

Provided for non-commercial research and education use.  
Not for reproduction, distribution or commercial use.



(This is a sample cover image for this issue. The actual cover is not yet available at this time.)

This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at SciVerse ScienceDirect

## Journal of Experimental Marine Biology and Ecology

journal homepage: [www.elsevier.com/locate/jembe](http://www.elsevier.com/locate/jembe)

## Acute toxicity and sub-lethal effects of common pesticides in post-larval and juvenile blue crabs, *Callinectes sapidus*

Joshua S. Osterberg<sup>1</sup>, Kelly M. Darnell<sup>2</sup>, T. Michelle Blickley<sup>3</sup>, Jocelyn A. Romano<sup>4</sup>, Dan Rittschof\*

Duke University Marine Laboratory, Nicholas School of the Environment, Duke University, 135 Duke Marine Lab Rd, Beaufort, NC 28516, USA

### ARTICLE INFO

#### Article history:

Received 15 January 2012  
Received in revised form 3 May 2012  
Accepted 7 May 2012  
Available online xxxx

#### Keywords:

Acephate  
Aldicarb  
*Callinectes sapidus*  
Imidacloprid  
Lambda-cyhalothrin  
Roundup

### ABSTRACT

The coastal plains of the southeastern United States support extensive agricultural operations that apply pesticides and herbicides. The shallow tidal creeks and ditches that directly drain agricultural fields are home to the young of the ecologically and economically important blue crab, *Callinectes sapidus* (Rathbun, 1896). Massive mortality observed by peeler crab fishers led us to investigate the acute toxicity of several commercial pesticide formulations and their active ingredients to blue crab megalopae and J1–J4 stage juveniles. Twenty-four hour acute toxicity assays were conducted with the organophosphate acephate (Orthene®), the carbamate aldicarb, the chloro-nicotinyl imidacloprid (Trimax™), the pyrethroid lambda-cyhalothrin (Karate® with Zeon Technology), and the glyphosate-based herbicide Roundup® Pro. LC<sub>50</sub> values ranged from 0.22 µg/L for megalopae exposed to lambda-cyhalothrin to 316,000 µg/L for juveniles exposed to Roundup. The acute toxicities of active ingredient insecticides to blue crabs followed the order: lambda-cyhalothrin > imidacloprid ≈ aldicarb > acephate. Megalopae were almost always more sensitive than juveniles and there was little difference between the LC<sub>50</sub>s of each commercial formulation and its active ingredient. Treatment of intermolt megalopae with LC<sub>20</sub> levels of Roundup resulted in significantly reduced time to metamorphosis (TTM) compared to estuarine water controls, while no differences resulted from treatment with the four active ingredient insecticides. Treatment with acephate, aldicarb, imidacloprid, and Roundup significantly increased the frequency of juveniles that died within 6 h of molting. The sensitivity of molting blue crabs to these pesticides makes frequently molting juveniles particularly vulnerable to pesticides in estuaries.

© 2012 Elsevier B.V. All rights reserved.

### 1. Introduction

The southeastern United States consists of extensive coastal plains drained by creeks, streams, and rivers that flow into estuaries and sounds. The land supports widespread agriculture, particularly cotton, corn, soybeans, and tobacco that forms the base of much of the local economy and is associated with abundant pesticide and herbicide application. The water bodies that drain this region make up the center of the U.S. blue crab fishery, which is often one of the

most economically important fisheries in the southeastern U.S. Herein we report the acute toxicities and sub-lethal effects of common pesticides in various blue crab life stages. We focused our study on the effects of pesticides commonly used around the North Carolina blue crab fishery.

The blue crab fishery is the most profitable and highest landing fishery in North Carolina with over 29.7 million pounds of hard blue crabs harvested in 2010, worth over \$23.8 million (NCDMF, 2011). This fishery is centered on the Albemarle and Pamlico Sounds, which are bordered by counties that host thousands of acres of cropland and a valuable agriculture industry. Over 225,000 acres of soybeans, corn, and cotton were harvested from Hyde, Beaufort, and Pamlico counties (NC) in 2010, contributing over \$161 million to the economy (NCDA&CS, 2011). Brackish tidal creeks that drain these agricultural fields are important habitats for juvenile and adult stage blue crabs (Posey et al., 2005; Ramach et al., 2009) and their prey and are frequently less than 10 cm deep and 2 m wide. With two of North Carolina's most important economic engines in close proximity, it is essential to understand the interaction and subsequent effects of pesticides on blue crabs.

After mating in spring and summer, gravid female blue crabs migrate downstream and out of the estuary to release larvae. Dispersed

\* Corresponding author. Tel.: +1 252 504 7634; fax: +1 252 504 7648.

E-mail addresses: [jso6@duke.edu](mailto:jso6@duke.edu) (J.S. Osterberg), [kellymdarnell@utexas.edu](mailto:kellymdarnell@utexas.edu) (K.M. Darnell), [twyla.blickley@nist.gov](mailto:twyla.blickley@nist.gov) (T.M. Blickley), [jaromano@ncsu.edu](mailto:jaromano@ncsu.edu) (J.A. Romano), [ritt@duke.edu](mailto:ritt@duke.edu) (D. Rittschof).

<sup>1</sup> Present address: Nicholas School of the Environment, Duke University, Box 90328, Durham, NC 27708, USA.

<sup>2</sup> Present address: Marine Science Institute, The University of Texas at Austin, 750 Channel View Dr., Port Aransas, TX 78373, USA.

<sup>3</sup> Present address: National Institute of Standards and Technology, 100 Bureau Dr., Gaithersburg, MD 20899, USA.

<sup>4</sup> Present address: Dept. of Population Health and Pathobiology, North Carolina State University College of Veterinary Medicine, 1060 William Moore Drive, Raleigh, NC 27607, USA.

zoea larvae then travel offshore to develop before returning onshore as settlement stage (post-larval) megalopae in late summer and fall. Megalopae use flood tide transport to migrate upstream (Welch et al., 1997) where they settle on submerged vegetation and metamorphose to the first juvenile stage. Secondary dispersal away from initial settlement sites to their broader estuarine habitat occurs in early juvenile stages (between J1 and J4; Reynolds and Eggleston, 2004).

The inspiration for this study was the precipitous die off of peeler crabs (juveniles ready to molt to adults) associated with spring rains as well as some permanent soft crabs in mid-summer observed by a Hyde County, NC shedder (peeler crab fisher). These events coincided with the approximate times of nearby aerial insecticide and/or herbicide applications to cotton. No specific causal agents were determined for that incident. The mortality suggests a potent toxicant while the shell hardening issues suggest some sort of endocrine disruption. We chose to address the mortality issue in this study and have focused this study on the acute toxicity of chemicals used in cotton agriculture. Blue crab juveniles are the most likely age group to be directly impacted by agricultural runoff and so are tested here. Blue crab megalopae are less likely to inhabit drainage ditches but are easily collected and maintained in the lab in large numbers and so make good test subjects. Blue crab peelers are too delicate and adults too large for these tests to be easily performed.

Cotton is usually planted in April and May and is often treated at planting with the carbamate aldicarb (2-methyl-2-(methylthio)propionaldehyde O-methylcarbamoyloxime) or the chloro-nicotinyl imidacloprid (*N*-[1-[(6-chloro-3-pyridyl)methyl]-4,5-dihydroimidazol-2-yl]nitramide) to prevent thrip infestations (J. Bachele personal communication; Bachele et al., 2005). Aldicarb is a moderately water soluble cholinesterase inhibitor that was applied to 35% of the North Carolina cotton acreage in 2007 at a rate of 0.756 lbs/A for a total of 132,000 lbs (USDA, 2008). Imidacloprid is a moderately water soluble acetylcholine receptor agonist (Gupta et al., 2002; Matsuda et al., 2001) that has been reported in water bodies at concentrations as high as 9 µg/L (DeLorenzo et al., 2012).

In the summer, cotton leaves are often sprayed with a pyrethroid insecticide such as lambda-cyhalothrin (λ-cyhalothrin; (RS)-alpha-cyano-3-phenoxybenzyl 3-(2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethylcyclopropanecarboxylate) or an organophosphate such as acephate (O,S-dimethyl acetylphosphoramidothioate) to prevent thrips, aphids, loopers, and lygus (Bachele et al., 2005). Lambda-cyhalothrin is not water soluble and acts by binding to and opening sodium channels on neuron axons, effectively paralyzing subjects (He et al., 2008). A total of 4000 lbs was applied to 17% of the NC cotton acreage in 2007 at a rate averaging 0.028 lbs/A with an additional 3000 lbs applied to soybean fields (USDA, 2008). Acephate is a highly water soluble insecticide that inhibits the breakdown of acetylcholine (Eto, 1974). A total of 122,000 lbs of acephate was used on North Carolina cotton fields in 2007 at a rate of 0.481 lbs/A (USDA, 2008).

The genetic modification of several crops including cotton, corn, and soybeans with glyphosate resistant genes has greatly changed which herbicides are used on crops with a trend towards glyphosate-based products. So called Roundup Ready® (Monsanto Company) cotton has been available since 1997 and nearly all North Carolina cotton is now of this variety. A total of 951,000 lbs of glyphosate-based products (including glyphosate-isopropylamine salt, the form used in Roundup) were applied to at least 86% of NC cotton fields in 2007 at a rate averaging 0.733 lbs/A per application (USDA, 2008). Glyphosate is a highly water soluble phosphonoglycine herbicide that inhibits an enzyme required to produce the amino acids tryptophan, phenylalanine, and tyrosine (Amrhein et al., 1980). It is often found in commercial products (Roundup) with a polyethoxylated tallow amine (POEA) surfactant that has proven more toxic than the active ingredient itself (Giesy et al., 2000).

Twenty-four hour acute toxicity experiments were performed on megalopae and juvenile blue crabs, *Callinectes sapidus*, with both commercial formulation pesticides and their active ingredients (Table 1). The effects of active ingredient compounds and commercial Roundup on the metamorphosis of megalopae to the first juvenile stage were also investigated.

