FWC/FWRI filecode F2498-05-06-F

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

SFWMD #OT050676

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Abstract

Pesticides targeting mosquitoes are increasing in use as the mosquito population continually poses a threat and a nuisance to human 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. As such, we tested the acute and chronic toxicity of the chemicals associated with mosquito control (i.e. naled and permethrin) in the Florida Keys on critical early life history stages of queen conch (*Strombus gigas*), a keystone species in the Florida Keys ecosystem. Specifically, we conducted bioassay experiments that mimicked the exposure regime that the animals would have experienced in the natural environment on embryos, autotrophic, heterotrophic, and settlement stage queen conch larvae. We found that there were little to no acute effects (i.e. mortality) on embryos and larvae exposed to the two pesticides. However, there were several sub-lethal or chronic effects seen. Queen conch embryos exposed to naled and permethrin hatched, but many were deformed and would probably not survive for very long in natural conditions. Autotrophic and heterotrophic larvae also experienced very little mortality, but, in general, larvae exposed to the higher pesticide concentrations showed slower growth (i.e. they were smaller in size). The low mortality was probably due to the low exposure times; nevertheless, the pesticides caused a sub-lethal effect that may have long-term consequences on survival. Larvae that take longer to reach competency (i.e. recruitment size) will remain in the water column longer and thus have a higher chance of being predated upon. Settlement stage larvae also had very little mortality; however, a higher percentage of larvae successfully underwent metamorphosis (i.e. recruitment) in the higher pesticide concentrations. This unexpected result can be explained by the fact that the pesticide acted as a false metamorphic cue, forcing the larvae to undergo metamorphosis. These results may have implications for continued coastal development in the Keys as the pesticides used in mosquito control may be negatively affecting the recovery of nearshore queen conch populations by reducing recruitment. Further studies on the long-term, chronic effects of pesticide exposure on queen conch embryos and larvae are warranted; for example: 1) how long can newly hatched larvae survive after pesticide exposure and 2) can larvae continue to grow normally, reach competency, and successfully recruit after pesticide exposure.

Introduction

The human population in south Florida and the Florida Keys has expanded rapidly because of the weather, the recreational opportunities and the high quality of life. However, as the human population has increased, so, too, has the burden on the natural resources that has resulted in the establishment of the Florida Keys National Marine Sanctuary. The human impact in this region has resulted in numerous reports implicating eutrophication and mosquito eradication in the degradation of the pristine waters of the Florida Keys (Lapointe et al. 1990; Lapointe and Clark 1992; Szmant and Forrester 1996; Pierce 1998). Both Lapointe and Clark (1992) and Szmant and Forrester (1996) found nutrient gradients from nearshore to offshore as well as elevated nutrients in nearshore developments. Szmant and Forrester (1996) concluded that nutrients originating on land probably were utilized by nearshore algal and seagrass communities. It is also reasonable to suspect that mosquito control pesticides may affect nearshore marine communities because of drift over and deposit into non-targeted areas (Hennessey et al. 1992; Bird et al. 1996; Pierce 1998). Drift is an even greater concern now that ultra-low volume spraying (ULV) has been incorporated into the mosquito control program.

There is an extensive body of literature relating to the toxicity of pesticides on a variety of invertebrates and vertebrates. It is possible locally sprayed pesticides may cause damage to the developing embryos and larvae of nearshore invertebrates, thereby causing mortality and a reduction in the larval supply. Dibrom® and Biomist 30/30® are the two pesticides sprayed in the Florida Keys by Monroe County Mosquito Control to control the mosquito population. Dibrom® is 85% naled (dimethyl 1,2-dibromo- 2,2- dichloroethyl phosphate), the remaining 15% is petroleum distillate solvent (Valent USA Corporation). Dibrom® is sprayed from an aircraft as an ultra-low volume spray and is applied at a rate of ½ oz per acre (Monroe County Mosquito Control). Naled is an organophosphate and, therefore, a cholinesterase inhibitor. Naled is toxic to most aquatic life (Hartley and Kidd 1983), birds (Tucker 1970; Tucker et al. 1987), and bees (Meister 1992). Naled has a half-life of 16 hours under a pH of 7 (Pierce 1998). Degradation decreases as pH rises (Pierce 1998). The primary by-product, dichlorvos (DDVP; 2,2- dichlorovinyl dimethyl phosphate), is highly toxic, insoluble in water, and has a half-life of 4 days in aquatic environments (Rumbold and Snedaker 1997).

The other adulticide currently used by Monroe County Mosquito Control is Biomist 30/30® (active ingredient permethrin), and 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 which paralyzes the nervous system with a mixture of both cis and trans isomers (Pierce 1998). It is ephemeral with a half-life of 14 days in seawater exposed to sunlight (Schimmel et al. 1983; Gonzalez-Doncel et al. 2003). Synthetic pyrethroids are toxic to fish and other aquatic organisms, including aquatic invertebrates (Anderson 1982; Hill 1985; Lee et al. 2002), hence the warning on the label to not apply this product within 30 meters of lakes and streams.

Naled and permethrin can 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 in contaminants including pesticides. Embryos and larvae have displayed developmental toxicity when exposed to contaminated microlayers (Rumbold and Snedaker 1997; Rumbold and Snedaker 1999).

The queen conch, *Strombus gigas*, is a marine gastropod that inhabits the tropical western Atlantic and is a keystone species indigenous to the Florida Keys ecosystem. It once supported a significant commercial and recreational fishery in south Florida (Stevely and Warner, 1978). However, because of a decline in the stock, all harvest was prohibited in 1985 in State waters and this ban was extended to Federal waters in 1986. The population has been slow to recover (Glazer and Berg 1994; Berg and Glazer 1995; Glazer and Delgado 2003). The prolonged recovery of queen conch in Florida has been attributed, in part, to limited recruitment and declining water quality. Queen conch larvae are positively phototropic and are therefore associated with the surface layers (Barile et al. 1994) where many contaminants accumulate. For example, at our experimental hatchery we demonstrated that water quality has a direct effect on larval conch; growth more than doubled and densities were increased by a factor of 20 after incoming water was ozonated to remove dissolved contaminants (e.g. nutrients, heavy metals, and pesticides) (Glazer et al. 1997; Glazer and Delgado 2003). In addition, in a pilot study, we found that the pesticides used for mosquito eradication in the Florida Keys had a statistically significant, acutely toxicological effect on embryogenesis and early larval development. Perivitellin space was used as a proxy for embryo development (as the embryo develops and takes up more room within the egg, perivitellin space is reduced). Embryo development was delayed in the Biomist experiment and embryos did not develop at all in the Dibrom experiment (McIntyre et al 2006). These observations suggest that mosquito spraying over the Florida Keys watershed may negatively affect queen conch recruitment in nearshore waters.

As such, we tested the acute and chronic toxicity of the chemicals associated with mosquito control (i.e. naled and permethrin) in the Florida Keys on critical early life history stages of the queen conch. Specifically, we conducted bioassay experiments on embryos, autotrophic, heterotrophic, and settlement stage (i.e. competent) queen conch larvae.

Methods

Exposure Regimes

Although there have been efforts to address the issue of toxicity of contaminants using traditional static exposure regimes, considerable concern has been expressed regarding the application of laboratory-derived toxicity data to field situations in an attempt to estimate environmental consequences. It has been argued that laboratory toxicity tests can only be extrapolated to field conditions if the exposure regime used in the laboratory is comparable to that found in the field. Most early standard acute toxicity tests utilized a static exposure to test a toxicant for a specified period of time (usually 96 hours). This type of toxicity test may be appropriate to evaluate chronic, continuous sources of pollutants, but the tests are considered to be poor estimators of episodic short duration events followed by rapid dilution, which is the case for most pesticide exposures in the marine environment (Wells et al. 1984; Kucklick 1994).

Therefore, a continuous-flow exposure system developed by Singer et al. (1990 a, b; 1991) and employed successfully by Wetzel (Wetzel and Van Vleet 2001; Van Vleet and Wetzel 1996, 1997) was used. This continuous-flow test provides a tool for testing toxicity under dynamic exposure regimes, more relevant to actual field conditions. The exposure system consists of 18 independent glass chambers that hold approximately 280 ml of exposure solution in each chamber (Fig. 1). Each exposure chamber was made of an upper and lower housing held together using a silicone O-ring and stainless steel U-clamp and had three glass ports fitted with gas-tight screw caps and fitted tubing (Fig. 2). Two of the ports allowed for the introduction and discharge of the test solution, while the third port allowed the injection of food for the test animals (Fig. 2). Delivery of the control water or test solution to each chamber was controlled using a multi-head peristaltic pump that delivers solution to each chamber independently. Silicone tubing was used for delivery of all solutions. The glass test chambers were arranged for triplicate runs of different test solutions including a series of control chambers.

