The Effects of Water Quality on Embryogenesis and Larval Development of Queen Conch: Implications for Recruitment to and Coastal Development of the Florida Keys

Analytical Chemistry Report
Mote Marine Laboratory

Dana L. Wetzel, Ph.D.
And
Richard H. Pierce, Ph.D.

February 2, 2006

FWC Grant #
9734-250-2498

Mote Marine Laboratory
Technical Report Number 1075

Scope of Work:
Mote Marine Laboratory will provide the following deliverables:
1. Assist in conducting laboratory experiments for FWC grant #2498 (SFWMD agreement #OT050676).
2. Acquire test chambers, chemicals, Teflon tubing and pump for experiments.
3. Provide FWC with quarterly reports of work completed on project.
4. Analyze water samples for pesticide concentrations and breakdown products as per the proposal.
5. Provide FWC with the data from the water sample analyses.

Methods:
Sample Handling

One liter samples of each exposure solution were collected either before (time zero) or during the exposure experiment, and placed into a clean, dichloromethane (DCM) rinsed amber bottled fitted with a Teflon lined cap. Approximately 100 ml of DCM was added to each sample to help preserve them and start the extraction process. The samples were transported on ice in coolers and stored immediately at 4^0C until they were extracted.

Method Summary

The water exaction method detailed below was based upon the modified DEP method GC-002 entitled "Standard Operation Procedure for: EXTRACTION OF ORGANOPHOSPHORUS, ORGANONITROGEN AND ORGANOHALIDE PESTICIDES, PCBs AND PAH's FROM WATER, WASTES, SEDIMENT, PUFs AND FILTERS FOR SUBSEQUENT ANALYSIS BY GC-ECD/NPD/FPD/FID/MS." Briefly, each exposure solution was extracted via liquid-liquid extraction three times with pesticide grade dichloromethane, as described below.

Samples were allowed to warm to room temperature. The samples were poured into separatory funnels and spiked with an internal standard (OCN). Approximately 100 ml of DCM was added to each separatory funnel and the mixture shaken for about 1 minute. The DCM fraction was dried over sodium sulfate in a funnel and collected into clean, DCM rinsed Rapid-Vap tubes. This process was repeated two more times with additional volumes of DCM. The sodium sulfate was rinsed three times with additional DCM. The samples were then reduced in volume using the Rapid-Vap (Labconco) system under a stream of high purity, nitrogen gas. The samples were evaporated and solvent exchanged into pesticide grade hexane and brought up to 1 ml final volume.

Sample Analysis

A Varian 3800 DC equipped with 30-m glass capillary DB-5 and DB-1 columns and dual ECD detectors coupled with a Turbochrome chromatography data processing system was used for the analysis of water samples. Briefly, the oven program had an initial temperature of 125^0C and was held for 2.25 minutes, then ramped at a rate of 15^0C/min to 325^0C and held at that temperature for 0.42 minutes. The samples were injected using a Varian 8200 auto-sampler and the syringe was washed with hexane for 60 seconds before and after each sample was processed. The samples were analyzed using a dual column configuration (DB-1 and DB-5), with one column for identification and quantification and the other for confirmation. Each

sample was analyzed a minimum of two times to determine any instrument variability. Blanks were run between samples to ensure that there was no sample carry-over. Calibration curves and relative response factors were calculated using pesticide standards. Response factors were calculated for internal standards and pesticide standards for each series of samples run to monitor any changes in instrument sensitivity. The lower limits of detection were determined to be approximately 10.0, 6.0, and 5.0 ng/ml respectively for dibrom, ddvp and Biomist

Data:

Table 1. Biomist Settlement Exposure (ug/l)

Nominal Concentrations	T= 0 hrs	T=12 hrs
Control	0.00	0.00
1.8	0.28	0.00
3.75	0.84	0.00
7.5	1.55	0.33
15	4.68	0.45
30	5.25	1.55

Table 2. Biomist Embryo Exposure (ug/l)

Nominal Concentrations	T= 0 hrs	T=12 hrs	T=24 hrs	T=48 hrs	T=96 hrs
Control	0.00	0.00	0.00	0.00	0.00
1.8	0.12	0.03	0.00	0.00	0.00
3.75	0.20	0.05	0.00	0.00	0.00
7.5	0.34	0.05	0.00	0.00	0.00
15	0.82	0.08	0.00	0.00	0.00
30	1.38	0.10	0.00	0.00	0.00