## 2. Methods

### 2.1. Blue crab collection

Blue crab (*C. sapidus* Rathbun) megalopae were collected from the water column as they migrated inshore during summer 2008 nighttime rising tides. Two 0.75 m diameter plankton nets were deployed for 1 h straddling the maximum flood tide current from the NOAA sampling platform located beneath the Pivers Island Bridge, Beaufort, North Carolina, USA. Plankton samples were coarsely sieved to remove ctenophores and visually identified blue crab megalopae were sorted out. Megalopae were maintained in filtered aged estuarine seawater (ASW; salinity 35, 25 °C) on an ambient light:dark cycle and reared in 6 in. diameter glass finger bowls. Each finger bowl contained 100–200 individuals in ~800 mL of ASW. Filtered and aged estuarine seawater was used to mimic natural conditions as closely as possible while minimizing any potential effects of chemical cues. Megalopae were fed with ~5 mL (~1500 individuals) of newly hatched brine shrimp (*Artemia*) nauplii following daily water changes. Newly molted juvenile (J1) blue crabs and dead megalopae were removed from the finger bowls daily. Megalopae were used in toxicity testing within three days of collection or allowed to molt to juveniles. This method of collecting megalopae from near-oceanic water ensured that the crabs had not been exposed to any significant amount of pesticide for several weeks.

Juvenile blue crabs were reared in similar conditions to megalopae but were kept at lower densities (~50 per 800 mL) and fed crushed shrimp pellets (dry fish food) daily. Strips of nylon

**Table 1**  
Pesticide properties and blue crab LC<sub>50</sub>s listed in order of decreasing toxicity.

Compound	Formulation	Class	24 h LC <sub>50</sub> (95% confidence interval) (µg/L)	
			Megalopae	Juveniles
Karate®	Comm.	Pyrethroid	0.5260 (0.351–0.789)	3.565 (1.721–7.385)
λ-Cyhalothrin	A.I.	Pyrethroid	0.2233 (0.1833–0.2720)	2.701 (2.215–3.294)
Trimax™	Comm.	Chloro-nicotinyl	312.7 (222.4–439.9)	816.7 (692.9–962.6)
Imidacloprid	A.I.	Chloro-nicotinyl	10.04 (6.381–15.79)	1112 (841.9–1468)
Aldicarb <sup>a</sup>	A.I.	N-methyl carbamate	311.6 (281.6–344.8)	291.1 (227.7–372.3)
Orthene®	Comm.	Organophosphate	61,210 (48,500–77,260)	191,300 (141,100–259,000)
Acephate	A.I.	Organophosphate	50,380 (44,300–57,300)	137,300 (132,800–141,900)
Roundup® Pro <sup>b</sup>	Comm.	Phosphonoglycine	6279 (5937–6640)	316,000 (167,000–595,200)

Comm., commercial; A.I., active ingredient.

<sup>a</sup> Aldicarb was only tested as A.I.

<sup>b</sup> Roundup Pro was only tested as a commercial product.



window screening were added to each bowl to provide structure and minimize interactions among juveniles, including cannibalism. First stage (J1) through fourth stage (J4) juveniles were reared and used in acute toxicity assays.

## 2.2. Chemicals

Active ingredient insecticides used for megalopae and juvenile acute toxicity and time to metamorphosis assays were acquired through Fisher Scientific and were manufactured by Chem Service (West Chester, PA). The four insecticides were dry powder acephate (99.3% purity), aldicarb (99.0% purity), imidacloprid (99.5% purity), and  $\lambda$ -cyhalothrin (99% mix of isomers). Experimental samples of granular Temik® 15G (15% aldicarb) and liquid Trimax® Pro (40.80% imidacloprid) were acquired from Bayer CropScience (Kansas City, MO). Karate® with Zeon Technology™ (Syngenta Crop Protection, Greenville, NC) was graciously donated by Open Grounds Farm (Carteret County, NC). Ortho Orthene® Fire Ant Killer (Ortho Group, Marysville, OH) with 50% acephate and Roundup® Pro Concentrate (Monsanto Company, St. Louis, MO; 50.2% glyphosate isopropylamine salt) were purchased from Lowe's Home Improvement. Karate and Temik 15G are restricted use pesticides and were obtained and used under North Carolina Pesticide Board Pesticide Applicator License number 026-26323.

## 2.3. Experimental solutions

All chemicals were kept in the dark to prevent photolysis. Working stocks were made immediately before use with nanopure water in glass vials and kept in a light-tight metal container for up to one week before being discarded. Lambda-cyhalothrin was the only compound that required a solvent so stocks were made with methanol so that dosing solutions never exceeded 0.05% methanol. Toxicant dilutions were made immediately before use and diluted with ASW. Concentrations of commercial products in this study are presented as nominal concentrations of active ingredient.

## 2.4. Acute toxicity

We performed 24 h acute toxicity assays on megalopae and juvenile crabs. Experiments were dosed once at the beginning of the experiment under still conditions (static, non-renewal). Between two and six assays were performed for each experimental toxicant, with each assay testing progressively narrower ranges of concentrations around the effective concentration for 50% mortality. Each assay included several concentrations of pesticide as well as aged seawater (ASW) control and a solvent control when needed. Test animals in a given round were haphazardly taken from all of the available rearing finger bowls and allocated to their respective containers with a small amount of ASW while pesticide dilutions were made. The ASW was then removed and replaced with toxicant solution. Megalopae were tested in glass test tubes containing 10 mL of test solution ( $n = 5$  individuals per tube, 3 replicates per concentration). Juveniles were tested in cell-culture-treated (hydrophilic) polystyrene 24-well microplates with one individual per well in 1.5 mL of test solution ( $n = 24$  individuals per concentration). These two container materials were chosen so that they had similar hydrophilicity. Test containers were covered to minimize evaporation and the number of dead crabs counted at 24 h. No feeding, aeration, or mixing took place during the 24 h assay. Death was judged by lack of movement of the appendages and antennae in response to shaking the container. Dead juveniles usually had an opaque coloration and were often upside down with legs curled.

Acute toxicity was modeled as four-parameter log dose–response nonlinear regressions using GraphPad Prism 5 (GraphPad Software, San Diego, CA). To avoid the logarithm of zero, ASW and solvent

controls were entered into models as concentration at least 3 log units below the lowest experimental concentration. This was 0.0001  $\mu\text{g/L}$  for  $\lambda$ -cyhalothrin and Karate; 0.001  $\mu\text{g/L}$  for imidacloprid, Trimax, and aldicarb; and 1  $\mu\text{g/L}$  for Orthene, acephate, and Roundup. Since 100% mortality was reached with all test solutions, model maxima were constrained to a value of 100%. If control mortality was zero for a toxicant then its model was constrained to a minimum of 0%. No lower constraints were placed on models of toxicants with control mortalities greater than zero.  $\text{LC}_{50}$  values with 95% confidence intervals were calculated from each model. Statistical differences between  $\text{LC}_{50}$  concentrations of megalopae and juveniles in the same toxicant and between the same ontogenetic stage in the commercial pesticide and its active ingredient were tested using one-way analysis of variance (ANOVA).

## 2.5. Time to metamorphosis (TTM)

After completing and modeling the 24 h acute toxicity assays, the concentration modeled to kill 20% of the crabs ( $\text{LC}_{20}$ ) was used to test the effects of pesticides on the time to metamorphosis from the megalopae stage to the J1 stage. Megalopae were collected as above and used within 24 h of collection. Only visually identified intermolt megalopae of a single cohort (Forward et al., 1996) were used in this assay. Intermolt megalopae were housed individually in glass test tubes filled with 2 mL of test solution ( $n = 30$  per concentration). Housing megalopae individually in glass eliminated any potential effects of plasticizers, cannibalism, or changes in density with removal of juveniles. Test solutions were made using estuarine seawater (ESW) so that any natural chemical cues would be present. These were static, non-renewal exposures on an ambient light:dark cycle. Water was not changed and megalopae were not fed during the experiment. Megalopae were checked approximately every 6 h until all megalopae had either died as megalopae or molted to juveniles. The time period in which any individual died or molted (or both) was recorded.

Two separate rounds of time to metamorphosis (TTM) assays were completed. The first round tested the ESW control (control 1), 32,000  $\mu\text{g/L}$  acephate, 240  $\mu\text{g/L}$  aldicarb, and 4.8  $\mu\text{g/L}$  imidacloprid. The second round tested the ESW control (control 2), 0.15  $\mu\text{g/L}$   $\lambda$ -cyhalothrin, and 5500  $\mu\text{g/L}$  Roundup Pro. Statistical analyses were performed using GraphPad Prism 5 between experimental results and those of their respective control; i.e. only within round 1 and within round 2. This was done because different cohorts of megalopae, collected on different days with different experiences, were used in each round.

Time to metamorphosis data were analyzed using three different methods: ANOVA on the mean TTM (Forward et al., 1994, 1996, 1997); Log-rank Mantel–Cox survival analysis (similar to Tankersley and Wieber, 2000); and ANOVA of regression-derived  $\text{ET}_{50}$ . For the first method, all of the times (h) of metamorphosis for each treatment were averaged together (Brumbaugh and McConaughy, 1995; Fitzgerald et al., 1998; Forward et al., 1994, 1996, 1997; Gebauer et al., 1999; O'Connor and Gregg, 1998; O'Connor and Judge, 1997; Rodriguez and Epifanio, 2000; Wolcott and De Vries, 1994). Only those megalopae that successfully molted to the juvenile stage, whether alive or dead when checked were included in the calculation of the mean time to metamorphosis. Two one-way ANOVAs (one for each round) with Dunnett's post-hoc tests were employed to compare the mean TTM.