Each pesticide (i.e. naled and permethrin) was exposed to queen conch embryos, autotrophic, heterotrophic, and settlement stage larvae separately. Conch were exposed in triplicate to five different concentrations of each of pesticide, plus a control of Instant Ocean™ synthetic seawater (Table 1). Embryos and larvae for use in the exposure experiments were obtained from egg masses collected from wild conch located on the back-reef of the Florida Keys reef tract. The egg masses were brought to the lab [or sent to Harbor Branch Oceanographic Institute (HBOI) for subsequent larval rearing] and disinfected using a 0.5% Clorox® solution (10 ml of 5% household strength Clorox® per 2 liters of artificial seawater). Each egg mass was placed in the solution for 30 seconds, removed, and dipped in 3 beakers of clean synthetic seawater for 10 seconds per dip (Davis 1994).

Prior to use, the synthetic seawater was filtered through a 5 μm charcoal filter, a 5 μm cellulose filter, and a 1 μm cellulose filter. Temperature, salinity, oxygen concentration, and pH were monitored in the test solutions every 24 hours using an YSI 600 XL sonde. We also used a qualitative test for *Vibrio* spp., a marine pathogen critical in evaluating the health of a larval culture. Plates made from TCBS (Thiamine Citrate Bile Salt Sucrose) agar, which is specific to *Vibrio*, were inoculated at exposure times 48 hours and 96 hours for each bioassay and colony counts were conducted. These tests were conducted to ensure that any effects seen on larval growth and survival were due to the pesticides and not due to disease.

The test solutions used technical grade mosquito control pesticides and were mixed with synthetic seawater in glass carboys using large magnetic stirrers. Test concentrations were 0 ppb (control), 1.75 ppb, 3.75 ppb, 7.5 ppb, 15 ppb, and 30 ppb (Mosquito Control spray concentration). Subsequent water testing was used to confirm the exposure concentrations (see below).

Pulsed exposure experiments, 96-hrs in duration, emulated real-life exposures for conch, and consisted of a 12-hour dosing period (flow rate of 2 ml/min) for each test chamber with the experimental analytes. Test solutions were introduced into each chamber and pumped through the chambers and attached Teflon™ tubing for a short period of time to allow the hydrophobic analytes to reach adsorption equilibrium with the walls of the chambers and tubing. The conch were then added to the test chambers. After 12-hours, contaminant-free seawater was pumped

into the test chambers to create a declining exposure condition. Fresh seawater was continuously provided via peristaltic pump to the test chambers for the next 84-hours. Exposure chamber effluents from each treatment concentration were collected at times: 0 hr, 12 hr, 24 hr, 48 hr, and 96 hr and analyzed for the pesticides and their degraded products. Temperature was maintained at 28°C by placing all chambers in a temperature controlled room. A total of eight exposure tests were completed; life stage, time of exposure, type of exposure, and sub-lethal endpoints are listed in Table 1. We evaluated survival and sub-lethal endpoints using a one-way ANOVA. All statistical tests were run on SPSS 11.0 (SPSS Inc.) for Windows. Results were considered significant if $P<0.05$.

Pesticide Concentration Testing

Pesticide concentrations were analyzed by gas chromatography using dual column electron capture detectors (GC-ECD). 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^{o}C at Mote Marine Laboratory until they were extracted. 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.

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^{0}C and was held for 2.25 minutes, then ramped at a rate of 15^{0}C/min to 325^{0}C 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 naled, DDVP, and permethrin.

Results

Pesticide Concentration Testing

Temperature, salinity, oxygen concentration, and pH were stable for all test solutions for all the bioassays. The qualitative tests for *Vibrio* suggested that any potential negative effects were probably due to pesticide exposure.

Exposure concentrations targeted for the experiments ranged from 1.75 to 30 ppb for both naled and permethrin. This nominal range would include and exceed the environmentally relevant concentrations found by Pierce et al. (2004).

In the naled exposures, actual concentrations (analytically determined) at time zero ranged from 1.59 to 28.86 ppb in the embryo exposure test, from 1.55 to 33.02 ppb in the autotrophic exposure test, from 3.47 to 22.20 ppb in the heterotrophic exposure test, and from 0.03 to 26.28 ppb in the settlement exposure test (Tables 2-5). Concurrently, DDVP levels ranged from 0.04 to 6.85 ppb during the same time period. Both pesticides continued to decrease in concentration during the subsequent water sampling times until there were no detectable amounts, usually after the 24 hour sampling event (Tables 2-5).

The solubility of permethrin was much less in seawater than naled and resulted in lower actual concentration levels than targeted. These concentrations ranged from 0.12 to 1.38 ppb for the embryo exposure, from 0.62 to 5.08 ppb for the autotrophic exposure, from 0.14 to 5.26 ppb for the heterotrophic exposure, and from 0.28 to 5.25 ppb for the settlement exposure (Tables 6-9). Although these values did not reach the targeted concentrations, they fall within the highest values of permethrin found by Pierce et al. (2004), approximately 0.07 ppb in the sub-surface water and 9 ppb in the sea-surface microlayer.

Naled - Embryos

There were significant differences in embryogenesis (i.e. perivitellin space) on all four days of the naled experiment; Day 1: ($F_{(5, 174)} = 12.888$, $P < 0.001$), Day 2: ($F_{(5, 174)} = 4.484$, $P = 0.001$), Day 3: ($F_{(5, 174)} = 4.715$, $P < 0.001$), Day 4: ($F_{(5, 174)} = 4.697$, $P < 0.001$). As one would expect pervitellin space decreased over time as the developing embryos took up more space in the egg capsules (Fig. 3). However, there were some unexpected results as the embryos in the pesticide treatments seemed to be taking up more space in their egg capsules than the controls, especially by Day 3 and Day 4 (Fig. 3) potentially indicating that they were further developed than the controls. However, this was not the case. The reason that the embryos in the pesticide treatments were taking up more space in their egg capsules was that they were sloughing off cells and were deformed.

Most of the embryos hatched; however, since we noticed that some of the embryos seemed to be deformed (e.g. missing the shell, cells sloughing off), we decided to use the

percentage of normal embryos instead of hatch success as our sub-lethal endpoint. There was no statically significant difference in the percentage of normal embryos at the end of the four day bioassay ($F_{(5, 12)} = 2.460$, $P = 0.093$) (Fig. 4). However, naled seemed to affect the mucous membrane of the egg strands as they tended to fall apart in the pesticide treatments.

Naled - Autotrophic Larvae

The control showed the highest survival at over 90% with lower survival in the naled treatments (Fig. 5). However, even the lowest survival was still over 70% (Fig. 5) and so there was no statistically significant difference in survival among the treatments ($F_{(5, 12)} = 1.527$, $P = 0.253$).

The sub-lethal endpoint of growth showed a statistically significant difference among the treatments ($F_{(5, 142)} = 3.457$, $P = 0.006$) (Fig. 6). Larvae were much larger in the control and lowest naled concentration (Fig. 6). Growth was lowest in the 1.55 ppb and the 7.42 ppb treatments (Fig. 6).

Naled - Heterotrophic Larvae

At the end of the four day bioassay, the heterotrophic larvae showed no significant difference in survival among the naled treatments ($F_{(5, 12)} = 0.575$, $P = 0.718$) (Fig. 7). Although, the larval survival in the highest concentrations was a little lower than in the rest of the treatments (Fig. 7).

The sub-lethal endpoint of growth showed a statistically significant difference ($F_{(5, 156)} = 2.649$, $P = 0.025$) (Fig. 8). However, there was no clear trend as larvae in the 3.47 ppb treatment were the largest, while the larvae in the 18.27 ppb treatment were the smallest and the control only a little larger than the 18.27 ppb treatment (Fig. 8).

Naled - Settlement Stage Larvae

The queen conch larvae used in this experiment experienced very little to no mortality and there was no significant difference in survival among the treatments ($F_{(5, 12)} = 0.686$, $P = 0.643$). However, there was a significant difference in the percentage of competent larvae that settled successfully ($F_{(5, 12)} = 8.028$, $P = 0.002$) (Fig. 9). The highest concentration of naled yielded the most settlement, while the two lowest concentrations had the least settlement (Fig. 9).

