The Effects of the Pesticides Biomist 30/30® and Dibrom® on Queen Conch (*Strombus gigas*) Embryos and Larvae: A Pilot Study

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ABSTRACT

Pesticides targeting mosquitoes are increasing in use as the mosquito population continually poses a threat and a nuisance to residents and animals. However, as the use of pesticides increases there is a need to investigate the effects that these pesticides have on the marine environment. Four bioassays were conducted to determine if the pesticides used for mosquito control in the Florida Keys, Biomist 30/30® and Dibrom®, are affecting the recovery of *Strombus gigas*. The bioassays tested the effects of each pesticide on embryogenesis and veliger survival. Three concentration levels were tested: the target spray concentration used by mosquito control, half the target concentration, and twice the target concentration. Embryonic development was determined by measuring the perivitellin space of developing embryos. Larval survival was determined by counting the number of veligers swimming in the water column after 48 hours of exposure to the pesticides. Embryos exposed to the pesticides exhibited delayed or abnormal development while embryos in the controls showed normal development. Larvae exposed to Biomist 30/30® had 100% mortality after 48 hours. Unfortunately, all the larvae (including those in the control) in our Dibrom® bioassay crashed after 24 hours. These results may have implications for continued coastal development in the Florida Keys, as the pesticides used in mosquito control may be negatively affecting the recovery of nearshore queen conch populations.

KEY WORDS: Queen conch, larvae, pesticides, Florida Keys

Los Efectos de los Pesticiads Biomist 30/30® y Dibrom® en los Embriones y Larvas del Caracol Rosado (*Strombus gigas*): Un Estudio Piloto

Pesticidas contra mosquitos estan aumentando en uso, como la población de mosquitos es una amenaza y un fastidio a residentes y animales. Sin embargo, como el uso de pesticidas aumenta hay una necesidad de investigar los efectos que estos pesticidas tienen en el ambiente marino. Cuatro experio-

mentos se realizaron para determinar si los pesticidas utilizadas para el control de mosquitos en los Cayos de la Florida, Biomist 30/30® y Dibrom®, afectan la recuperación de *Strombus gigas*. Los experimentos pruebaron los efectos de cada pesticida sobre la sobrevivencia de embriones y larvas. Tres concentraciones de pesticidas se probaron: la concentración que se aplica por el control del mosquito, la mitad de esa concentración, y el doble de esa concentración. El desarrollo embrionario fue determinado midiendo el espacio de perivitellin. Sobrevivencia de larvas fue determinada contando el número de larvas nadando en la columna de agua después de 48 horas de la exposición a los pesticidas. Los embriones expusieron a los pesticidas mostraron desarrollo retrasado o abnormal mientras embriones en los controles mostraron desarrollo normal. Las larvas expusieron a Biomist 30/30® tuvo 100% de mortalidad después de 48 horas. Desgraciadamente, todas las larvas (inclusive ésos en el control) en nuestro experimento con Dibrom® se murieron después de 24 horas. Estos resultados pueden tener implicaciones para el desarrollo costero en los Cayos de la Florida como los pesticidas utilizaron en el control de los mosquitos pueden estar afectando negativamente la recuperación de poblaciones de *Strombus gigas* en aguas costeras.

PALABRAS CLAVES: *Strombus gigas*, larvas, pesticidas, Cayos de la Florida

INTRODUCTION

Queen conch (*Strombus gigas*) once supported a significant commercial fishery in the Florida Keys, but overfishing and habitat degradation caused a vast decline in the number of queen conch in the area. Due to the large reduction in the Florida Keys conch population, the commercial fishery was closed in 1976 and the recreational fishery was closed in 1986 (Glazer and Berg 1994, Glazer et al. 2003). Despite protection for more than 17 years in the state of Florida, there has been very little recovery of the population since the fishery closure (Glazer and Berg 1994, Stoner et al. 1997, Glazer et al. 2003). The queen conch is not currently threatened with extinction, but it is listed by the Convention of International Trade in Endangered Species (Ray-Culp and Stoner 2000).

The Florida Keys population of queen conch resides on the backside of the Florida Keys reef tract. Conch reproduce during the summer months by laying an encapsulated egg strand that may contain up to 500,000 embryos (Davis 1994). The strand is wrapped around itself and covered with sand for protection. After four days of development the larvae emerge from the egg mass. Larvae can be found in water up to 100 m deep, but most are found in the upper 5 m (Stoner 1997). Veligers spend 3-4 weeks in the water column before settling to the ocean floor. During this time larvae may travel large distances from where they hatched. Past surveys have shown an infrequent and irregular supply of larvae in the Florida Keys area (Stoner 1997). The irregular supply of larvae may partially account for the lack of recovery among the queen conch population (Stoner et al. 1997).