A survival analysis on metamorphosis was employed as the second method of TTM analysis similar to Tankersley and Wieber (2000). "Death" was replaced in the traditional survival analysis with "metamorphosis." This method has the benefit of taking into account megalopae that die during the experiment when calculating the percentage of megalopae or juveniles at each point. A log-rank

Mantel–Cox test (Peto and Peto, 1972) was employed for each round of testing.

The third method of TTM analysis mirrored our acute toxicity analysis. TTM was modeled using a four-parameter curve similar to the dose–response curve. The percentage of megalopae metamorphosed at each time point, as calculated during the survival analysis (above), was used as the input data. The times modeled to produce 50% molting for each treatment ( $ET_{50}$ : effective time 50) were compared using two separate one way ANOVAs with Bonferroni-corrected pairwise post-hoc comparisons.

Fisher's exact tests were used to compare the frequencies of megalopae that molted to J1 for each treatment. Likewise, the

frequencies of molted juveniles found dead when first checked after molting were tested. These tests were performed using SigmaPlot 11 (Systat Software, San Jose, CA).

### 3. Results

#### 3.1. Acute toxicity

##### 3.1.1. Karate and $\lambda$ -cyhalothrin

Karate with Zeon technology and its active ingredient  $\lambda$ -cyhalothrin were the most potent of the insecticides tested.  $LC_{50}$  values for megalopae exposed to Karate was 0.5260  $\mu\text{g/L}$  and 3.565  $\mu\text{g/L}$  for juveniles (Fig. 1,

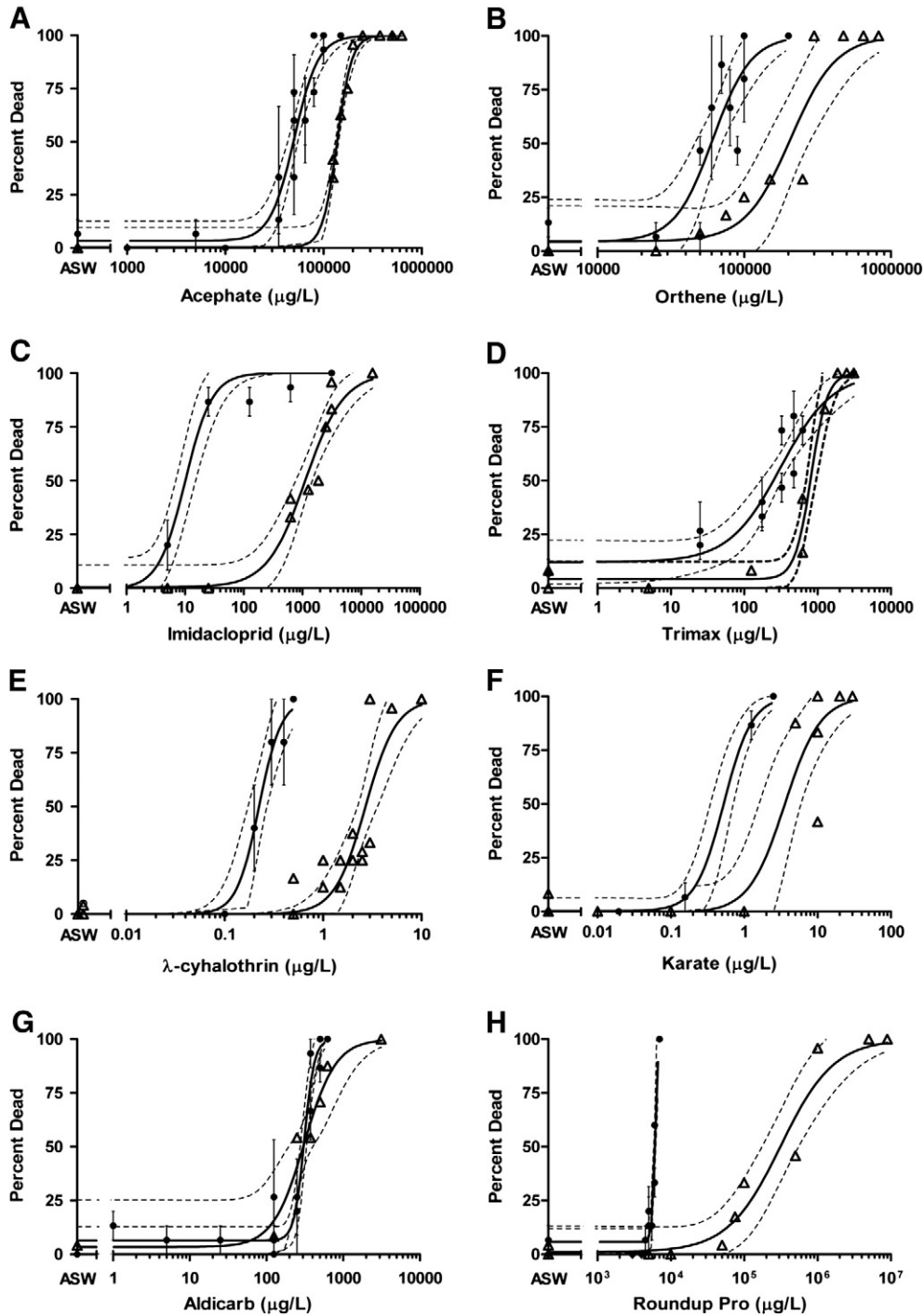


Fig. 1. Mortality of megalopae (●) and juveniles (Δ) exposed to pesticides. Commercial formulations are presented in the left column and active ingredients in the right. Best fit model (solid line) and 95% confidence interval (dotted line) are drawn. N.B. panels G and H are different active ingredients.

Table 1). Treatment with  $\lambda$ -cyhalothrin produced similar results with a megalopa  $LC_{50}$  of 0.2233  $\mu\text{g/L}$  and a juvenile  $LC_{50}$  of 2.701  $\mu\text{g/L}$ . The  $LC_{50}$  for juveniles exposed to  $\lambda$ -cyhalothrin was significantly greater than that for megalopae ( $p < 0.0001$ ,  $F_{1,29} = 68.7$ ). There was no significant difference between Karate and  $\lambda$ -cyhalothrin  $LC_{50}$  values for either megalopae ( $p = 0.075$ ,  $F_{1,23} = 3.478$ ) or juveniles ( $p = 0.4413$ ,  $F_{1,28} = 0.6101$ ).

### 3.1.2. Trimax and imidacloprid

Trimax and its active ingredient imidacloprid were the next most potent insecticides after Karate and  $\lambda$ -cyhalothrin. The  $LC_{50}$ s for megalopae and juveniles exposed to Trimax were 312.7  $\mu\text{g/L}$  and 816.7  $\mu\text{g/L}$ , respectively (Fig. 1, Table 1). Imidacloprid was significantly more toxic than Trimax ( $p < 0.0001$ ,  $F_{1,51} = 42.68$ ) to megalopae with a  $LC_{50}$  value 10.04  $\mu\text{g/L}$ . Exposure of juveniles to imidacloprid produced a  $LC_{50}$  of 1112  $\mu\text{g/L}$ , which was not significantly different than that for Trimax ( $p = 0.0969$ ,  $F_{1,17} = 3.087$ ). Both Trimax ( $p = 0.002$ ,  $F_{1,44} = 17.07$ ) and imidacloprid ( $p < 0.0001$ ,  $F_{1,24} = 42.87$ ) were significantly more toxic to megalopae than to juveniles.

### 3.1.3. Aldicarb

We had difficulty getting pelletized Temik 15G into solution so we only present results for its active ingredient aldicarb. Aldicarb exhibited toxicity to blue crabs similar to the toxicity of Trimax and imidacloprid.  $LC_{50}$ s for megalopae and juveniles treated with aldicarb were 311.6  $\mu\text{g/L}$  and 291.1  $\mu\text{g/L}$ , respectively (Fig. 1, Table 1). Aldicarb was the only tested insecticide that showed no significant difference in toxicity between megalopae and juveniles ( $p = 0.8554$ ,  $F_{1,55} = 0.0335$ ). The dose-response curve for megalopae treated with aldicarb was particularly steep and had a Hill slope of 6.66 compared to the more gradual Hill slope of 2.05 for juveniles treated with aldicarb (Fig. 1).

### 3.1.4. Orthene and acephate

Orthene and its active ingredient acephate were by far the least potent of the insecticides tested. The  $LC_{50}$  for Orthene-exposed megalopae was 61,210  $\mu\text{g/L}$ , between two and four orders of magnitude less potent than the other insecticides (Fig. 1, Table 1). The  $LC_{50}$  of Orthene for blue crab juveniles (191,300  $\mu\text{g/L}$ ) was significantly higher than for megalopae ( $p = 0.0069$ ,  $F_{1,42} = 8.077$ ). The toxicity of acephate to megalopae ( $LC_{50} = 50,380 \mu\text{g/L}$ ) did not differ significantly from that of Orthene ( $p = 0.1151$ ,  $F_{1,90} = 2.531$ ). Acephate was significantly more toxic to megalopae than to juveniles ( $p = 0.004$ ,  $F_{1,64} = 8.892$ ) with a juvenile  $LC_{50}$  of 137,300  $\mu\text{g/L}$ . Acephate was significantly more toxic to juveniles than Orthene ( $p = 0.0149$ ,  $F_{1,18} = 7.247$ ).

### 3.1.5. Roundup

The toxicity of the herbicide Roundup Pro was generally very low but significantly higher to megalopae than to juveniles ( $p < 0.0001$ ,  $F_{1,41} = 48.63$ ). The  $LC_{50}$  for megalopae exposed to Roundup was 6279  $\mu\text{g/L}$  and that for juveniles was 316,000  $\mu\text{g/L}$  (Fig. 1, Table 1). Similar to the results of aldicarb exposure, roundup exposure produced the particularly large difference in Hill slopes of 11.41 for megalopae and 1.2 for juveniles (Fig. 1).