Table 3. Biomist Heterotrophic Exposure (ug/l)

Nominal Concentrations	T= 0 hrs	T=12 hrs	T=24 hrs	T=48 hrs	T=96 hrs
Control	0.14	0.03	0.00	0.00	0.00
1.8	0.47	0.10	0.00	0.00	0.00
3.75	0.40	0.08	0.00	0.00	0.00
7.5	0.54	0.08	0.00	0.00	0.00
15	3.68	0.42	0.00	0.00	0.00
30	5.26	0.54	0.00	0.00	0.00

Table 4. Biomist Autotrophic Exposure (ug/l)

Nominal Concentrations	T= 0 hrs	T=12 hrs	T=24 hrs	T=48 hrs	T=96 hrs
Control	0.00	0.00	0.00	0.00	0.00
1.8	0.62	0.00	0.00	0.00	0.00
3.75	0.60	0.01	0.00	0.00	0.00
7.5	1.07	0.03	0.00	0.00	0.00
15	3.07	0.05	0.00	0.00	0.00
30	5.08	0.30	0.00	0.00	0.00

Table 5. Dibrom/DDVP Settlement Exposure (ug/l)

Nominal Concentrations	T= 0 hrs dibrom	T=0 hrs DDVP	T= 12 hrs dibrom	T=12 hrs DDVP
Control	0.00	0.00	0.00	0.00
1.8	0.03	0.04	0.00	0.00
3.75	1.05	0.06	0.17	0.00
7.5	4.09	1.46	0.89	0.38
15	9.77	3.80	3.86	4.13
30	26.28	6.30	6.59	5.70

Table 6. Dibrom Embryo Exposure (ug/l)

Nominal Concentrations	T=0hrs dibrom	T=0hrs DDVP	T=12hrs dibrom	T=12hrs DDVP	T=24hrs dibrom	T=24hrs DDVP	T=48hrs dibrom	T=48hrs DDVP	T=96hrs dibrom	T=96 hrs DDVP
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.8	1.59	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3.75	3.31	0.09	0.91	0.72	0.00	0.00	0.00	0.00	0.00	0.00
7.5	5.28	0.21	1.22	0.48	0.00	0.00	0.00	0.00	0.00	0.00
15	11.40	2.71	1.60	2.08	0.00	0.07	0.00	0.00	0.00	0.00
30	28.86	4.92	2.23	6.23	0.00	0.11	0.00	0.00	0.00	0.00

Table 7. Dibrom Heterotrophic Exposure (ug/l)

Nominal Concentrations	T=0hrs dibrom	T=0hrs DDVP	T=12hrs dibrom	T=12hrs DDVP	T=24hrs dibrom	T=24hrs DDVP	T=48hrs dibrom	T=48hrs DDVP	T=96hrs dibrom	T=96 hrs DDVP
Control	0.00	0.00	5.08	0.00*	0.00	0.00	0.00	0.00	0.00	0.00
1.8	3.47	0.28	6.45	0.59*	0.00	0.00	0.00	0.00	0.00	0.00
3.75	11.86	0.53	5.45	0.51*	0.29	0.00	0.00	0.00	0.00	0.00
7.5	11.63	0.54*	5.72	0.84*	0.25	0.00	0.00	0.00	0.00	0.00
15	18.27	2.43*	4.35	0.66*	0.63	0.00	0.00	0.00	0.00	0.00
30	22.20	6.59*	8.09	3.77*	0.39	0.00	0.00	0.00	0.00	0.00
*Trace permethrin residues at < 0.09 ppb										

Table 8. Dibrom Autotrophic Exposure (ug/l)

Nominal Concentrations	T=0hrs dibrom	T=0hrs DDVP	T=12hrs dibrom	T=12hrs DDVP	T=24hrs dibrom	T=24hrs DDVP	T=48hrs dibrom	T=48hrs DDVP	T=96hrs dibrom	T=96 hrs DDVP
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.8	1.55	1.02	0.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3.75	1.33	1.12	0.44	0.25	0.00	0.00	0.00	0.00	0.00	0.00
7.5	7.42	1.95	5.06	1.81	0.34	0.81	0.00	0.00	0.00	0.00
15	22.94	2.62	6.13	2.23	0.60	1.35	0.00	0.00	0.00	0.00
30	33.02	6.85	7.77	4.75	0.84	2.22	0.00	0.00	0.00	0.00