Permethrin - Embryos

There were significant differences in embryogenesis (i.e. perivitellin space) on three of the four days of the permethrin experiment (Day 4 being the exception); Day 1: ($F_{(5, 174)} = 8.781$, $P < 0.001$), Day 2: ($F_{(5, 174)} = 4.101$, $P = 0.002$), Day 3: ($F_{(5, 174)} = 2.654$, $P = 0.024$), Day 4: ($F_{(5, 174)} = 1.929$, $P = 0.092$). As one would expect pervitellin space decreased over time as the developing embryos took up more space in the egg capsules (Fig. 10). As with the naled exposure, there were some unexpected results as the embryos in the permethrin treatments seemed to be taking up more space in their egg capsules than the controls, especially on Day 4 (Fig. 10) potentially indicating that they were further developed than the controls. However, this was not the case for the same reason as before…the embryos in the pesticide treatments were deformed and were sloughing off cells.

As with the naled exposure, we used the percentage of normal embryos at the end of the four day experiment as our sub-lethal endpoint because most of the embryos hatched whether they were normal or deformed. There was a significant difference in the percentage of normal embryos among the permethrin treatments ($F_{(5, 12)} = 5.174$, $P = 0.009$) (Fig. 11). Nearly 100% of the embryos in the control were normal; however, the percentage of normal embryos in the pesticide treatments ranged from as low as 5% to about 50% (Fig. 11).

Permethrin - Autotrophic Larvae

Like the naled exposure, the control in the permethrin bioassay showed the highest survival (over 80%) with lower survival in the pesticide treatments (Fig. 12). However, this was not statistically significant ($F_{(5, 12)} = 1.236$, $P = 0.351$).

Unlike the naled exposure, the sub-lethal endpoint of growth in the autotrophic permethrin bioassay showed no statistically significant difference among the treatments ($F_{(5, 128)} = 0.599$, $P = 0.701$) (Fig. 13). Larvae were all very similar in size at the end of the two day experiment (Fig. 13). Although, there was a slight trend for decreasing size with increasing pesticide concentration except for the fact that the highest permethrin concentration had the largest queen conch larvae (Fig. 13).

Permethrin - Heterotrophic Larvae

At the end of the four day bioassay, larval survival was lowest in the control and in the lowest concentration of permethrin (Fig. 14). However, this slight difference in survival was not statistically significant ($F_{(5, 12)} = 2.291$, $P = 0.111$) (Fig. 14).

The sub-lethal endpoint of growth showed a statistically significant difference ($F_{(5, 147)} = 2.706$, $P = 0.023$) (Fig. 15). Unlike the naled experiment, there was a clear trend in this bioassay. The larvae in the highest permethrin concentrations showed the least amount of growth (Fig. 15).

Permethrin - Settlement Stage Larvae

The queen conch larvae used in this experiment experienced very little to no mortality and there was no significant difference in survival among the treatments ($F_{(5, 12)} = 0.800$, $P = 0.571$). However, there was a significant difference in the percentage of competent larvae that settled successfully ($F_{(5, 12)} = 5.738$, $P = 0.006$) (Fig. 16). Like the naled experiment, the highest concentrations of permethrin yielded the most settlement, while the 0.84 ppb concentration had the least settlement (Fig. 16). Curiously, the control had the second lowest settlement at just over 40% (Fig. 16).

Conclusions

The Monroe County Mosquito Control District uses naled and permethrin mosquito adulticides in the Florida Keys. Naled is a fast acting, short residual organophosphate insecticide; however, its primary degradation product, DDVP is more toxic and more persistent than naled. Organophosphate pesticides are used extensively in coastal areas for control of salt marsh mosquitoes (Pierce et al. 1996) and are known for their environmental persistence and global occurrence. These pesticides are highly toxic to aquatic invertebrates acting as a nerve poison

interfering with acetyl cholinesterase activity (Pierce et al. 2000) and as such could cause major complications for coastal marine invertebrate communities. Permethrin is a neurotoxin that acts as a sodium channel blocker; aquatic ecosystems are particularly vulnerable to the impact of permethrin (J. of Pesticide Reform 2002).

Naled Exposures

The naled embryo experiment showed very little mortality under the 12 hour exposure regime and our perivitellin space measurements suggested that the embryos exposed to the pesticides developed just as well as the controls (Fig. 3). However, this was not the case. The mucous membrane of the egg strands tended to fall apart in the pesticide treatments and many of the embryos in the pesticide treatments were deformed (although this was not statistically significant). Even though these deformed embryos were alive and hatched, they could not be considered viable and in the wild would probably die very quickly as a consequence of the exposure or due to predation.

The naled exposures for autotrophic and heterotrophic larvae also had very little mortality; nevertheless, there was a non-significant trend for decreased survival with increasing naled concentration (Figs. 5 and 7). Although, there was no acute effect, there seemed to be a potential long-term effect of naled exposure on autotrophic and heterotrophic queen conch larvae. Some of the pesticide concentrations had statistically smaller queen conch larvae (Figs. 6 and 8). In general, larvae exposed to the higher pesticide concentrations showed slower growth (i.e. they were smaller in size). The low mortality was probably due to the low exposure times; however, that short-term pesticide exposure may have long-term consequences on survival. Larvae that take longer to reach competency (i.e. recruitment size) will remain in the water column longer and thus have a higher chance of being predated upon.

Settlement stage larvae also had very little mortality; however, a higher percentage of larvae successfully underwent metamorphosis (i.e. recruitment) in the higher pesticide concentrations (Fig. 9). This unexpected result can be explained by the fact that the pesticide acted as a false metamorphic cue, forcing the larvae to undergo metamorphosis. The mode of action in naled targets the nervous system.

All of the targeted ranges (i.e. concentrations) for the naled exposures were reached (Tables 2-5). We used Mosquito Control's target spray concentration with subsequent 50% dilutions to mimic as best as we could the exposure regime the embryos and larvae would experience. Aerial application of pesticides may impact non-targeted locations; a study of naled/DDVP was conducted in a mangrove community and residential water collection systems on Grand Cayman Island following applications over residential areas as well as coastal mangrove forests (Pierce and Henry 1988). Although no naled or DDVP was detected in the mangrove community following application, residues of both were recovered from cistern water up to 16 hours after application (Pierce and Henry, 1988). Similarly, a study to monitor the distribution and persistence of mosquito adulticides in the Florida Keys National Marine Sanctuary showed aerial drift of naled into the leeward side of the application area, with some tidal transport out of canals into the adjacent waters of the sanctuary (Pierce 1998). Naled was applied as ultra low volume technical product from aircraft to reach a broad area of mosquito infestation. Concentrations of naled in the water column were below toxicity threshold levels,

however, the highly toxic degradation product, DDVP was found in five out of ten water column samples collected between 2 and 4 hours after application. The concentrations ranged from <0.2 ppb to 0.56 ppb, exceeding the acute toxicity threshold (no observable effects level), indicating a potential ecological hazard for some aquatic invertebrates (Pierce 1998). The no effects level for DDVP cholinesterase inhibition in larvae of the lobster, *Homarus gammarus*, was reported to be 0.16 ppb (McHenery et al. 1996) and the LC_{50} for the sand shrimp and also the sea urchin was 4.0 ppb (EXTOXNET 2004). Our DDVP concentrations were much higher than that in the highest naled treatments (Tables 2-5).

Permethrin Exposures

The permethrin embryo experiment showed very little mortality under the 12 hour exposure regime and our perivitellin space measurements suggested that the embryos exposed to the pesticides developed just as well as the controls (Fig. 10). However, as in the naled experiment, this was not the case. There were a statistically significant lower percentage of normal embryos in the permethrin treatments (Fig. 11). Even though the deformed embryos were alive and hatched, they could not be considered viable and in the wild would probably die very quickly as a consequence of the exposure or due to predation.

The permethrin exposures for autotrophic and heterotrophic larvae also had very little mortality (not significant among treatments) (Figs. 12 and 14). Although, there was no acute effect, there seemed to be a potential long-term effect of permethrin exposure on autotrophic and heterotrophic queen conch larvae. Autotrophic larvae were all very similar in size at the end of the two day experiment; however, there was a slight trend for decreasing size with increasing pesticide concentration except for the fact that the highest permethrin concentration had the largest queen conch larvae (Fig. 13). Heterotrophic larvae showed a statistically significant trend with the larvae in the highest permethrin concentrations experiencing the least amount of growth (Fig. 15). The low mortality was probably due to the low exposure time of only 12 hours; however, that short-term pesticide exposure may have long-term consequences on survival. Larvae that take longer to reach competency (i.e. recruitment size) will remain in the water column longer and thus have a higher chance of being predated upon.

Settlement stage larvae also had very little mortality, and as in the naled exposure, a higher percentage of larvae successfully underwent metamorphosis (i.e. recruitment) in the higher permethrin concentrations (Fig. 16). This may be explained by the fact that the pesticide targets the nervous system and molluscan larvae are known to undergo metamorphosis in response to neurologically active compounds.