## 3.2. Time to metamorphosis (TTM)

Roundup was the only treatment that significantly reduced mean TTM ( $q = 2.646$ ,  $p < 0.05$ ) and  $ET_{50}$  ( $p = 0.002$ ; Fig. 2) but its survival curve was not significantly different than controls (Fig. 3). Round 1 tested acephate, aldicarb, and imidacloprid. There was no significant difference in mean TTM ( $F_{3,85} = 0.0653$ ,  $p = 0.978$ ; Fig. 2), survival curves ( $\chi^2 = 1.428$ , 3 df,  $p = 0.6990$ ; Fig. 4), or  $ET_{50}$ s ( $F_{3,35} = 2.265$ ,  $p = 0.091$ ; Fig. 2). Mean TTM ranged from 64.14 h for aldicarb to

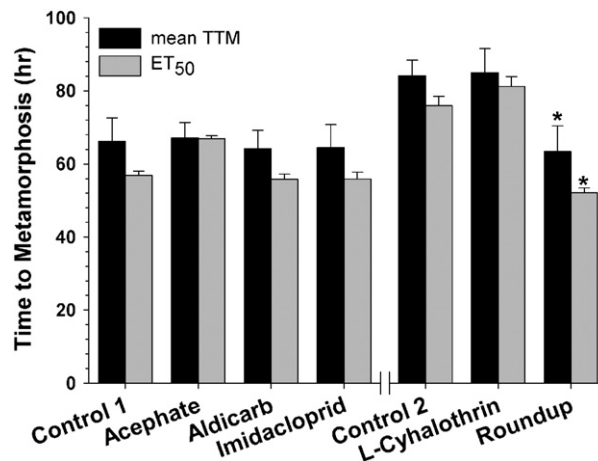


Fig. 2. Mean time to metamorphosis ( $\pm$  S.E.M.) and modeled  $ET_{50}$ . An axis break separates round 1 from round 2 and statistical analyses were only performed within groups. Significance: \* $p < 0.05$ .

67.13 h for acephate.  $ET_{50}$ s ranged from 55.81 h for aldicarb to 66.90 h for acephate.

Round 2 tested  $\lambda$ -cyhalothrin and Roundup. There were significant differences in mean TTM ( $F_{2,68} = 4.362$ ,  $p = 0.0165$ ; Fig. 4) and  $ET_{50}$  ( $F_{2,19} = 6.515$ ,  $p = 0.0070$ ; Fig. 2) but no differences among survival curves ( $\chi^2 = 2.212$ , 2 df,  $p = 0.3309$ ; Fig. 3). Mean TTM for round 2 ranged from 63.38 h for Roundup to 85.03 h for  $\lambda$ -cyhalothrin.  $ET_{50}$  for Roundup and  $\lambda$ -cyhalothrin were 52.06 h and 81.25 h, respectively. For both rounds all  $ET_{50}$  values were lower (quicker) than their corresponding mean TTM.

Of the 30 intermolt megalopae per treatment, 86.2% of control 1 and 96.7% of control 2 molted to juveniles. Only treatment with

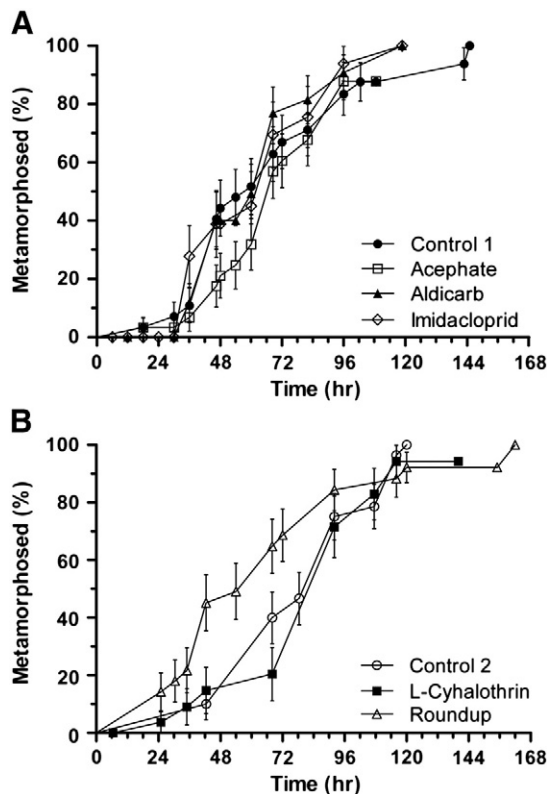


Fig. 3. Metamorphosis curves (with S.E.M.) for round 1 (A) and round 2 (B). Only those megalopae still alive or successfully molted to the J1 stage (alive or dead) at each check point are included in the percentage calculation.



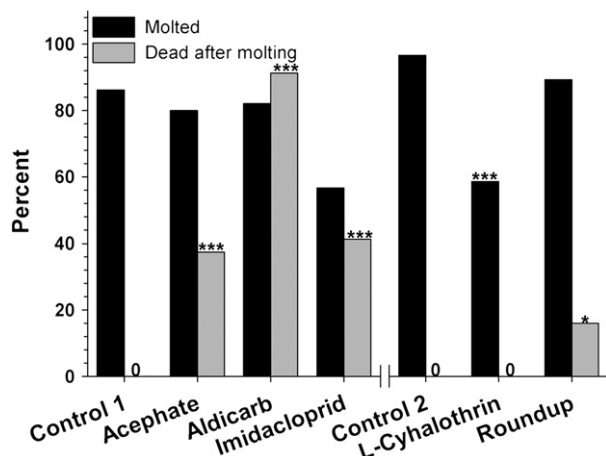


Fig. 4. The percentages of megalopae successfully molting to the J1 stage and the percentages of those successfully molting that were found dead as J1 stage crabs during TTM experiments. An axis break separates round 1 from round 2 and statistical analyses were only performed within rounds. Significance: \* $p < 0.05$ , \*\*\* $p < 0.0005$ .

imidacloprid (56.7%;  $p = 0.0204$ ) and  $\lambda$ -cyhalothrin (58.6%;  $p = 0.0004$ ) resulted in significantly fewer megalopae molting than their respective controls (Fig. 4). None of the newly molted juveniles in either control treatments was dead when first checked after molting. Treatment with the three insecticides acephate, aldicarb, and imidacloprid significantly increased the frequency of juveniles that died after molting compared to control 1 (Fig. 4). Aldicarb proved most deadly to the molting crabs with 91.3% ( $p < 0.0001$ ) of juveniles dead when first checked after molting. Treatment with acephate and imidacloprid resulted in 37.5% ( $p = 0.0005$ ) and 41.2% ( $p = 0.0007$ ) of the juveniles dying after metamorphosis, respectively. The commercial herbicide product Roundup Pro ( $p = 0.04$ ) significantly increased mortality after molting compared to control 2.

#### 4. Discussion

##### 4.1. Acute toxicity

The acute toxicities of the tested active ingredient insecticides to blue crab megalopae and juveniles result in the pattern:  $\lambda$ -cyhalothrin > imidacloprid  $\approx$  aldicarb > acephate (Fig. 5). The commercial herbicide formulation Roundup was similar to acephate in its toxicity to juvenile blue crabs, but was much more toxic to megalopae.

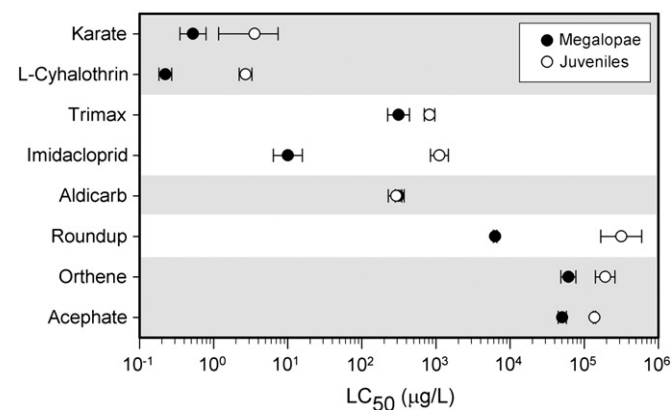


Fig. 5.  $LC_{50}$ s ( $\pm 95\%$  confidence interval) for megalopae (●) and juveniles (○) treated with the various pesticides. Background shading groups commercial products with their active ingredient.

Lambda-cyhalothrin ( $\lambda$ -cyhalothrin) is an extremely potent pyrethroid insecticide whose mode of action is to bind to and open sodium channels on neuron axons, effectively paralyzing subjects (He et al., 2008). Lambda-cyhalothrin, alone and in its commercial formulation Karate, was by far the most acutely toxic compound tested (Fig. 5). Treatment of blue crabs with  $\lambda$ -cyhalothrin produced a megalopa  $LC_{50}$  of 0.2233  $\mu\text{g/L}$  and a juvenile  $LC_{50}$  of 2.701  $\mu\text{g/L}$ .  $LC_{50}$  values for blue crab megalopae and juveniles exposed to the commercial formulation Karate were 0.526  $\mu\text{g/L}$  and 3.565  $\mu\text{g/L}$ , respectively. Our values are similar to 24 h  $LC_{50}$  reported for other crustaceans treated with  $\lambda$ -cyhalothrin-based pesticides such as 0.183  $\mu\text{g/L}$  for the fairy shrimp *Streptocephalus sudanicus* (Lahr et al., 2001) and 0.87  $\mu\text{g/L}$  for the freshwater shrimp *Caridina laevis* (Sucahyo et al., 2008). Mokry and Hoagland (1990) reported similar 48 h  $LC_{50}$  values for *Daphnia magna* and *Cerrodaphnia dubia* first instars (Table 2).