It is possible that locally sprayed pesticides may cause damage to the

developing embryos and larvae, thereby, causing mortality and a reduction in the larval supply. Dibrom® and Biomist 30/30® are sprayed throughout Monroe County to control the mosquito population. Dibrom® is 85% Naled (Dimethyl 1,2-dibromo- 2,2- dichloroethyl phosphate), the remaining 15% is a petroleum distillate solvent (Valent USA Corporation). Dibrom®is applied by aerial application to 289,531.96 acres in the Florida Keys (Monroe County Mosquito Control). It is applied at a rate of ½ oz per acre (Monroe County Mosquito Control). Naled has a half-life of 16 hours under a pH of 7, degradation decreases as the pH rises (Pierce 1998). The primary by-product is dichlorvos, DDVP (2,2- Dichlorovinyl dimethyl phosphate) is highly toxic and insoluble in water (Snedaker and Rumbold 1997). Dichlorvos does not bind to sediments and has a half-life of four days in aquatic environments (Extension Toxicology Network).

Biomist 30/30®, (active ingredient permethrin), is sprayed in an ultra-low volume form from a sprayer mounted on the back of a truck (Monroe County Mosquito Control). Permethrin ((3- Phenoxyphenyl) methyl (+) cis, trans- 3-(2,2- dichloroethenyl)- 2,2- dimethyl cyclopropanecarboxylate) is a synthetic pyrethroid with a mixture of both cis and trans isomers. It is ephemeral with a half-life of 14 days in seawater exposed to sunlight (Schimmel et al. 1983 and Gonzalez-Doncel et al. 2003). Synthetic pyrethroids are toxic to fish and other aquatic organisms, including aquatic invertebrates (Lee et al. 2002). Biomist 30/30® is combined with the synergist piperonly butoxide (Butylcarbityl- 6-propyliperonyl ether. Piperonly butxide is considered toxic to fish and aquatic invertebrates (National Pesticide Telecommunications Network).

Dibrom® and Biomist 30/30® enter the water by drift, runoff, and leaching. Tests indicate that DDVP appears to enter the water by tidal flushing of pesticide residue from residential canal systems where Dibrom® is sprayed (Pierce 1998). Sea surface microlayers frequently become enriched with contaminants, including pesticides. Embryos and larvae have displayed developmental toxicity when exposed to contaminated microlayers (Snedaker and Rumbold 1997). Snedaker and Rumbold (1999) demonstrated that sea surface microlayers collected off the Florida Keys adversely affect the embryogenesis of invertebrates and fish. The present study will test the effects of the two pesticides used in mosquito control in the Florida Keys on queen conch embryos and larvae to determine if they play any role in limiting recruitment and, thus, the recovery of the conch population in the Florida Keys.

METHODS

Conch egg masses were collected from breeding aggregations located on the back-reef of the Florida Keys reef tract. The egg masses were brought to the lab and disinfected using a 0.5% Clorox® solution. The 0.5% Clorox® solution was prepared by placing 10 milliliters of 5% household strength Clorox® per 2 liters of filtered seawater. Each egg mass was placed in the solution for 30 seconds, removed, and dipped in 3 beakers of clean seawater for 10 seconds per dip as per the method of Davis (1994). There were four bioassays conducted, Biomist 30/30® (Clark Mosquito Control) was tested

using embryos and larvae; Dibrom® (Amvac Chemical Corporation) was also tested using embryos and larvae. There were five treatments (with three replicates of each treatment) for each run of the experiments. Treatments consisted of a control using Instant Ocean® made with deionized water, filtered nearshore seawater, and three concentrations of Dibrom® and Biomist 30/30®. The three concentrations of Biomist 30/30® were 5.05051E-08 ml, 2.52525E-08 ml (target concentration), and 1.26382E-08 ml per 1 ml of artificial seawater. The three concentrations of Dibrom® were 4.45931E-08 ml, 2.22965E-08 ml (target concentration) and 1.11483E-08 ml per 1 ml of artificial seawater. The salinity of the artificial seawater was adjusted to 35‰ and was vacuum filtered through 50 micron filter pads to remove any particles prior to use. The nearshore water was collected from Florida Bay and filtered through a 0.45 micron Millipore filter prior to use. The effects of nearshore water on embryos and larvae were tested in conjunction with the Biomist 30/30® assays.