The solubility of permethrin was much less in seawater than naled and resulted in lower actual concentration levels than targeted (Tables 6-9). Although we did not reach the targeted concentrations, the actual values fall within the highest values of permethrin found by Pierce et al. (2004), approximately 0.07 ppb in the sub-surface water and 9 ppb in the sea-surface microlayer; therefore, our results are still ecologically relevant. To illustrate the toxic nature of permethrin and for comparative purposes, the LC_{50} and EC_{50} of lobster and oyster larvae to permethrin are <1.0 ppb and <1.0 ppm, respectively (McLeese et al. 1980; Schimmel et al. 1984).

Our findings 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 by reducing recruitment. This has consequences for FWC's queen conch restoration program. FWC has been translocating nearshore conch into offshore spawning aggregations in an effort to boost larval production and recruitment (Delgado et al. 2004). If increased recruitment is the ultimate goal of the queen conch restoration program, larvae must survive and be retained within the Florida Keys. Mechanisms for larval retention in the Florida Keys have been described by Lee and Williams (1999). If larvae are retained within the Florida Keys system, any increase in local larval production will increase larval supply and may increase recruitment. However, it is unknown what effects aerial drift of mosquito control pesticides may be having on nearshore larval recruitment. Further studies on the long-term, chronic effects of pesticide exposure on queen conch embryos and larvae are warranted; for example: 1) how long can newly hatched larvae survive after pesticide exposure and 2) can larvae continue to grow normally, reach competency, and successfully recruit after pesticide exposure. These studies might also include determining the effects of multiple mosquito control pesticides acting synergistically on several life stages.

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Table 1. Exposure tests on the different larval stages of queen conch: All exposure tests consisted of five concentrations and one control (a total of six treatments). Each treatment had three replicates. Ten larvae were randomly placed into each replicate. Because of the handling associated with measuring perivitellin space for the embryos, fifty embryos were randomly placed into each replicate (ten for each day). The first 40 embryos were used to test the effects of the pesticide on embryogenesis (i.e. perivitellin space) over time; the last ten embryos were used as a measure of hatch success. Pervitellin space (i.e. area) was measured using Image Pro Plus imaging software. Autotrophic larvae were exposed for only 48 hours because they become heterotrophic during the next 24 hours. Heterotrophic larvae were fed algae during the exposures. Larval metamorphosis exposures were static tests performed in glass dishes and were only 12 hours in duration as metamorphosis is the shortest stage in the larval cycle.

Naled Exposures	**Exposure Regime**	**Exposure Time**	**Sub-lethal Endpoints**
1a.embryo	Spiked constant flow	96 hrs	Embryogenesis
1b.embryo	Spiked constant flow	96 hrs	Hatch success
2.autotrophic larvae	Spiked constant flow	48 hrs	Growth
3.heterotrophic larvae	Spiked constant flow	96 hrs	Growth
4.larval metamorphosis	Static	12 hrs	Metamorphic Success

Permethrin Exposures	**Exposure Regime**	**Exposure Time**	**Sub-lethal Endpoints**
1a.embryo	Spiked constant flow	96 hrs	Embryogenesis
1b.embryo	Spiked constant flow	96 hrs	Hatch success
2.autotrophic larvae	Spiked constant flow	48 hrs	Growth
3.heterotrophic larvae	Spiked constant flow	96 hrs	Growth
4.larval metamorphosis	Static	12 hrs	Metamorphic Success

Table 2. Naled embryo exposure actual concentrations (ppb).

Nominal Concentrations	T=0hrs naled	T=0hrs DDVP	T=12hrs naled	T=12hrs DDVP	T=24hrs naled	T=24hrs DDVP	T=48hrs naled	T=48hrs DDVP	T=96hrs naled	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
1.75	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 3. Naled autotrophic exposure actual concentrations (ppb).

Nominal Concentrations	T=0hrs naled	T=0hrs DDVP	T=12hrs naled	T=12hrs DDVP	T=24hrs naled	T=24hrs DDVP	T=48hrs naled	T=48hrs DDVP	T=96hrs naled	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
1.75	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

Table 4. Naled heterotrophic exposure actual concentrations (ppb).

Nominal Concentrations	T=0hrs naled	T=0hrs DDVP	T=12hrs naled	T=12hrs DDVP	T=24hrs naled	T=24hrs DDVP	T=48hrs naled	T=48hrs DDVP	T=96hrs naled	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
1.75	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 5. Naled settlement exposure actual concentrations (ppb).

Nominal Concentrations	T=0 hrs naled	T=0 hrs DDVP	T=12 hrs naled	T=12 hrs DDVP
Control	0.00	0.00	0.00	0.00
1.75	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. Permethrin embryo exposure actual concentrations (ppb).

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
1.75	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 7. Permethrin autotrophic exposure actual concentrations (ppb).

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
1.75	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 8. Permethrin heterotrophic exposure actual concentrations (ppb).

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
1.75	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 9. Permethrin settlement exposure actual concentrations (ppb).

Nominal Concentrations	**T=0 hrs**	**T=12 hrs**
Control	0.00	0.00
1.75	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

Figure 1. Exposure system schematic exhibiting flow patterns and main system components: a) cartridge filters; b) source water head tank; c) peristaltic delivery pump; d) eighteen cartridge pump heads; e) exposure chambers; f) waste ports; and g) waste water collection flasks (Singer et al. 1991).

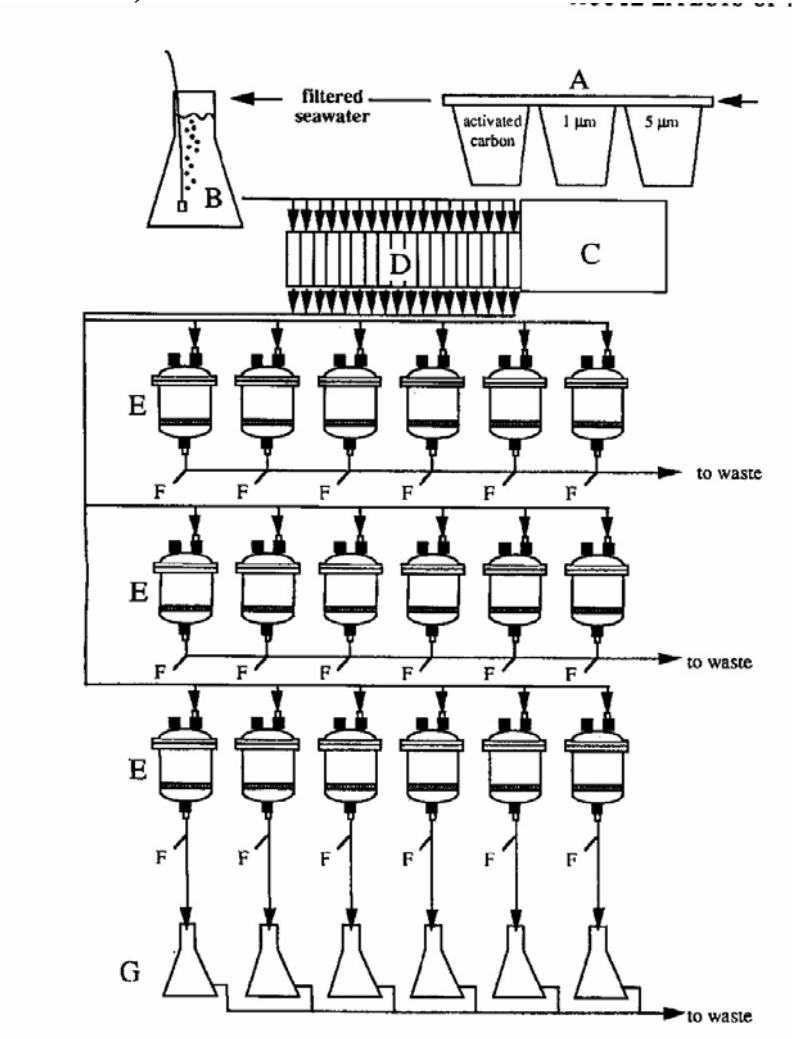

Figure 2. Schematic of the toxicity test exposure chamber: a) chemistry sampling arm; b) syringe for food introduction; c) diluent inlet; d) threaded glass fittings with phenolic caps; e) silicone O-ring sealed glass flange with clamp; f) fritted glass disk; and g) discharge outlet (Singer et al. 1991).