Imidacloprid is a chloro-nicotinyl insecticide and acetylcholine receptor agonist (Matsuda et al., 2001). We report a two order of magnitude difference between megalopae and juvenile blue crab imidacloprid  $LC_{50}$  values and over a one order of magnitude difference between megalopae values for imidacloprid and the commercial formulation Trimax. Imidacloprid toxicity in other crustaceans ranges broadly with 24 h  $LC_{50}$  values as low as 103  $\mu\text{g/L}$  for the amphipod *Gammarus pulex* (404 nmol/L; Ashauer et al., 2011) to 1122  $\mu\text{g/L}$  for the rice paddy ostracod *Ilyocypris dentifera* to as high as >320,000  $\mu\text{g/L}$  for *D. magna* (Sánchez-Bayo and Goka, 2006). 48 h  $LC_{50}$  values show a similarly large range in other crustacean models (Table 2). The blue crab megalopae and juveniles are more sensitive to imidacloprid than the model crustacean *D. magna* by many orders of magnitude but are similar in sensitivity to the widely used amphipod *G. pulex* (Ashauer et al., 2011).

Aldicarb is an N-methyl carbamate and cholinesterase inhibitor that is highly acutely toxic to a broad range of organisms, especially crustaceans. 24 h  $LC_{50}$  values of aldicarb to blue crab megalopae and juveniles (Table 1) are similar to that for *G. pulex* (Ashauer et al., 2011) but are two orders of magnitude less than for 72 h old *Artemia salina* nauplii (Barahona and Sánchez-Fortún, 1999) (Table 2). In 2010, the U.S. Environmental Protection Agency and Bayer CropScience agreed to reduce and ultimately discontinue the production of aldicarb as well as eliminate all aldicarb product registrations by the end of 2014 (Federal Register, 2010).

Acephate is a broad spectrum organophosphate and a known neurotoxin that inhibits the breakdown of acetylcholine (Eto, 1974). Acephate and Orthene were by far the least toxic insecticides tested with  $LC_{50}$ s over an order of magnitude higher than for any other pesticide tested here (Table 1), similar to results from other studies comparing toxicity of acetylcholinesterase inhibitors (Printes and Callaghan, 2004). Venkateswara et al. (2007) reported 24 h  $LC_{50}$  values for 72 h old *A. salina* nauplii treated with acephate (Table 2) even higher than the  $LC_{50}$ s for blue crab megalopae and juveniles reported here. Interestingly the acephate metabolite methamidophos (O,S-Dimethyl phosphoramidothioate) is highly toxic to crustaceans with 24 h  $LC_{50}$  values for *D. magna* of 108.7  $\mu\text{g/L}$  (Lin et al., 2006) and 0.16  $\mu\text{g/L}$  for mysis stage *Penaeus stylirostris* shrimp (Tamaron@ 600; Juarez and Sanchez, 1989).

Glyphosate is a phosphonoglycine herbicide that inhibits an enzyme required to produce certain amino acids (Amrhein et al., 1980) and was only tested here as the commercial product Roundup that includes the POEA surfactant that has proven toxic by itself (Brausch and Smith, 2007; Brausch et al., 2007; Folmar et al., 1979; Giesy et al., 2000). Blue crab megalopae were much more sensitive to Roundup than juveniles (Table 1). Different glyphosate-based products produced drastically different 48 h  $LC_{50}$  values for *D. magna* with Roundup (Folmar et al., 1979) being much more toxic than Rodeo® (Henry et al., 1994) (Table 2). The amphipods *Gammarus pseudolimnaeus* and *Hyalella azteca* were not very sensitive to glyphosate-based herbicides with a reported 48 h  $LC_{50}$  of

Table 2

Acute toxicities of the tested pesticides in crustaceans.

A.I.	Formulation	Species	Organism type	Duration	LC <sub>50</sub> (µg/L)	Ref.
λ-Cyhalothrin	Karate	<i>Streptocephalus sudanicus</i>	FW fairy shrimp	24 h	0.183	1
	Matador	<i>Caridina laevis</i>	FW shrimp	24 h	0.87	2
	Karate	<i>Daphnia magna</i>	FW cladoceran	48 h	1.04	3
Imidacloprid	Karate	<i>Cerrodaphnia dubia</i>	FW cladoceran	48 h	0.3	3
	A.I.	<i>Gammarus pulex</i>	FW amphipod	24 h	103	4
	A.I.	<i>Ilyocypris dentifera</i>	FW ostracod	24 h	1122	5
	A.I.	<i>Daphnia magna</i>	FW cladoceran	24 h	> 320,000	5
	A.I.	<i>Ilyocypris dentifera</i>	FW ostracod	48 h	301	5
	A.I.	<i>Daphnia magna</i>	FW cladoceran	48 h	17,360	5
	A.I.	<i>Artemia salina</i>	SW brine shrimp	48 h	361,000	6
Aldicarb	A.I.	<i>Gammarus pulex</i>	FW amphipod	24 h	658	4
	A.I.	<i>Artemia salina</i>	SW brine shrimp	24 h	60,122	7
Acephate	A.I.	<i>Artemia salina</i>	SW brine shrimp	24 h	2,350,000	8
	Methamidophos <sup>a</sup>	<i>Daphnia magna</i>	FW cladoceran	24 h	108.7	9
	Methamidophos	<i>Penaeus stylirostris</i>	SW shrimp	24 h	0.16	10
Glyphosate	Roundup	<i>Daphnia magna</i>	FW cladoceran	48 h	3000	11
	Roundup	<i>Gammarus pseudolimnaeus</i>	FW amphipod	48 h	62,000	11
	Rodeo	<i>Daphnia magna</i>	FW cladoceran	48 h	218,000	12
	Rodeo	<i>Hyalella azteca</i>	FW amphipod	96 h	720,000	12
	Roundup	<i>Acartia tonsa</i>	SW copepod	48 h	177,000	13

References: 1: Lahr et al. (2001); 2: Suchayo et al. (2008); 3: Mokry and Hoagland (1990); 4: Ashauer et al. (2011); 5: Sánchez-Bayo and Goka (2006); 6: Song et al. (1997); 7: Barahona and Sánchez-Fortún (1999); 8: Venkateswara et al. (2007); 9: Lin et al. (2006); 10: Juarez and Sanchez (1989); 11: Folmar et al. (1979); 12: Henry et al. (1994); 13: Tsui and Chu (2003).

A.I., active ingredient; FW, freshwater; SW, salt water.

<sup>a</sup> An acephate metabolite.

62,000 µg/L (Folmar et al., 1979) and a 96 h LC<sub>50</sub> of 720,000 µg/L (Henry et al., 1994), respectively. Tsui and Chu (2003) reported a Roundup 48 h LC<sub>50</sub> of 177,000 µg/L for the marine copepod *Acartia tonsa*. Blue crab megalopae are similarly sensitive to Roundup as *Daphnia magna*, but juvenile blue crab sensitivity is even less than amphipods and copepods.

All tested pesticides and formulations, except aldicarb, were significantly more toxic to megalopae than to juveniles (Fig. 5). There was no statistical difference between the aldicarb 24 h LC<sub>50</sub> values for megalopae and juvenile blue crabs. Foran et al. (1985) reported significantly greater toxicity of aldicarb to juvenile *Daphnia laevis* than adults. Alternatively, Barahona and Sánchez-Fortún (1999) showed decreased 24 h LC<sub>50</sub>s in 24 to 72 h old brine shrimp *A. salina* exposed to aldicarb. This could be more a function of the species than the toxicant since *A. salina* showed increasing sensitivity with age to several other pesticides as well (Sánchez-Fortún et al., 1995, 1996). Pesticides like mirex and kepone have shown a decrease in toxicity with increasing ontogenetic stages in blue crabs (Bookhout and Costlow, 1975; Bookhout et al., 1980). The greatest difference between LC<sub>50</sub>s for megalopae and juvenile blue crabs was seen with imidacloprid, which was 100-fold more toxic to megalopae than juveniles. Similarly, in the shrimp *P. stylirostris*, acephate metabolite methamidophos-based Tamaron® (Bayer CropScience) LC<sub>50</sub>s decreased approximately one order of magnitude in *P. stylirostris* from nauplius stage through mysis stage larvae (Juarez and Sanchez, 1989). The Roundup LC<sub>50</sub> for juvenile blue crabs was ~50-fold higher than for megalopae. The dose–response curve Hill slope for Roundup-treated megalopae was the steepest curve in the study and drastically higher than the slope for Roundup-treated juveniles (Fig. 1).

The toxicities of the tested commercial formulations and of their respective active ingredients were generally very similar. Exceptions to this trend were imidacloprid, which was significantly more toxic to megalopae than its commercial formulation Trimax, and acephate, which was significantly more toxic to juveniles than Orthene (Fig. 5). Since commercial products often contain adjuvants and surfactants the commercial products are often more toxic than the active ingredients alone. *D. magna*, *Danio rerio* zebrafish (Tisler et al., 2009), and green frog *Rana clamitans* tadpoles (Puglis and Boone, 2011) are significantly more sensitive to commercial formulations than active ingredient imidacloprid alone.