Biomist 30/30® was tested on queen conch embryos to determine its effects on embryonic development. The disinfected egg mass was broken into small sections and placed in 1,000 ml beakers containing 500 ml of one of the five treatment solutions. All beakers were covered with a watch glass to minimize contamination and evaporation. Beakers were placed in a water bath maintained at a constant temperature of 28 $\pm$ 1°C. The temperature was maintained using an aquarium heater and thermostat and was recorded at three locations in the water bath every 24 hours. The experiment ran for four days (96 hours). On days 2 and 4, cultures were taken of all five treatments to test for *Vibrio* contamination. The pH and oxidation/reduction potential were also measured for each treatment and recorded. The egg mass sections were examined using a dissecting microscope. The scope was equipped with a camera, and five pictures were taken of each section. Within each picture, two eggs were used to determine average perivitellin space (i.e. the space in-between the egg capsule and the developing embryo). We used perivitellin space as our performance measure because as the embryo develops normally within the egg capsule, the perivitellin space will decrease over time. The vertical and horizontal distance in millimeters of each egg and each embryo within the egg was measured (Figure 1). Perivitellin space was determined by taking the mean of the vertical and horizontal measurements of the egg and then subtracting the mean of the vertical and horizontal measurements of the embryo. A nested ANOVA was used to calculate differences in perivitellin space among the different treatments for each day.

In the second bioassay, Biomist 30/30® was used to test the effects on queen conch larvae mortality and growth. Larvae were hatched from egg masses collected in the wild. The egg masses were disinfected as described above. The larval experiment consisted of five treatments (with three replicates in each treatment). The same concentrations as the previous bioassay were used. Upon hatching, 10 larvae were placed in each replicate and covered with a watch glass to prevent contamination and evaporation. They were placed in a water bath maintained at a temperature of 28 $\pm$ 1°C. The experiment ran for 48 hours. On days 1 and 2 cultures were taken of all five treatments to test for *Vibrio* contamination. Every 24 hours the temperature,

pH, and oxidation/reduction potential was measured and recorded. The larvae were examined and mortality rates were determined for each treatment by counting the number of dead larvae versus the number of living larvae. Mortality was determined by position in the water column. Veligers that were able to maintain position in the water column by rotation or retraction of the velum were considered alive. Veligers that were resting on the bottom, with or without beating of a larval heart, were considered dead (Rumbold and Snedaker 1997). A chi-square test was used to analyze the data for each day.

Dibrom® was tested on queen conch embryos and larvae as described in the embryo and larval tests conducted with Biomist 30/30®. The experiment consisted of four treatments (an artificial seawater control and three pesticide concentrations) with three replicates of each treatment. The concentrations used were 4.45931E-08 ml, 2.22965E-08 ml (target concentration) and 1.11483E-08 ml per 1 ml of artificial seawater.

RESULTS

Temperature was constant throughout the study. We were unable to analyze pH and ORP because of faulty equipment. On day 2 of the first experiment the pH and ORP probes were no longer taking accurate readings.

Experiment 1: Queen Conch Embryogenesis and Biomist 30/30®

The first day of this experiment showed no significant difference ($F_{(4, 10)}$ = 0.944, p = 0.478) among any of the treatments (Figure 1). However, Day 2 showed a significant difference between the control and the treatments ($F_{(4, 10)}$ = 13.054, p = 0.001) as the embryos in the synthetic seawater control began to develop normally (Figure 2). Day 3 continued to show a significant difference between the control and the three pesticide concentrations; the nearshore treatment also began to show a slight difference compared with the three pesticide treatments ($F_{(4, 10)}$ = 6.234, p = 0.009) (Figure 1). However, on Day 4, there was no difference among the treatments ($F_{(4, 10)}$ = 3.20, p = 0.062) even though the embryos exposed to the pesticide concentrations were not as far along in their development as the conch in the synthetic seawater control or those in the nearshore water treatment (Figure 2).