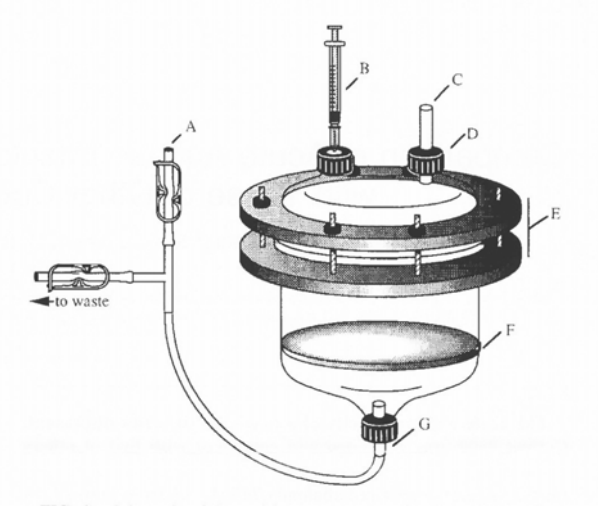

Figure 3. Graph of perivitellin space measurements (± one standard error) for each of the six treatments during the four days of the naled bioassay. Concentrations (ppb) are the actual naled concentrations that the embryos were initially exposed to.

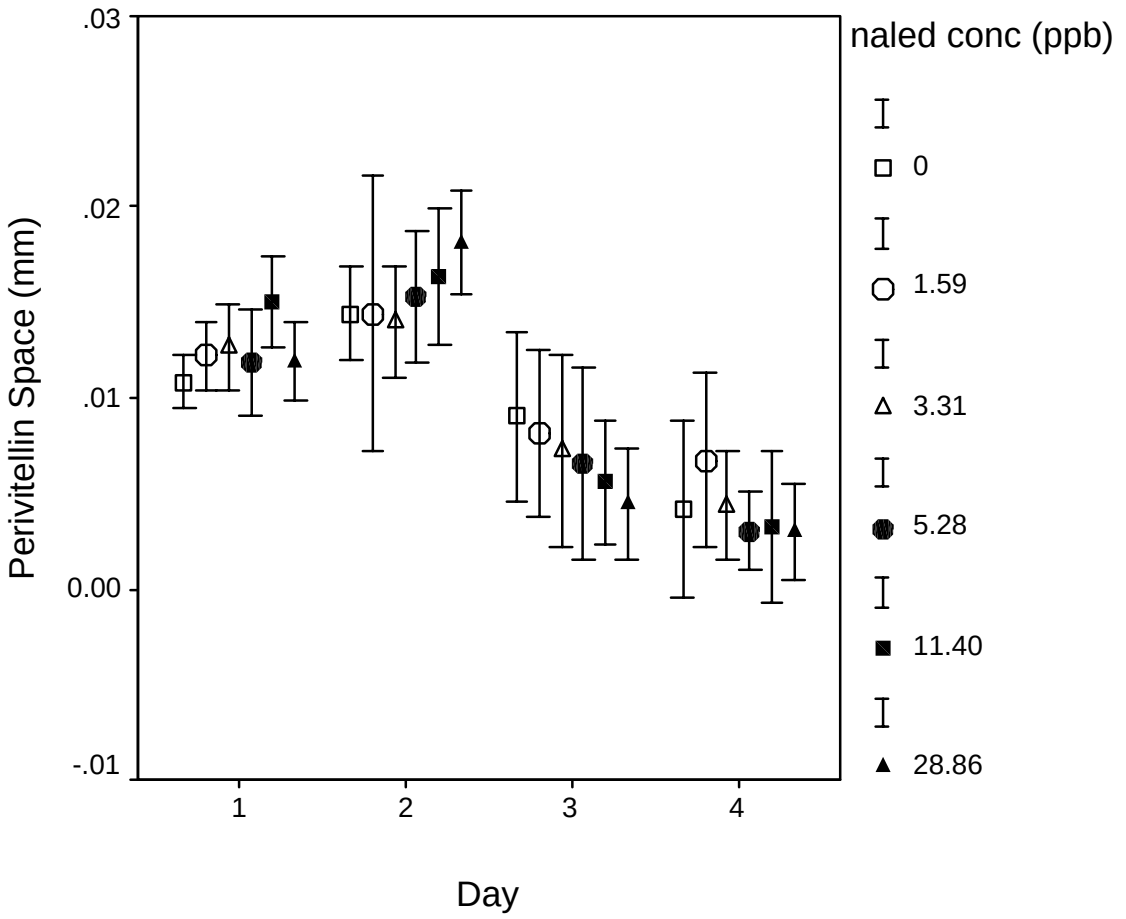

Figure 4. The percentage of normal embryos (± one standard error) at the completion of the four day (96 hour) bioassay versus the actual naled concentrations (ppb) that they were initially exposed to.

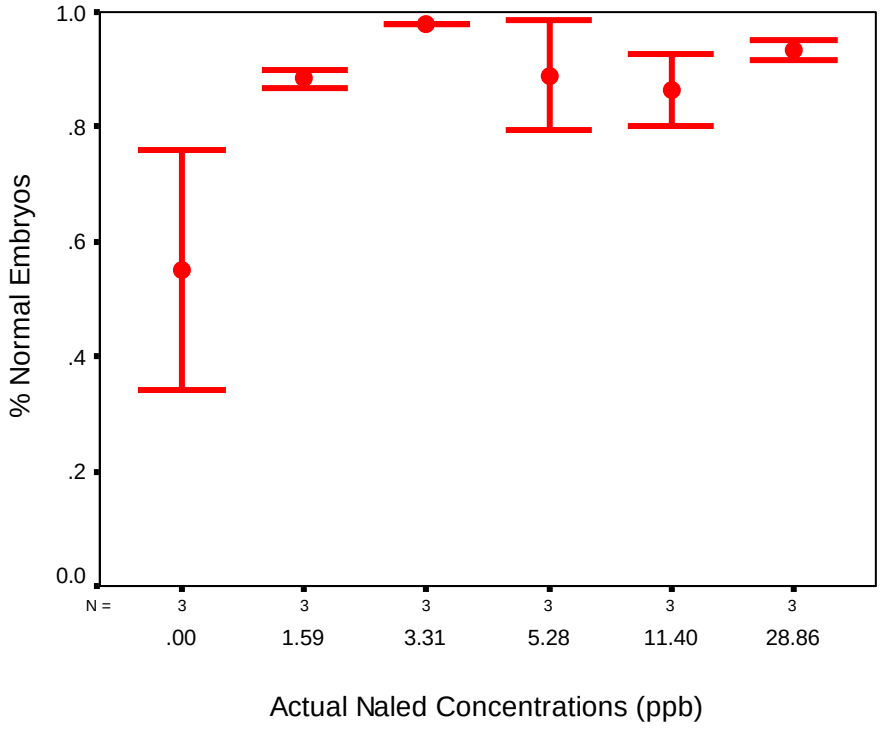

Figure 5. Percent survival of autotrophic larvae (± one standard error) at the completion of the two day (48 hour) bioassay versus the actual naled concentrations (ppb) that they were initially exposed to.

