Third Progress Report
For the period, 10/1/2013 to 3/31/2014
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Florida Keys National Marine Sanctuary Water Quality Protection:
Assess the effects of mosquito control pesticides on
non-targeted organisms in the
Florida Keys National Marine Sanctuary

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PROJECT PARTICIPANTS, 10/1/13 to 3/31/14:

1. Mote Marine Laboratory: Mote personnel prepared standard operating procedures for coral and lobster larvae exposure studies, prepared pesticide exposure solutions from the technical formulations used by the FKMCD, worked with partners for applying appropriate dosing concentrations for exposure studies, and provided HPLC-MS/MS analysis of standards and technical formulations to verify exposure concentrations for each set of toxicity tests.
   a) Mote Ecotoxicology Research Program: R. Pierce, PI.; M Henry, Staff Scientist; P. Blum, Senior Biologist; College Student Interns, Ariana Augustines (FSU) and Jon Scriven (Univ. South Hampton, UK), Samantha Harlow, (USF).
   b) Mote Marine Microbiology Research Program: Kimberly Ritchie, Co-PI, coral larvae collection, design and monitoring larval exposure studies;
   c) Mote Ocean Acidification Research Program, Emily Hall, Co-PI, Planning and interpretation of coral larvae exposure tests.

2. FWCC, FWRI, Marathon: FWRI personnel collected lobster larvae, set up and implemented each set of lobster larvae exposure studies. T. Matthews, Associate Research Scientist; G. Renchen, Biological Scientist I; Casey Butler, Biological Scientist I.

3. FKMCD: Personnel provided samples of technical pesticide formulations for exposure studies and provided advice and coordination regarding appropriate sites for monitoring pesticide residues in the NMS, resulting from field applications of mosquito control pesticides. Andrea Leal, Operations Director/Entomologist, Emilio Posada, upper keys field coordinator.

INTRODUCTION:

The overall goal of this study is to address EPA Region 4 Priority Area for Special Research Projects by assessing the effects of mosquito control pesticides on non-target organisms in the Florida Keys National Marine Sanctuary (FKNMS) and to provide resource managers with empirical data to preserve and enhance the living resources of the FKNMS. Objectives to accomplish this goal include:

Objective 1: Monitor routine field applications of mosquito control pesticides to determining the source, distribution, concentration and persistence of mosquito control pesticides in near-shore NMS.

Objective 2: Measure toxic effects of expected environmental concentrations (EEC) of naled, permethrin and malathion to early life stages of the scleractinian coral (Porites astreoides) and Spiny lobster (Panulirus argus) from puerulus larval stage through critical stages of metamorphosis that occur naturally in near-shore waters of the NMS

Objective 3: Provide empirical data for the responsible agencies and FKMCD to preserve and enhance living resources of the FKNMS, while protecting the public health and economic well being of the Florida Keys.
Activities proposed for the third 6 months of the project included:
1. Continued range-finder and definitive mosquito control pesticide exposure studies for lobster and coral larvae
2. Conduct field monitoring of pesticide distribution following FKMCD applications.
3. Monitor pesticide distribution from residential pesticide misting application systems.

Activities accomplished:

1. Exposure studies accomplished:

Lobster larvae:
Acute lethal toxicity (LC-50, 96-hr) and sublethal toxicity (development to juvenile) exposure tests included:
- Puerulus larvae exposed to naled; 12/6/2013.
- Puerulus larvae exposed to Permethrin 1/7/2014.
- Juvenile lobster exposed to Permethrin 2/6/2014.
- Puerulus larvae exposed to Malathion 3/7/2014.

Lobster puerulus collection devices were deployed by FWRI staff approximately one month prior to each set of toxicity tests and retrieved one day before each test. Viable larvae were collected, separated into four developmental stages; clear (youngest), semi-pigmented and pigmented (last stage before metamorphosis into juvenile), and juvenile. Specimens were then acclimated overnight in the test seawater before initiating each exposure test. Pueruli (clear) larva were selected for the larval exposure tests. The tests included seawater control, acetone dosing solution control and multiple exposure concentrations of selected mosquito control pesticides in the technical formulation applied by the FKMCD. Exposure tests were conducted in 1L glass exposure chambers (beakers), in 5 replicate, 500ml exposure solutions for each concentration and 5 larvae per beaker, 25 larvae for each exposure concentration, when sufficient larvae were collected, as described below in Results.

Coral larvae:
Porites astreoides coral spawn from April through June. Therefore, no coral larvae were available for exposure studies during this project period.

2. Field monitoring of pesticide distribution from FKMCD application:
The distribution and concentration of naled, applied from aerial application, was monitored in the Snake Creek area and the Venetian Shores canals on October 1, 2013. Water samples were collected from 12 sited in the canals, Snake Creek and adjacent FL Bay and Atlantic sides of the Creek at three times:
- Pre-application to detect any existing pesticide contamination
- Two hours post application to observe pesticide concentrations to which organisms are initially exposed.
- Five hours post application to observe tidal distribution and persistence.
3. Pesticide extraction and analysis:

- Exposure Studies: Technical pesticide formulations were prepared in acetone solution for subsequent dilution in seawater dosing solution to ensure that the concentration of acetone added to each exposure container was equal to or less than 5µl/L. Pesticide solutions were prepared at Mote and transported to FWRI, Marathon, for each set of toxicity tests. Representative samples of each pesticide standard and each exposure concentration were analyzed by HPLC-MS/MS at Mote to verify exposure concentrations and stability of the standards.