Experiment 2: Queen Conch Embryogenesis and Dibrom®

Day 1 did not show a significant difference among the treatments ($F_{(3, 8)}$ = 3.13, p = 0.088) (Figure 3). Day 2 continued to show no difference among the treatments ($F_{(3, 8)}$ = 2.12, p = 0.176) (Figure 3). However, Day 3 showed a significant difference among the treatments ($F_{(3, 8)}$ = 14.482, p = 0.001) as the control animals began to develop normally and the embryos exposed to the pesticide concentrations were either developing abnormally or not developing at all (Figure 3). Day 4 showed an even greater disparity in perivitellin space between the control and the three pesticide concentrations with the control showing normal development and the pesticide treatments exhibiting no change in perivitellin space over time (Figure 3). However, the ANOVA for Day 4 was not significant ($F_{(3, 8)}$ = 2.19, p = 0.167); this was probably due to the large deviations about the mean found in the pesticide concentrations.

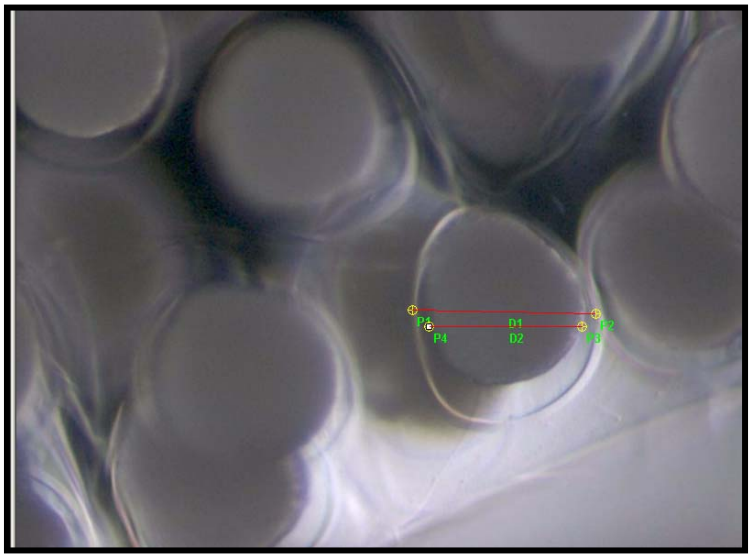

Figure 1. Picture of perivitellin space measurements being taken using the computer program Image Pro Plus. Horizontal and vertical measurements of the eggs and embryos were used to determine perivitellin space for each embryo.

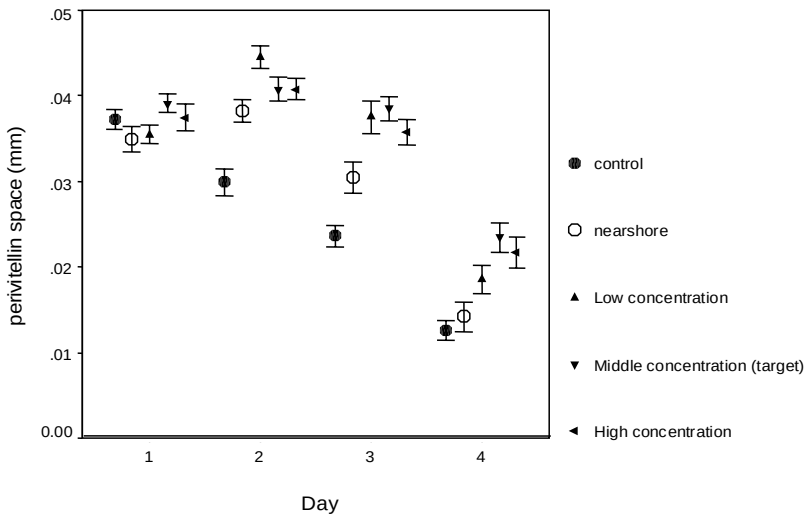

Figure 2. Graph of perivitellin space measurements for all five treatments of the Biomist30/30® bioassay (experiment 1). The graph shows the mean perivitellin space for each treatment on all four days of the bioassay. The error bars represent ± one standard error.

