larvae Exposure to Naled and Parmethrin

Summary of Coral Larvae 96hr Acute Toxicity Tests:
Naled & Permethrin Results:
- NOEL Naled > 8 ug/L of Naled;
- NOEL Permethrin > 8 ug/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

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

**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)