Juvenile crabs occur throughout estuaries and routinely inhabit and forage in very shallow water (Ramach et al., 2009) including shallow drainage areas. Thus they and their prey may be routinely exposed directly to overspray and run-off. Assuming a ditch, stream, or other body of water near agriculture has vertical sides, it can be modeled simply as a cuboid (3D rectangle). The depth a water body would need to be for a direct overspray of the tested compounds at the 2007 average rate for cotton (USDA, 2008) to equal the juvenile 24 h LC<sub>50</sub> is easily calculated. Since application rates can be converted to the units g/m<sup>2</sup>, the depth in meters can be calculated by dividing the LC<sub>50</sub> (converted to units of g/m<sup>3</sup>) by the application rate. Lambda-cyhalothrin and Karate were the most potent insecticide assayed and Karate was applied at a rate of 0.028 lbs a.i./A in 2007. A body of water would need to be greater than 87.7 cm (95% CI: 42–267 cm) deep for Karate application and greater than 1.15 m (0.95–1.41 m) deep for λ-cyhalothrin application to ensure the concentration remains below juvenile blue crab 24 h LC<sub>50</sub>s. This does not take into account any adsorption of λ-cyhalothrin to suspended particles, which would likely be significant. Leistra et al. (2004) reported 24–40% of λ-cyhalothrin remained in the water column in experimental vegetated ditches after one day. If this range of adsorption rates is taken into account our theoretical ditches would need to be greater than 21.1 to 35.5 cm deep for Karate application and greater than 27.8 to 46.4 cm deep for λ-cyhalothrin application. Since many ditches and creeks are less than 20 cm deep (Rittschof, personal observation), many direct oversprays with Karate or λ-cyhalothrin would likely kill over 50% of the blue crab juveniles in the ditch in the first 24 h. Direct overspray by imidacloprid or Trimax at the manufacturer's recommended 0.046 lbs a.i./A would require a water body deeper than 4.1 cm (3.1–5.4 cm) or 5.6 cm (4.7–6.6 cm), respectively, to maintain lower than LC<sub>50</sub> concentrations. These depths are common in eastern NC, so direct overspray with Trimax or imidacloprid has a good chance to be acutely toxic to any blue crabs there. Aldicarb is a seed treatment and it will be off the market by the end of 2014 (Federal Register, 2010) so a direct overspray is less likely. Overspray with acephate, Orthene, or Roundup at average NC rates would require a ditch less than 1 mm deep to surpass the juvenile LC<sub>50</sub> and so are unlikely to acutely impact blue crabs by direct overspray.

Since tidal creeks and marshes are forage areas for blue crabs as well as nursery areas for many important estuarine species, lethal



and sub-lethal effects here could have serious implications for the broader estuarine ecosystem. Changes to lower trophic levels will ultimately impact higher trophic levels throughout the estuary even if they are not directly influenced by the pollutants themselves. Predator–prey interactions have been shown to change in response to pollution (e.g. Brooks et al., 2009; Clements et al., 1989). Blue crabs and mummichogs (*Fundulus heteroclitus*) along with lower trophic level grass shrimp (*Palaemonetes pugio*) showed reduced densities in estuaries polluted with the organochlorine endosulfan (Scott et al., 1992). If prey populations are reduced in creeks developing blue crabs may be forced to forage in areas that are less safe from predators.

#### 4.2. Time to metamorphosis

Blue crab megalopae can delay metamorphosis until chemical cues indicating suitable juvenile habitat are sensed (reviewed by Forward et al., 2001). Cues such as ammonium and predator odor delay metamorphosis in estuarine crabs, as do extremes in salinity and oxygen concentration (Forward et al., 1994, 1996; Welch et al., 1997). Delays of metamorphosis can result in smaller and later molting crabs with consequently reduced fitness (Gebauer et al., 1999). Others have noted increased duration of estuarine crab larval development in response to pesticides (Bookhout and Costlow, 1975; Bookhout et al., 1972, 1976, 1980), with the exception of the stone crab *Menippe mercenaria* treated with the chlorinated hydrocarbon mirex (Bookhout et al., 1972). The acephate metabolite methamidophos slowed development in the shrimps *Macrobrachium rosenbergii* and *P. stylirostris* (Juarez and Sanchez, 1989). We therefore anticipated that all tested pesticides would increase TTM in blue crab megalopae. Since megalopae were collected from the field, different cohorts had different larval experiences and could have been of slightly different ages, which impact TTM, as seen in the difference between the respective controls for round 1 and 2 (Figs. 2–4). For that reason we only performed statistics within cohorts and not across cohorts.

Roundup was the only tested compound that significantly changed the rate of metamorphosis (Figs. 2 and 3). The mean TTM and the modeled  $ET_{50}$  of Roundup were significantly accelerated compared estuarine water. Most other studies of crab metamorphosis used offshore seawater as the negative control. Those studies have not tested any compounds that have been able to significantly accelerate metamorphosis beyond estuarine water (positive control) (Forward et al., 1994). Roundup and its POEA surfactant alone also significantly effected TTM in the frog *Rana pipiens*, but in contrast, it significantly increased TTM (Howe et al., 2004). Weis and Mantel (1976) reported that fiddler crabs treated with the organochlorine insecticide DDT (1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane) increased the rate of limb regeneration and reduced the time to the next molt. They noted no such differences after treatment with the organophosphates malathion (diethyl 2-[(dimethoxyphosphorothioyl)sulfanyl]butanedioate) and parathion (O,O-Diethyl O-(4-nitrophenyl) phosphorothioate), or the carbamate carbaryl (1-naphthyl methylcarbamate; Sevin®, Bayer).

Weis and Mantel (1976) suggested the excitation of the central nervous system by the neurotoxic DDT played a role in the acceleration of molting in fiddler crabs. Glyphosate, however, is not a neurotoxin and so some other mechanisms are more likely to be responsible. Surfactants are detergents and can alter cell membrane and sensory cell characteristics, increase uptake of extracellular molecules (Riechers et al., 1994), and alter thresholds for neuronal vesicle binding (Zhu and Stevens, 2008). We suggest that the tallow amine surfactant, POEA, might be altering sensory cell membranes and activating the same pathway as humic acids, or allowing natural chemical cues in the estuarine seawater medium to act at lower concentrations. Future studies of the effect of surfactants, and POEA in particular, on settlement, molting, and metamorphosis are warranted.

Survival curve analysis and the  $ET_{50}$  modeling take into the account the megalopae that die by including them into the calculation of percentage molted until they die. This perhaps makes these two methods of analysis more suitable for time to metamorphosis assays with toxic compounds than traditional mean TTM. The inclusion of dead megalopae in the  $ET_{50}$  calculation is responsible for making those values lower than their respective mean TTM. Most megalop's mortality occurred at the beginning of experiments and thus any remaining successful molts accounted for an increased percentage change in the percent molted value. This lead to steeper curves than would be modeled if only the final numbers of molted juveniles were used in the calculation of percentages. Steeper lines produced lower  $ET_{50}$  values.

Treatment with imidacloprid and  $\lambda$ -cyhalothrin resulted in a significantly reduced frequency of successful molting (Fig. 4). Only 56.67% of imidacloprid and 58.62% of  $\lambda$ -cyhalothrin treated megalopae molted compared to all others above 80%. Both of these pesticides showed similar trends in death and molting. Most of the megalopae that died under both treatments occurred within the first two days and before any megalopae molted. The timing of metamorphosis and death under the other treatments were much more interspersed. Where imidacloprid and  $\lambda$ -cyhalothrin differed was survival of the molted juveniles (Fig. 4). All of the molted juveniles treated with  $\lambda$ -cyhalothrin were found alive while 41.18% of imidacloprid treated juveniles were found dead. Most striking was the case of aldicarb, which resulted in a molting rate of 82.14%, but 91.3% of those were found dead as juveniles. It is possible that much of the  $\lambda$ -cyhalothrin had become adsorbed to the glass test tube (Ali and Baugh, 2003) after 2–3 days and therefore was not bioavailable to affect molting megalopae.

Unlike other pesticides on the market, none of those tested here target ecdysone, the hormone that controls molting in arthropods (Clare et al., 1992). Imidacloprid,  $\lambda$ -cyhalothrin, and aldicarb all affect the nervous system and induce neurotoxicity but belong to different chemical classes and operate by slightly different modes of action (Gupta et al., 2002; He et al., 2008; Kegley et al., 2011; Matsuda et al., 2001). Only aldicarb is a suspected endocrine disruptor, while the others are unknown (Kegley et al., 2011). The possible ability of aldicarb to affect both nervous and endocrine systems could be the reason it is so toxic to molting blue crabs.

Molting is an especially sensitive time for crustaceans (McCahon and Pascoe, 1988; Mortimer and Connell, 1994; Rebach and French, 1996). This makes the megalopae and juvenile stages of crustaceans particularly sensitive to environmental toxicants since they molt often. Blue crab megalopae usually molt in submerged vegetation soon after entering the estuaries using tidal stream transport (Forward et al., 1994; Welch et al., 1997). It is the juvenile stages that then disperse throughout estuaries, into tidal creeks and the upper estuary (Reyns and Eggleston, 2004). Early stage juveniles can molt as often as once a week effectively resulting in increased sensitivity to pesticide toxicity.

Lambda-cyhalothrin is the only tested compound that is not water soluble and so should have a lower risk of run-off. The adsorption and desorption of pesticides with dissolved or particulate organic matter is a complex issue controlled by several physical and chemical factors. None of those factors were explicitly tested in this project. All of the experiments presented here were performed in sediment-free environments with filtered seawater. Pesticides such as  $\lambda$ -cyhalothrin that have low water solubility and strongly adsorb to sediment (Ali and Baugh, 2003) can still impact blue crabs and their prey. It has been shown that the concentration of freely dissolved  $\lambda$ -cyhalothrin in water is the fraction that is toxic (Amweg et al., 2005; Hamer et al., 1999; Maund et al., 1998). The process of adsorption can also inhibit the breakdown of the pesticide, therefore prolonging its persistence in the environment (Schwarzenbach et al., 1993). Thus creek sediments can be repositories of hydrophobic pesticides for years

beyond their original application. Blue crab juveniles and adults bury in the sediment to hide and to prevent the growth of fouling organisms on parts of their carapace they cannot reach. Buried blue crabs and other benthic infauna are exposed to the pore water, and thus could be exposed to low pesticide concentrations for extended periods of time. The prolonged phase of desorption of pesticides into the pore water makes this habitat particularly vulnerable to chronic toxicity and sub-lethal effects. Maul et al. (2008) reported significant reductions in growth of larval *Chironomus tenans* at  $\lambda$ -cyhalothrin concentrations 4.3 times lower than their LC<sub>50</sub> when exposed in 10 day sediment-water experiments. Rasmussen et al. (2008) reported changed ecosystem function and decreased mobility and feeding in the amphipod *G. pulex* and the stone fly *Leuctra nigra* when treated with 0.1  $\mu\text{g/L}$   $\lambda$ -cyhalothrin in experimental stream channels. If blue crabs respond similarly, decreased growth could have significant reproductive and ecological effects. Future studies of  $\lambda$ -cyhalothrin toxicity in blue crabs should focus on sub-lethal effects of chronic low level exposures from spiked sediment.