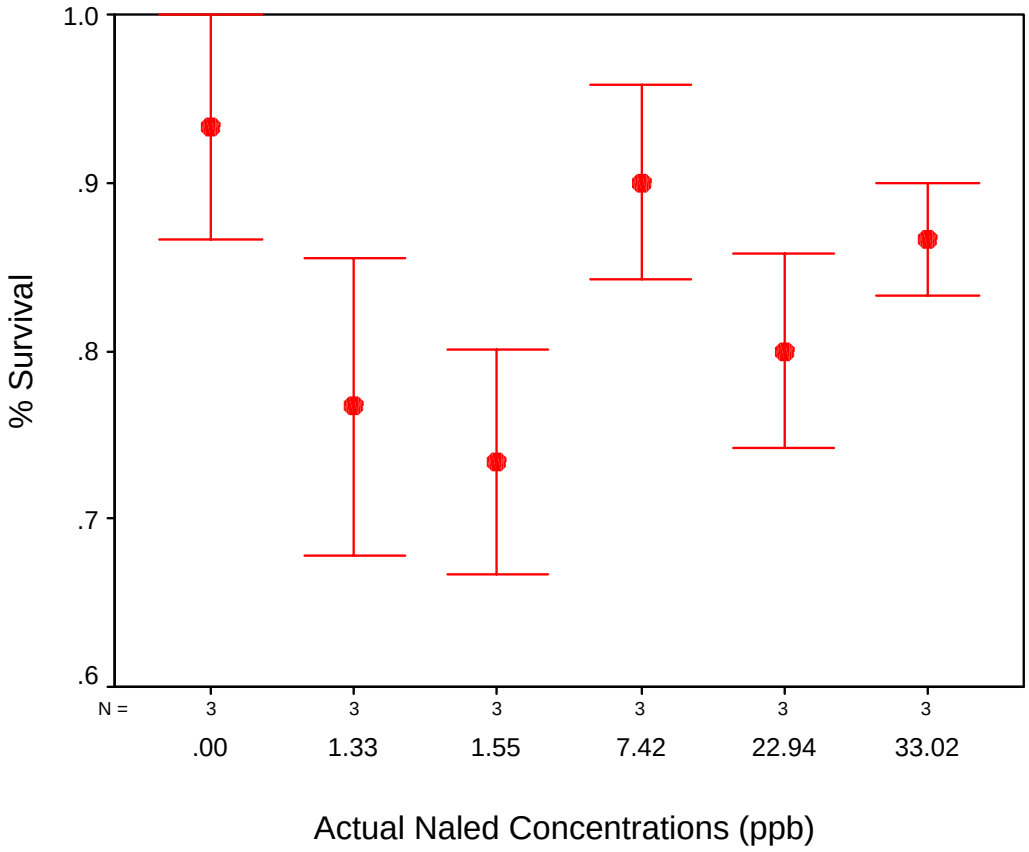

Figure 6. Mean length (mm) of autotrophic larvae (± one standard error) at the completion of the two day (48 hour) bioassay versus the actual naled concentrations (ppb) that they were initially exposed to.

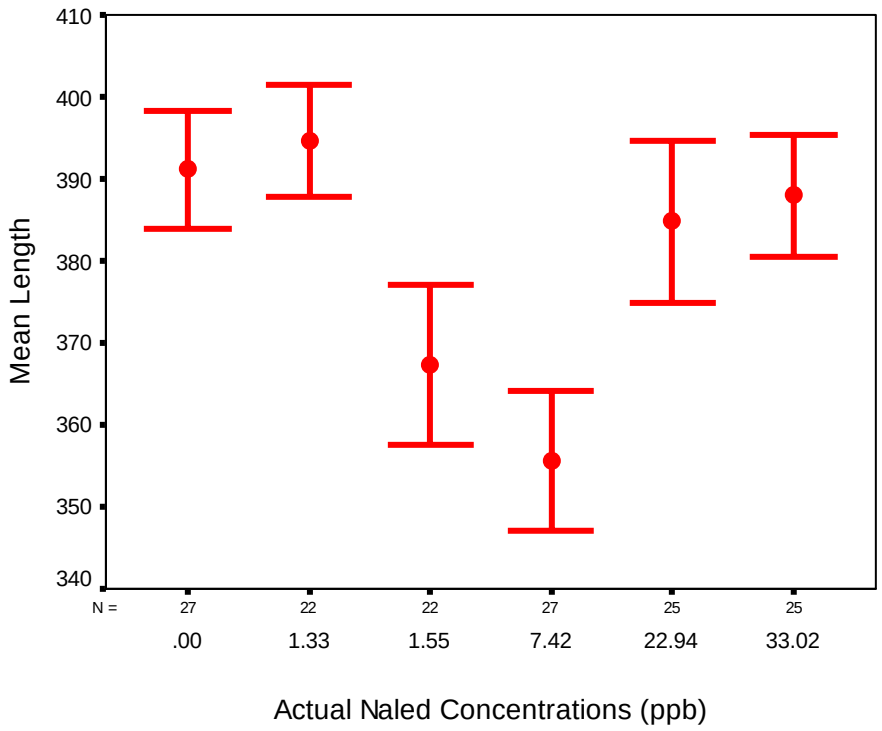

Figure 7. Percent survival of heterotrophic larvae (± one standard error) at the completion of the four day (96 hour) bioassay versus the actual naled concentrations (ppb) that they were initially exposed to.

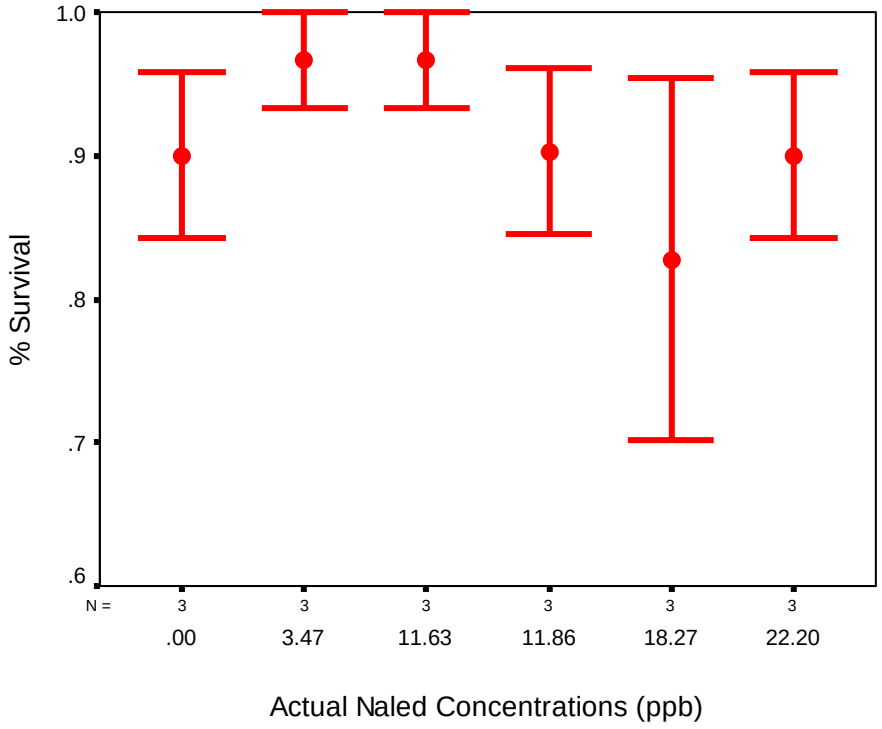

Figure 8. Mean length of heterotrophic larvae (± one standard error) at the completion of the four day (96 hour) bioassay versus the actual naled concentrations (ppb) that they were initially exposed to.

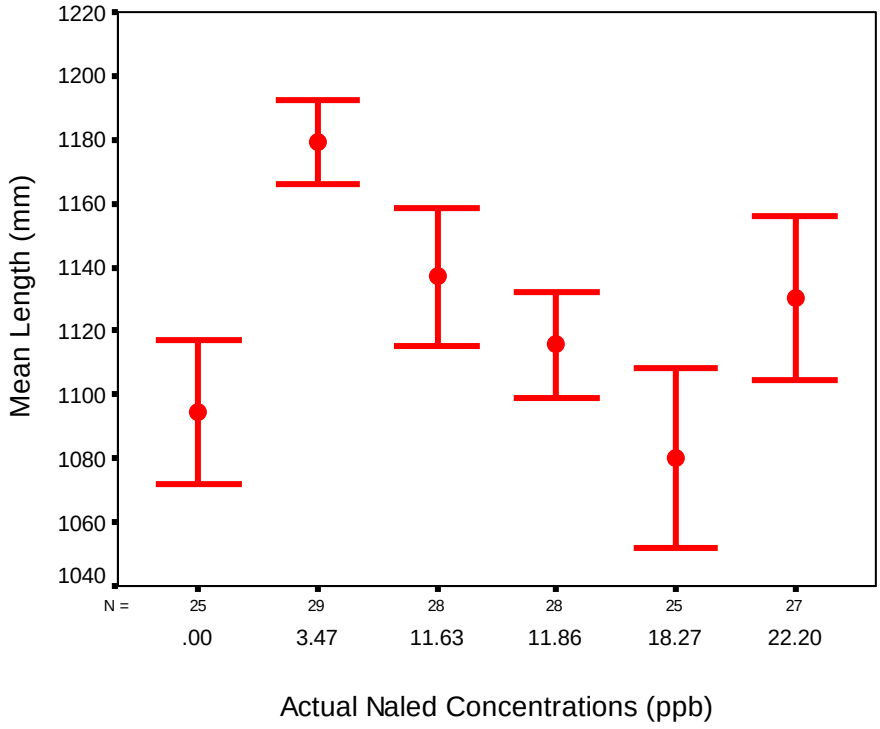

Figure 9. The percentage of competent larvae that settled successfully (± one standard error) versus the actual naled concentrations (ppb) that they were initially exposed to.