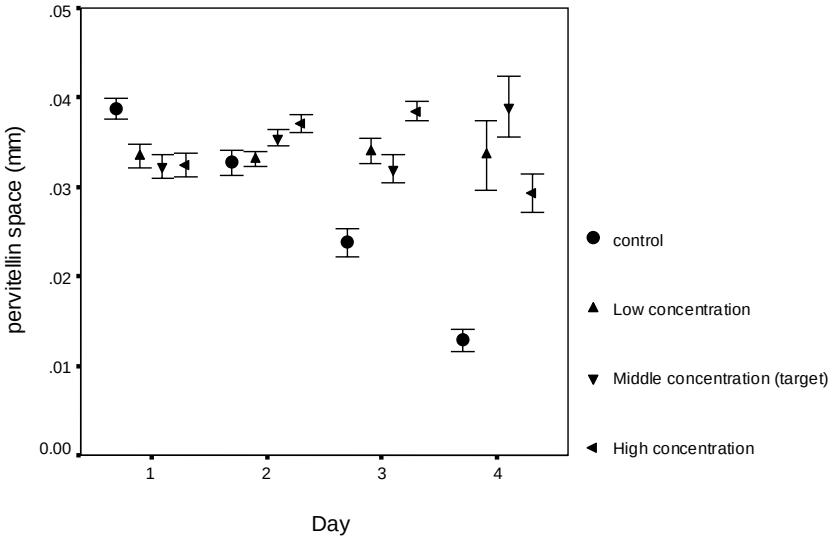

Figure 3. Graph of perivitellin space measurements for all five treatments of the Dibrom® bioassay (experiment 1). The graph shows the mean perivitellin space for each treatment on all four days of the bioassay. The error bars represent ± one standard error

Experiment 3: Queen Conch Larvae and Biomist 30/30®

Queen conch larvae exposed to Biomist 30/30® experienced a high degree of mortality in all three treatment concentrations after 48 hours (Figure 4). The chi-square test for Day 1 showed a significant difference in mortality (X^2 = 57.32, $p < 0.001$) with mortality in the control and the nearshore water treatment hovering around 20% while the mortality in the pesticide treatments ranged from about 50% to 95% (Figure 4). On Day 2, mortality in the pesticide treatments jumped to almost 100%, whereas mortality was much lower in the control and nearshore water treatment (Figure 4). The chi-square test results for Day 2 were significant ($X^2 = 48.33$, $p < 0.001$).

Experiment 4: Queen Conch Larvae and Dibrom®

Due to 100% mortality among all of the treatments and the control after the first 24 hours, no statistical analyses were run for this experiment.

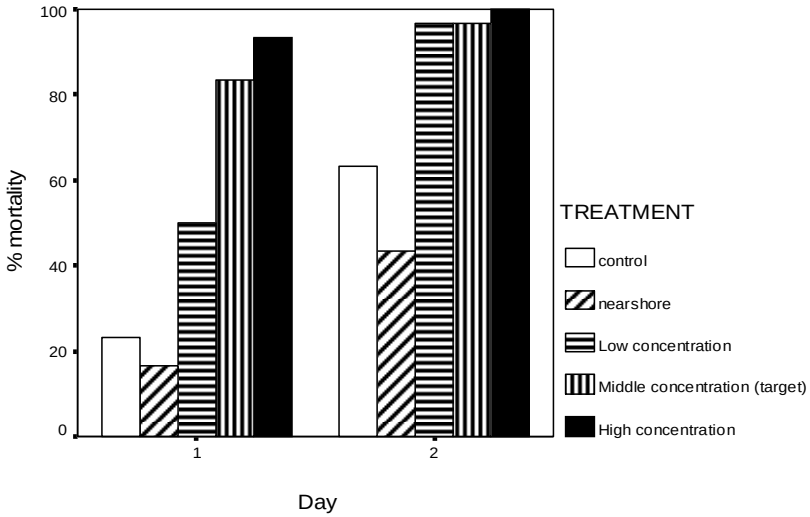

Figure 4. The percentage of mean larval mortality in all five treatments of Biomist 30/30® within 48 hours.

DISCUSSION

Our results showed that Biomist 30/30® and Dibrom® have significant toxicological effects on the development and survival of queen conch embryos and larvae. Biomist 30/30® proved to be toxic to embryos at all three concentrations. The greatest number of effects was seen at the target concentration and higher. Abnormalities were noted during embryogenesis, with slow development seen in all the pesticide treatments (Figure 2). It was also noted that several embryos appeared to be shedding cells. A great number of larvae died during the first 24 hours of the Biomist 30/30® larval bioassay (Figure 4). All of the pesticide treatments had 100% mortality within 48 hours. There were also deaths among the control and nearshore treatments. These deaths may be attributed to the fact that it is difficult to keep larvae alive in the lab without large tanks, water exchanges, or aeration (Davis 1994). Death may have also occurred due to the extraction method used on the veligers from the initial beaker. A 10 ml pipette was used to transfer each veliger out of the beaker and into one of the treatments.