- Field Monitoring: One-Liter water samples were collected from each of the 12 sites, 50ml of dichloromethane (DCM) was added immediately to initiate extraction and stop degradation. QA/QC included duplicate samples at two of the 12 sites and three water samples spiked with known concentrations of naled to verify extraction efficiency. Results = 73 ± 11% recovery. Samples were transported to Mote on ice and analyzed by HPLC-MS/MS.

RESULTS:

Lobster larvae:

1. Puerulus larvae exposed to Naled:
   - Definitive exposure test:
     - Exposure concentrations: Target = 1.0, 5.0, 10, 20, 40 µg/L
     - LC-MS/MS verification: naled = 0.6, 2.8, 6.7, 9.3, 15 µg/L
       DDVP = 0.3, 0.3, 0.5, 0.5, 0.6 µg/L
     - Exposure concentration corrected for 73 ± 11 % extraction recovery:
       Corrected Naled dose = 0.9, 4.0, 9.5, 13, 21 µg/L
       - LC-50 96hr no significant difference between control and exposures through 9.5 µg/L concentrations, 100% mortality at 21 µg/L.
   - (Note: the lower LC-MS measured concentration is due to an average of ~70% recovery of standard naled from spiked exposure water samples.
   - Sublethal, development impacts exhibited no difference between control and exposed larvae through 9.3 µg/L. 100% mortality at 21 µg/L.

2. Puerulus larvae exposed to Permethrin 1/7/2014:
   - Definitive exposure test:
     - Exposure concentrations: Target = 0.5, 1.0, 2.0, 3.0, 4.0 µg/L
     - LC-MS/MS verification: Permethrin = 1.5, 1.0, 1.6, 1.8, 3.0 µg/L
     - Corrected for 79 ± 8 % standard recovery;
       Corrected Permethrin concentration = 1.9, 1.2, 2.0, 2.3, 3.8 µg/L
     - LC-50 96hr ; % mortality
       - <5% mortality for controls, 1.2, 1.9 and 2.0 µg/L;
       - 16 ± 26% mortality at 2.3 µg/L
       - 44 ± 46% mortality at 3.8 µg/L
     - Results indicate LC-50, 96hr to be about 4 µg/L
   - Sublethal, development impacts exhibited no difference between control and remaining live exposed larvae.
   ● Samples processed, data interpretation and assessment in progress.

   ● Definitive exposure test:
     - Exposure concentrations: Target malathion; \(= 10, 50, 100\) - Exposure concentrations: Target malathion; \(= 10, 50, 100 \mu g/L\)
     - LC-MS/MS verification: Malathion \(=10, 11, 14 \mu g/L\)
       (note, % standard recovery tests under revision due to instrument malfunction)
   ● LC-50 96 hr; % mortality; No mortality up to 14 \(\mu g/L\) malathion concentration.
   ● Sublethal development; No difference between controls and exposed larvae.

5. Field monitoring of pesticide distribution from FKMCD application:
   ● Field concentrations of naled:
     - Pre-appl: \(< 0.001 \mu g/L\) all stations (std recovery from seawater = 82 \pm 3\%, \(n=3\))
     - 2-hr post appl: range; canals, 0.24 to 3.14 \(\mu g/L\); Creek mouth 0.03 to 0.04 \(\mu g/L\);
       FL Bay, 0.09 to 0.60 \(\mu g/L\); Atlantic, 0.01 to 0.05 \(\mu g/L\).
     - 5 hr post appl: range; canals, 0.02 to 0.19 \(\mu g/L\); Creek mouth, 0.03 to 9.09 \(\mu g/L\);
       FL Bay, 0.01 to 0.07 \(\mu g/L\); Atlantic, 0.09 to 0.17 \(\mu g/L\).

ACTIVITIES SCHEDULED FOR THE NEXT SIX MONTHS:

1. Field applications of mosquito control pesticides in cooperation with the Florida Keys Mosquito Control District.
2. Continued toxicity studies with coral larvae, in cooperation with K. Ritchie and E. Hall at Mote’s Tropical Research Lab on Summerland Key (starting April, 2014).
3. Final review of lobster exposure data with T. Matthews, G. Renchen and C. Butler at FWRI facility in Marathon, FL. Implementation of additional pueruli toxicity tests to fill data gaps if needed.
   - Conduct % recovery for all pesticides at different concentrations to provide accurate corrections throughout the intended exposure range.
   - Evaluate and report persistence of pesticides at 24 hours in exposure chambers.
4. Monitor the distribution and persistence of permethrin applied from a residential automated permethrin sprayer system.

FINANCIAL PROGRESS:

The project is on budget with approximately 75% of the overall Project budget having been spent as of 3/31/2014.