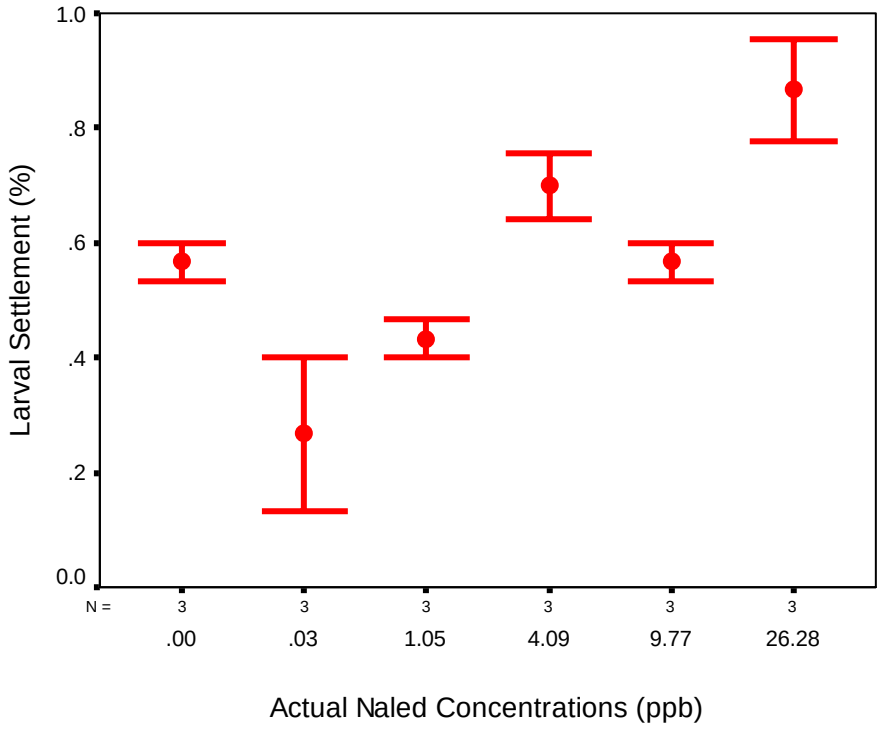

Figure 10. Graph of perivitellin space measurements (± one standard error) for each of the six treatments during the four days of the permethrin bioassay. Concentrations (ppb) are the actual permethrin concentrations that the embryos were initially exposed to.

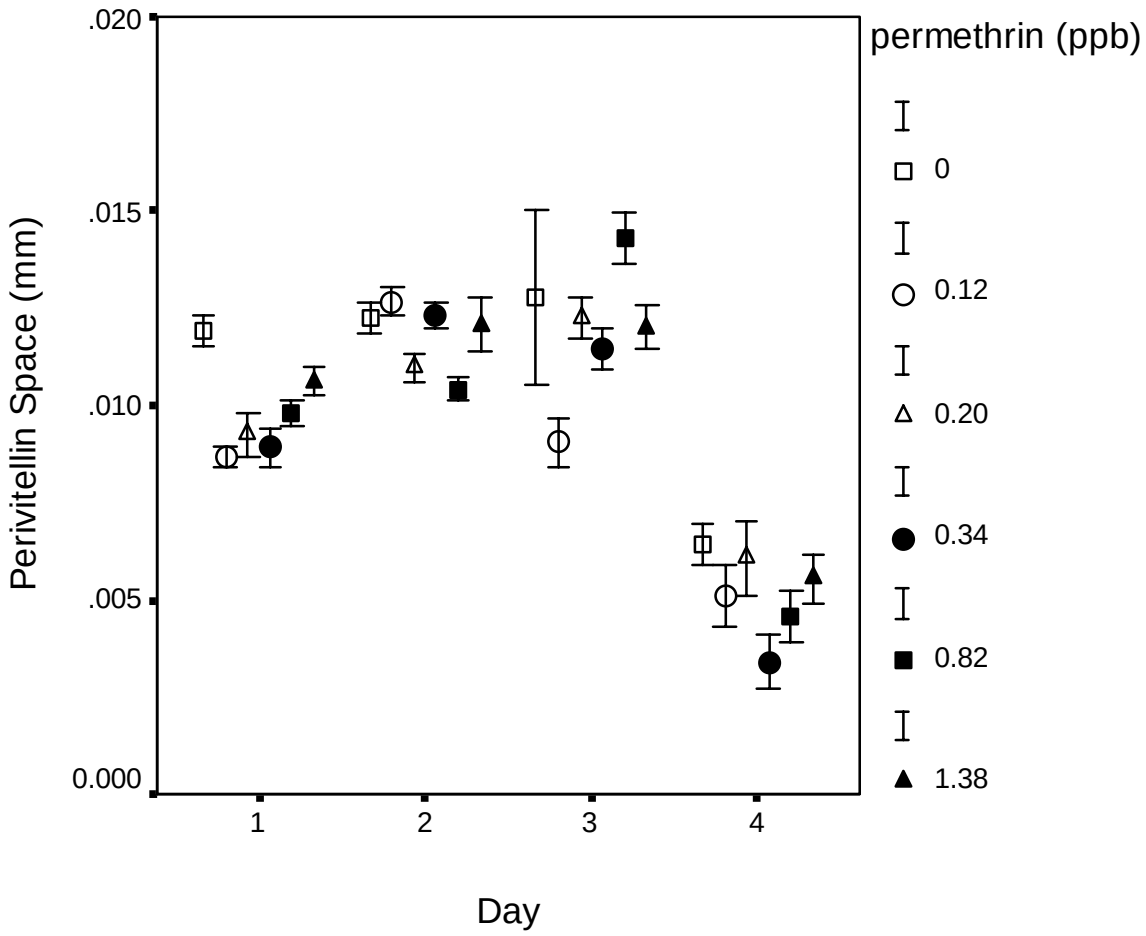

Figure 11. The percentage of normal embryos (± one standard error) at the completion of the four day (96 hour) bioassay versus the actual permethrin concentrations (ppb) that they were initially exposed to.

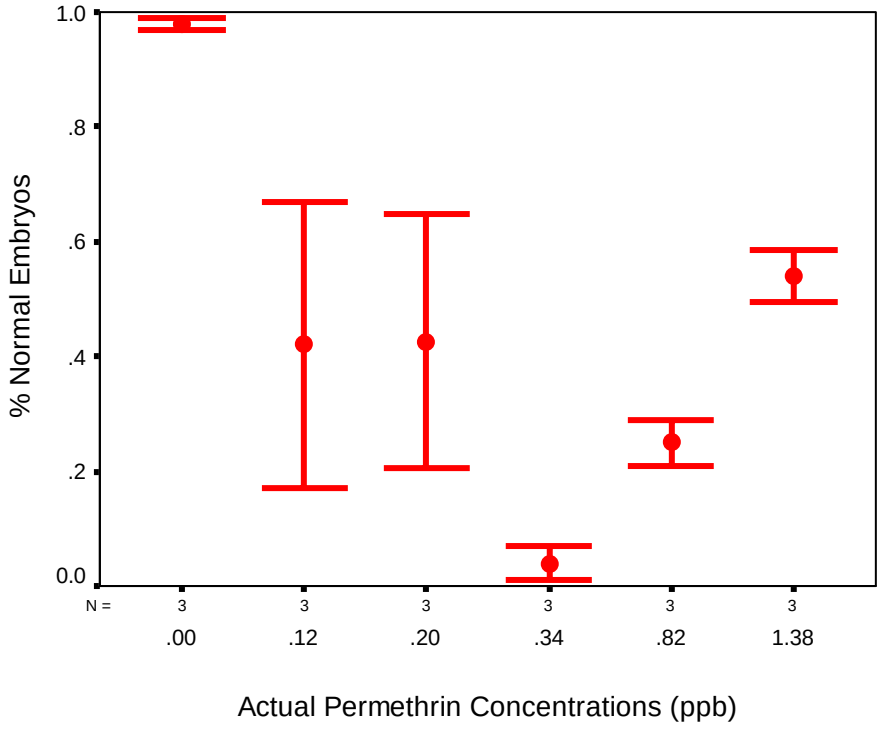

Figure 12. Percent survival of autotrophic larvae (± one standard error) at the completion of the two day (48 hour) bioassay versus the actual permethrin concentrations (ppb) that they were initially exposed to.