The effects caused by Biomist 30/30® may only be a portion of the effects permethrin has on queen conch embryogenesis. Permethrin does not dissolve in water and therefore binds to other sources such as glass and plastic (Lee et al. 2002). The bioassays were static, as such this would allow for the permethrin to bind readily to the glass and may have reduced the amount of pesticide effecting the embryos and larvae. Unfortunately, there were not enough funds to run chemical analyses to test the actual concentration at the end of the bioassays; therefore, the amount of pesticide that remained in the water could

not be determined. Degradation could be estimated by the half-life of either pesticide; degradation can fluctuate with the pH level, and it would be necessary to determine the pH to estimate the amount of pesticide remaining in the water. Unfortunately, as stated in the Results, the pH meter malfunctioned and pH levels could not be determined at the end of the experiment.

With Dibrom® having such a high toxicity, it was not surprising to get such a vast difference in perivitellin space between the control and the pesticide treatments (Figure 3). All three treatment concentrations seemed to have the same effect. Many of the embryos exhibited major deformities during development, and many did not undergo embryogenesis, but instead began shedding cells until an embryo was no longer visible (Figure 5). Unfortunately, this affected the accurate measurement of the perivitellin space since the ultimate goal was to measure the growth of the embryo as it developed. Many of the embryos that shed cells took up a large portion of the egg capsule even though the embryos were not developing and were not viable, thus, explaining our non-significant result on Day 4 of the experiment.

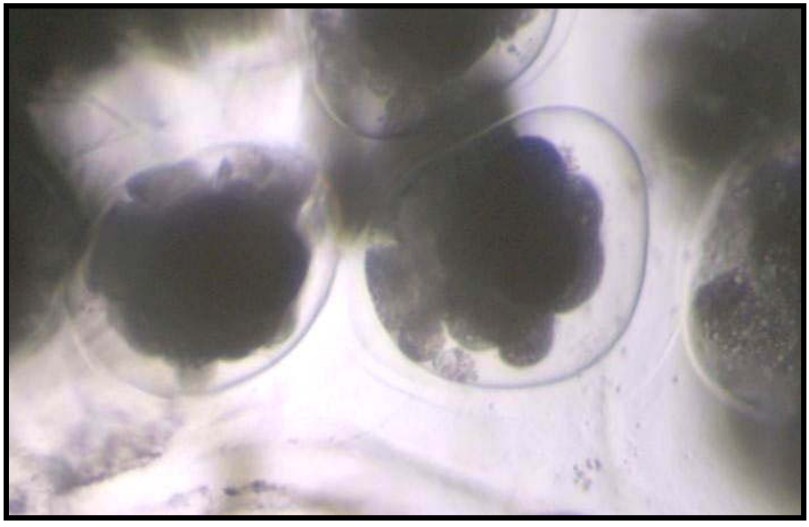

Figure 5. Image of embryos in Dibrom® concentration 2.22965E-08 ml (target concentration). Embryos are displaying the observed shedding of cells.

Many factors may have influenced the failure of the final bioassay testing larval survival after exposure to Dibrom®. As stated previously, it is difficult to raise veligers in the lab. Nevertheless, cross contamination is the likely culprit. Another possible mode of contamination is based on the structure of the pesticide. Naled is mixed with petroleum products making Dibrom® an aromatic hydrocarbon; therefore, it is possible that the control beakers were contaminated in this way.

It is unknown if Biomist 30/30® and Dibrom® reach breeding aggregations offshore; although, larvae undoubtedly drift into nearshore areas where they could encounter either of these pesticides. Pierce (1998) reported that only a small percentage of DDVP and permethrin sprayed in the Florida Keys can be found in surface and subsurface water. Further studies are needed to determine the amount of pesticide remaining in the water column after spraying. Benthic substrates should also be tested to determine if permethrin or Dibrom® bind to them since they are insoluble in water. In addition, studies have shown that outflow from Florida Bay and Biscayne Bay transport pollutants from the Florida mainland (Rumbold and Snedaker 1999). Therefore, other pesticides or pollutants might interfere with queen conch development and growth.

This pilot study has provided evidence that the pesticides used for mosquito control in the Florida Keys may be negatively affecting the queen conch population. However, further testing of Biomist 30/30® and Dibrom® would be beneficial. For example, using flow-through chambers (instead of static chambers) to better mimic what the embryos and larvae are exposed to in the natural environment and testing more environmentally relevant pesticide concentrations (along with chemical analyses to confirm actual test concentrations) would provide a more accurate picture of how these mosquito control agents affect queen conch embryos and larvae and in-turn how they may affect the recovery of the queen conch population in the Florida Keys.

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