## 5. Conclusion

Blue crabs are ecologically and economically vital estuarine species in the coastal southeastern United States and often live in shallow creeks that can experience elevated pesticide loads. The pesticides assayed here showed toxicity to blue crab megalopae and juveniles in the order of  $\lambda$ -cyhalothrin > imidacloprid  $\approx$  aldicarb > acephate with the herbicide Roundup showing low toxicity to young blue crabs similar to acephate. The potential for direct oversprays of  $\lambda$ -cyhalothrin and imidacloprid to reach acutely toxic concentrations in shallow tidal creeks makes these two insecticides the most dangerous to developing blue crabs. The metamorphosis-associated mortality at LC<sub>20</sub> levels of all but  $\lambda$ -cyhalothrin suggests that frequently molting juvenile blue crabs are at an elevated risk of pesticide toxicity beyond what is suggested by the LC<sub>50</sub>s alone. Future studies on this subject should include evaluating the sub-lethal effects in blue crabs from exposures to environmentally-relevant concentrations of individual pesticides, pesticide mixtures, and their adjuvants or surfactants. Furthermore, the role that pesticides play in the availability or toxicity of blue crab prey species should be explored.

## Acknowledgments

This research was funded by North Carolina Sea Grant Blue Crab Research Program to D. Rittschof and D. Newman. We wish to thank M.Z. Darnell, T. Schultz, F. Lian, and E. Varela-Acevedo for help with the collection and rearing of crabs, and NOAA Beaufort Lab for use of their sampling platform. This paper was improved with the help of two anonymous reviewers. Restricted use pesticides were obtained and used under North Carolina Pesticide Board Pesticide Applicator License number 026-26323 to T.M. Blickley. [SS]

## References

- Ali, M.A., Baugh, P.J., 2003. Sorption-desorption studies of six pyrethroids and mirex on soils using GC/MS-NICI. *Int. J. Environ. Anal. Chem.* 83, 923–933.
- Amrhein, N., Schab, J., Steinrücken, H.C., 1980. The mode of action of the herbicide glyphosate. *Naturwissenschaften* 67, 356–357.
- Amweg, E.L., Weston, D.P., Ureda, N.M., 2005. Use and toxicity of pyrethroid pesticides in the Central Valley, California, USA. *Environ. Toxicol. Chem.* 24, 966–972.
- Ashauer, R., Hintermeister, A., Potthoff, E., Escher, B.I., 2011. Acute toxicity of organic chemicals to *Gammarus pulex* correlates with sensitivity of *Daphnia magna* across most modes of action. *Aquat. Toxicol.* 103, 38–45.
- Bacheler, J.S., Edmissten, K.L., Koening, S.R., York, A.C., Toth, S.J., 2005. Crop profile for cotton in North Carolina. [www.impcenters.org/cropprofiles/docs/nccotton.html](http://www.impcenters.org/cropprofiles/docs/nccotton.html) 2005.
- Barahona, M.V., Sánchez-Fortún, S., 1999. Toxicity of carbamates to the brine shrimp *Artemia salina* and the effect of atropine, BW284c51, iso-OMPA and 2-PAM on carbaryl toxicity. *Environ. Pollut.* 104, 469–476.
- Bookhout, C.G., Costlow Jr., J.D., 1975. Effects of Mirex on the larval development of blue crab. *Water Air Soil Pollut.* 4, 113–126.
- Bookhout, C.G., Wilson, A.J., Duke, T.W., Lowe, J.J., 1972. Effects of Mirex on the larval development of 2 crabs. *Water Air Soil Pollut.* 1, 165–180.
- Bookhout, C.G., Costlow Jr., J.D., Monroe, R., 1976. Effects of methoxychlor on larval development of mud-crab and blue crab. *Water Air Soil Pollut.* 5, 349–365.
- Bookhout, C.G., Costlow Jr., J.D., Monroe, R., 1980. Kepone effects on larval development of mud-crab and blue-crab. *Water Air Soil Pollut.* 13, 57–77.
- Brausch, J.M., Smith, P.N., 2007. Toxicity of three polyethoxylated tallowamine surfactant formulations to laboratory and field collected fairy shrimp, *Thamnocephalus platyurus*. *Arch. Environ. Contam. Toxicol.* 52, 217–221.
- Brausch, J.M., Beall, B., Smith, P.N., 2007. Acute and sub-lethal toxicity of three POEA surfactant formulations to *Daphnia magna*. *Bull. Environ. Contam. Toxicol.* 78, 510–514.
- Brooks, A.C., Gaskell, P.N., Maltby, L.L., 2009. Sublethal effects and predator-prey interactions: implications for ecological risk assessment. *Environ. Toxicol. Chem.* 28, 2449–2457.
- Brumbaugh, R.D., McConaughy, J.R., 1995. Time to metamorphosis of the blue crab *Callinectes sapidus* megalopae: effects of benthic macroalgae. *Mar. Ecol. Prog. Ser.* 129, 113–118.
- Clare, A.S., Rittschof, D., Costlow, J.D., 1992. Effects of the nonsteroidal ecdysone mimic RH-5849 on larval crustaceans. *J. Exp. Zool.* 262, 436–440.
- Clements, W.H., Cherry, D.S., Cairns, J., 1989. The influence of copper exposure on predator-prey interactions in aquatic insect communities. *Freshw. Biol.* 21, 483–488.
- DeLorenzo, M.E., Thompson, B., Cooper, E., Moore, J., Fulton, M.H., 2012. A long-term monitoring study of chlorophyll, microbial contaminants, and pesticides in a coastal residential stormwater pond and its adjacent tidal creek. *Environ. Monit. Assess.* 184, 343–359.
- Eto, M., 1974. *Organophosphorus Pesticides: Organic and Biological Chemistry*. CRF Press, Cleveland.
- Federal Register, 2010. Aldicarb; notice of receipt of request to voluntarily cancel a pesticide registration. *Fed. Regist.* 75 (194), 62129–62131.
- Fitzgerald, T.P., Forward Jr., R.B., Tankersley, R.A., 1998. Metamorphosis of the estuarine crab *Rhithropanopeus harrisi*: effect of water type and adult odor. *Mar. Ecol. Prog. Ser.* 165, 217–223.
- Folmar, L.C., Sanders, H.O., Julin, A.M., 1979. Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. *Arch. Environ. Contam. Toxicol.* 8, 269–278.
- Foran, J.A., Germuska, P.J., Delfino, J.J., 1985. Acute toxicity of aldicarb, aldicarb sulfoxide, and aldicarb sulfone to *Daphnia laevis*. *Bull. Environ. Contam. Toxicol.* 35, 546–550.
- Forward Jr., R.B., Frankel, D.A.Z., Rittschof, D., 1994. Molting of megalopae from the blue crab *Callinectes sapidus*: effects of offshore and estuarine cues. *Mar. Ecol. Prog. Ser.* 113, 55–59.
- Forward Jr., R.B., De Vries, M.C., Rittschof, D., Frankel, D.A.Z., Bischoff, J.P., Fisher, C.M., Welch, J.M., 1996. Effects of environmental cues on metamorphosis of the blue crab *Callinectes sapidus*. *Mar. Ecol. Prog. Ser.* 131, 165–177.
- Forward Jr., R.B., Tankersley, R.A., Blondel, D., Rittschof, D., 1997. Metamorphosis of the blue crab *Callinectes sapidus*: effects of humic acids and ammonium. *Mar. Ecol. Prog. Ser.* 157, 277–286.
- Forward Jr., R.B., Tankersley, R.A., Rittschof, D., 2001. Cues for metamorphosis of brachyuran crabs: an overview. *Am. Zool.* 41, 1108–1122.
- Gebauer, P., Paschke, K., Ander, K., 1999. Costs of delayed metamorphosis: reduced growth and survival in early juveniles of an estuarine grapsid crab, *Chasmagnathus granulata*. *J. Exp. Mar. Biol. Ecol.* 238, 271–281.
- Giesy, J.P., Dobson, S., Solomon, K.R., 2000. Ecological risk assessment for Roundup herbicide. *Rev. Environ. Contam. Toxicol.* 167, 35–120.
- Gupta, S., Gajbhiye, V.T., Agnihotri, N.P., 2002. Leaching behavior of imidacloprid formulations in soil. *Bull. Environ. Contam. Toxicol.* 68, 502–508.
- Hamer, M.J., Goggin, U.M., Muller, K., Maund, S.J., 1999. Bioavailability of lambda-cyhalothrin to *Chironomus riparius* in sediment-water and water-only systems. *Aquat. Ecosyst. Health Manage.* 2, 403–412.
- He, L.-M., Troiano, J., Wang, A., Goh, K., 2008. Environmental chemistry, ecotoxicity, and fate of lambda-cyhalothrin. *Rev. Environ. Contam. Toxicol.* 195, 71–91.
- Henry, C.J., Higgins, K.F., Buhl, K.J., 1994. Acute toxicity and hazard assessment of Rodeo, X-77 Spreader, and Chem-Trol to aquatic invertebrates. *Arch. Environ. Contam. Toxicol.* 27, 392–399.
- Howe, C.H., Berrill, M., Pauli, B.D., Helbing, C.C., Werry, K., Veldhoen, N., 2004. Toxicity of glyphosate-based pesticides to four North American frog species. *Environ. Toxicol. Chem.* 23, 1928–1938.
- Juarez, L.M., Sanchez, J., 1989. Toxicity of the organophosphorus insecticide metamidophos (O, S-dimethyl phosphoramidothioate) to larvae of the freshwater prawn *Macrobrachium rosenbergii* (De Man) and the blue shrimp *Penaeus stylirostris* Stimpson. *Bull. Environ. Contam. Toxicol.* 43, 302–309.
- Kegley, S.E., Hill, B.R., Orme, S., Choi, A.H., 2011. PAN Pesticide Database, Pesticide Action Network, North America (San Francisco, CA). <http://www.pesticideinfo.org>. Accessed 8 October 2011.
- Lahr, J., Badji, A., Marquenie, S., Schuiling, E., Ndour, K.B., Diallo, A.O., Everts, J.W., 2001. Acute toxicity of locust insecticides to two indigenous invertebrates from Sahelian temporary ponds. *Ecotoxicol. Environ. Saf.* 48, 66–75.
- Leistra, M., Zweers, A.J., Warinton, J.S., Crum, S.J., Hand, L.H., Beltman, W.H., Maund, S.J., 2004. Fate of the insecticide lambda-cyhalothrin in ditch enclosures differing in vegetation density. *Pest Manag. Sci.* 60, 75–84.
- Lin, K., Zhou, S.S., Xu, C., Liu, W.P., 2006. Enantiomeric resolution and biotoxicity of methamidophos. *J. Agric. Food Chem.* 54, 8134–8138.
- Matsuda, K., Buckingham, S.D., Kleiler, D., Rauh, J.J., Gruaso, M., Sattelle, D.B., 2001. Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. *Trends Pharmacol. Sci.* 22, 573–580.