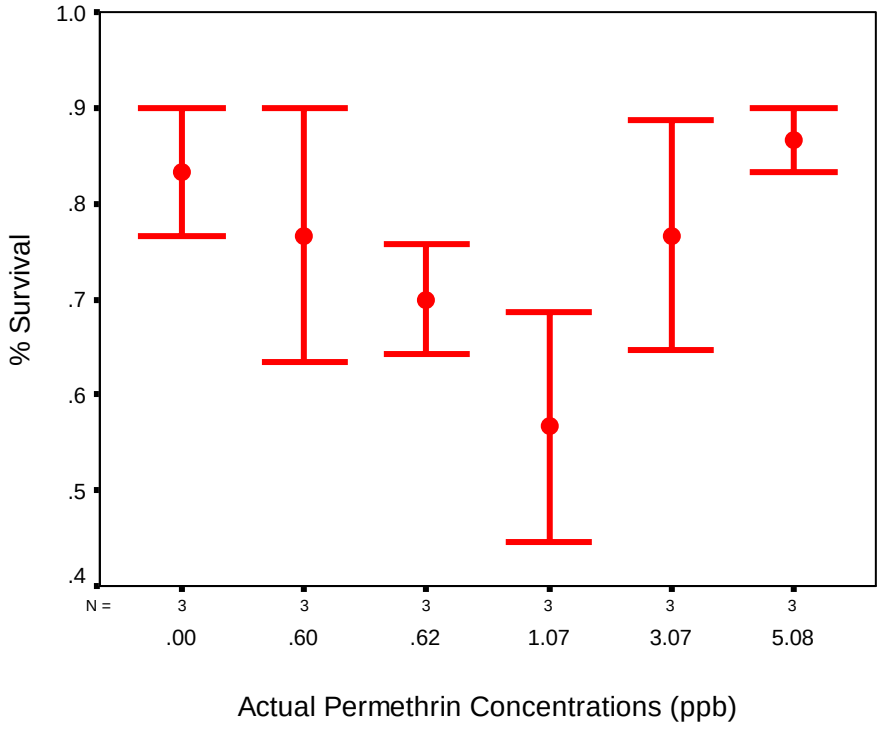

Figure 13. Mean length of autotrophic larvae (± one standard error) at the completion of the two day (48 hour) bioassay versus the actual permethrin concentrations (ppb) that they were initially exposed to.

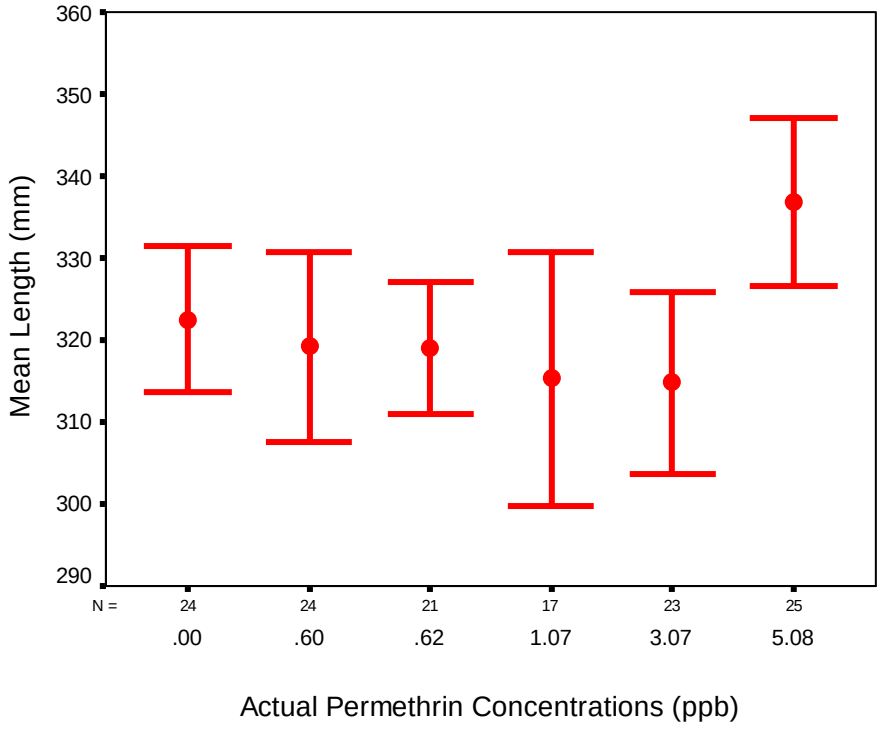

Figure 14. Percent survival of heterotrophic larvae (± one standard error) at the completion of the four day (96 hour) bioassay versus the actual permethrin concentrations (ppb) that they were initially exposed to.

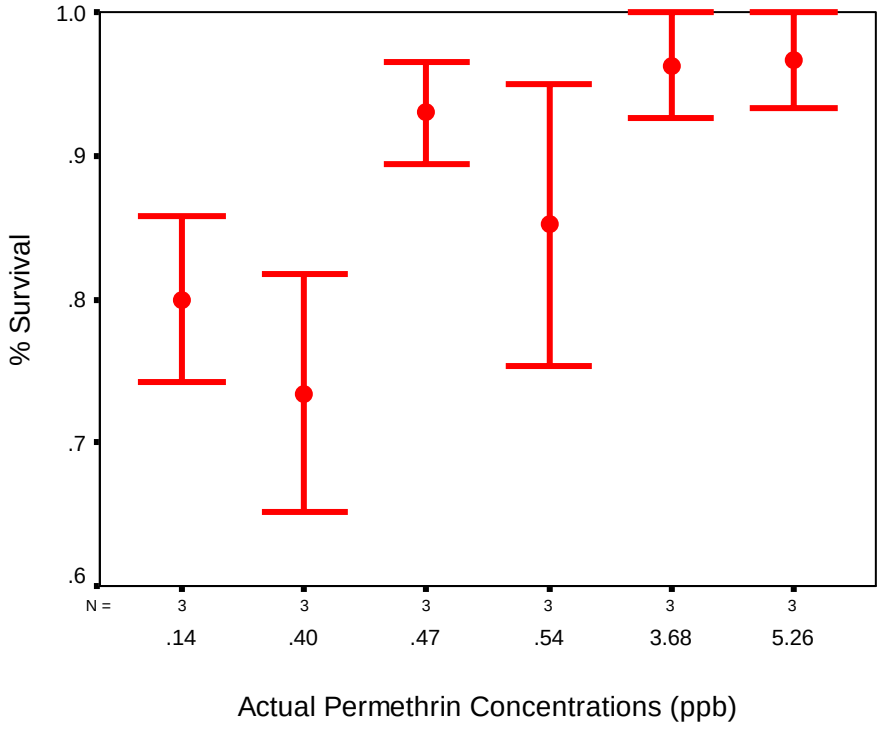

Figure 15. Mean length of heterotrophic larvae (± one standard error) at the completion of the four day (96 hour) bioassay versus the actual permethrin concentrations (ppb) that they were initially exposed to.

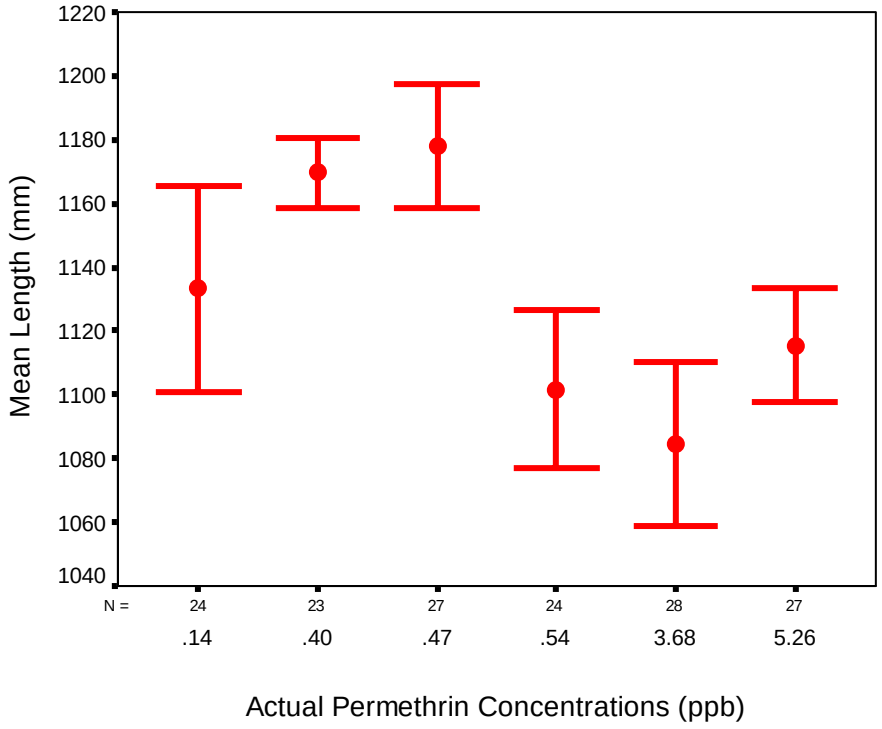

Figure 16. The percentage of competent larvae that settled successfully (± one standard error) versus the actual permethrin concentrations (ppb) that they were initially exposed to.