Discussion:

Previous studies of residues of the mosquito control pesticide, dibrom, its break down product ddvp and permanone, in water monitored for 39 hours after application (Pierce et al., 2005) showed a range of concentrations for the three types of environments monitored (i.e., surface and subsurface waters from Atlantic, bayside, and canal sites). Only one station at the 14-16 hour sampling time had a measurable amount of dibrom detected (0.19ug/l) although a few of the other stations had measurable concentrations of the dibrom breakdown product, ddvp, at ranges from 0.05 -0.56 ug/l found during the 14-16 hour sampling time. There were trace amounts of permethrin found in two of the sub-surface samples collected from the Atlantic, but no other detectable levels were found in the remaining bayside and Atlantic samples. However, 3-6 hours after an application of permanone to some interior canals, levels of permethrin ranged from 3.13-9.41 ug/l in the sea surface microlayer. There were no detectable levels found in the sub-surface water samples for the same time (Pierce et al., 2005).

Exposure solution concentrations targeted for this conch exposure work ranged from 1.8 ug/l to 30 ug/l for both dibrom and Biomist. This nominal range would include and exceed the environmentally relevant concentrations found by Pierce et al.

In the dibrom exposure studies, actual concentrations (analytically determined) for the time zero assays ranged from 0.03 to 26.28 ug/l in the settlement exposure test, from 1.59 to 28.86 ug/l in the embryo exposure test, from 3.47-22.20 ug/l in the heterotrophic exposure test and from 1.55 to 33.02 in the autotrophic exposure test (Tables 5-8). Concurrently, ddvp levels ranged from 0.04 to 6.85 ug/l during the same time zero period. Both pesticides continued to decrease in concentration during the subsequent water sampling times until there were no detectable amounts (Tables 5-8), usually after the 24 hour sampling event.

Solubility of Biomist (permethrin) was much less in seawater than dibrom and resulted in lower concentration levels detected. These concentrations ranged from 0.28 to 5.25 ug/l for the settlement study, from 0.12 to 1.38 ug/l for the embryo study, from 0.14 to 5.26 ug/l for the heterotrophic study, and from 0.62 to 5.08 ug/l for the autotrophic exposure study. Although these values did not reach the targeted concentrations, the high values of permethrin found in the Pierce et al. (2005) study were for sea-surface microlayer and not the sub-surface water. Because of the insolubility of Biomist, it may be difficult to solublize enough of this pesticide to reach a concentration of 30 ug/l, even with the addition of acetone to enhance solubility.

Recommendations:

All of the targeted ranges for exposure for the dibrom exposure studies were reached. Therefore, no further suggestions for this part of the project are included in this report. The issue of solubility for Biomist (permethrin) may not allow for making a seawater-soluble fraction at the concentration of 30 ug/l. However, we can attempt to combine each of the four life stages, embryo, heterotrophic, autotrophic and settlement into one exposure study targeting the highest concentrations of 15 and 30 ug/l. A study to determine the maximum concentration that can be created by altering the amount of acetone, stirring regime etc. would need to be done however, before the exposure study could begin. We would be willing to analyze the additional samples required for this additional study at no cost so that we can complete what we believe is a very important study.

Some possible future research directions would enhance the understanding of the effects of mosquito control pesticides on the different life stages of the conch. These studies might include determining the effects of multiple mosquito control pesticides acting

synergistically on several life stages. In addition, a study designed to simulate deposition of permethrin and dibrom directly on to settled larvae or juveniles that are exposed during low tide or as the tide recedes, would eliminate the solubility problem and would be equivalent to amounts collected at the water surface microlayer found by the Pierce et al. study may be very relevant.

Reference:

Pierce R.H., M.S. Henry, T.C. Blum and E.M. Mueller. 2004. Aerial and tidal transport of mosquito control pesticides into the Florida Keys National Marine Sanctuary. Rev. Biol. Trop. 53:117-125.