- Maul, J.D., Brennan, A.A., Harwood, A.D., Lydy, M.J., 2008. Effect of sediment-associated pyrethroids, fipronil, and metabolites on *Chironomus tentans* growth rate, body mass, condition index, immobilization, and survival. *Environ. Toxicol. Chem.* 27, 2582–2590.
- Maund, S.J., Hamer, M.J., Warinton, J.S., Kedwards, T.J., 1998. Aquatic ecotoxicology of the pyrethroid insecticide lambda-cyhalothrin: considerations for higher-tier aquatic risk assessment. *Pestic. Sci.* 54, 408–417.
- McCahon, C.P., Pascoe, D., 1988. Cadmium toxicity to the freshwater amphipod *Gammarus pulex* (L.) during the molt cycle. *Freshw. Biol.* 19, 197–203.
- Mokry, L.E., Hoagland, K.D., 1990. Acute toxicities of five synthetic pyrethroid insecticides to *Daphnia magna* and *Ceriodaphnia dubia*. *Environ. Toxicol. Chem.* 9, 1045–1051.
- Mortimer, M.R., Connell, D.W., 1994. Critical internal and aqueous lethal concentration of chlorobenzenes with the crab *Portunus pelagicus* (L.). *Ecotoxicol. Environ. Saf.* 28, 298–312.
- NCDA&CS, 2011. 2011 North Carolina Agriculture Statistics. North Carolina Department of Agriculture & Consumer Services in coordination with the United States Department of Agriculture National Agriculture Statistics Service. <http://www.ncagr.gov/stats/2011AgStat/AgStat2011.pdf>. Accessed 7 November 2011.
- NCDMF, 2011. Commercial Statistics, Crabs, Blue, Hard, 2010. North Carolina Division of Marine Fisheries. [www.ncfisheries.net/statistics/comstat/index.html](http://www.ncfisheries.net/statistics/comstat/index.html). Accessed 7 November 2011.
- O'Connor, N.J., Gregg, A.S., 1998. Influence of potential habitat cues on duration of the megalopal stage of fiddler crab *Uca pugnax*. *J. Crust. Biol.* 18, 700–709.
- O'Connor, N.J., Judge, M.L., 1997. Flexibility in timing of molting of fiddler crab megalopae: evidence from in situ manipulation of cues. *Mar. Ecol. Prog. Ser.* 146, 55–60.
- Peto, R., Peto, J., 1972. Asymptotically efficient rank invariant test procedures. *J. R. Stat. Soc. A* 132, 185–207.
- Posey, M.H., Alphin, T.D., Harwell, H., Allen, B., 2005. Importance of low salinity areas for juvenile blue crabs, *Callinectes sapidus* Rathbun, in river-dominated estuaries of southeastern United States. *J. Exp. Mar. Biol. Ecol.* 319, 81–100.
- Printes, L.B., Callaghan, A., 2004. A comparative study on the relationship between acetyl cholinesterase activity and acute toxicity in *Daphnia magna* exposed to anticholinesterase insecticides. *Environ. Toxicol. Chem.* 23, 1241–1247.
- Puglis, H.J., Boone, M.D., 2011. Effects of technical-grade active ingredient vs. commercial formulation of seven pesticides in the presence or absence of UV radiation on survival of green frog tadpoles. *Arch. Environ. Contam. Toxicol.* 60, 145–155.
- Ramach, S.M., Darnell, M.Z., Avissar, N.G., Rittschof, R., 2009. Habitat use and population dynamics of blue crabs, *Callinectes sapidus*, in a high-salinity embayment. *J. Shellfish. Res.* 28, 635–640.
- Rasmussen, J.J., Friberg, N., Larsen, S.E., 2008. Impact of lambda-cyhalothrin on a macroinvertebrate assemblage in outdoor experimental channels: implications for ecosystem functioning. *Aquat. Toxicol.* 90, 228–234.
- Rebach, S., French, D.P., 1996. Effects of Dimilin on the blue crab, *Callinectes sapidus*, in shallow-water habitats. *Estuaries* 19, 279–287.
- Reyns, N.B., Eggleston, D.B., 2004. Environmentally-controlled, density-dependent secondary dispersal in a local estuarine crab population. *Oecologia* 140, 280–288.
- Riechers, D.E., Wax, L.M., Liebl, R.A., Bush, D.R., 1994. Surfactant-increased glyphosate uptake into plasma membrane vesicles isolated from common lambsquarters leaves. *Plant Physiol.* 105, 1419–1425.
- Rodriguez, R.A., Epifanio, C.E., 2000. Multiple cues for induction of metamorphosis in larvae of the common mud crab *Panopeus herbstii*. *Mar. Ecol. Prog. Ser.* 195, 221–229.
- Sánchez-Bayo, F., Goka, K., 2006. Influence of light in acute toxicity bioassays of imidacloprid and zinc pyriithione to zooplankton crustaceans. *Aquat. Toxicol.* 78, 262–271.
- Sánchez-Fortún, S., Sanz, F., Barahona, M.V., 1995. Acute toxicities of selected insecticides to the aquatic arthropod *A. salina*. *Bull. Environ. Contam. Toxicol.* 54, 76–82.
- Sánchez-Fortún, S., Sanz, F., Barahona, M.V., 1996. Acute toxicity of several organophosphorous insecticides and protection by cholinergic antagonists and 2-PAM on *A. salina* larvae. *Arch. Environ. Contam. Toxicol.* 31, 391–398.
- Schwarzenbach, R.P., Gschwend, P.M., Imboden, D.M., 1993. *Environmental Organic Chemistry*. Wiley, New York.
- Scott, G.I., Fulton, M.H., Crosby, M., Key, P.B., Daugomah, J.W., Waldren, J., Strozier, E.S., Loudon, C., Chandler, G.T., Bidleman, T.F., Jackson, K., Hampton, T., Huffman, T., Schulz, A., Bradford, M., 1992. Agricultural Insecticide Runoff Effects on Estuarine Organisms; Correlating Laboratory and Field Toxicity Test, Ecophysiology Bioassays and Ecotoxicological Biomonitoring (Final Report). U.S. EPA, Gulf Breeze, FL.
- Song, M.Y., Stark, J.D., Brown, J.J., 1997. Comparative toxicity of four insecticides, including imidacloprid and tebufenozide, to four aquatic arthropods. *Environ. Toxicol. Chem.* 16, 2494–2500.
- Sucahyo, D., van Straalen, N.M., Krave, A., van Gestel, C.A.M., 2008. Acute toxicity of pesticides to the tropical freshwater shrimp *Caridina laevis*. *Ecotoxicol. Environ. Saf.* 69, 421–427.
- Tankersley, R.A., Wieber, M.G., 2000. Physiological responses of postlarvae and juvenile blue crabs *Callinectes sapidus* to hypoxia and anoxia. *Mar. Ecol. Prog. Ser.* 194, 179–191.
- Tisler, T., Jemec, A., Mozetic, B., Trebse, P., 2009. Hazard identification of imidacloprid to aquatic environment. *Chemosphere* 76, 907–914.
- Tsui, M.T.K., Chu, L.M., 2003. Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. *Chemosphere* 52, 1189–1197.
- USDA, 2008. North Carolina 2007 Chemical Use Estimates. United States Department of Agriculture, National Agricultural Statistics Service, North Carolina Field Office. <http://www.ncagr.gov/stats/environmental/ChemUse2007.pdf>. Accessed 4 January 2012.
- Venkateswara, Rao J., Kavitha, P., Jakka, N.M., Sridhar, V., Usman, P.K., 2007. Toxicity of organophosphates on morphology and locomotor behavior in brine shrimp, *Artemia salina*. *Arch. Environ. Contam. Toxicol.* 53, 227–232.
- Weis, J.S., Mantel, L.H., 1976. DDT as an accelerator of limb regeneration and molting in fiddler crabs. *Estuar. Coast. Mar. Sci.* 4, 461–466.
- Welch, J.M., Rittschof, D., Bullock, T.M., Forward Jr., R.B., 1997. Effects of chemical cues on settlement behavior of blue crab (*Callinectes sapidus*) postlarvae. *Mar. Ecol. Prog. Ser.* 1544, 143–153.
- Wolcott, D.L., De Vries, M.C., 1994. Offshore megalopae of *Callinectes sapidus*: depth of collection, molt stage and response to estuarine cues. *Mar. Ecol. Prog. Ser.* 109, 157–163.
- Zhu, Y., Stevens, C.F., 2008. Probing synaptic vesicle fusion by altering mechanical properties of the neuronal surface membrane. *Proc. Natl. Acad. Sci. U. S. A.* 105, 18018–18022.