Vol. 392: 157–165, 2009
doi: 10.3354/meps08205

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published October 19

Ambient Malathion concentrations modify behavior and increase mortality in blue crabs

Christina M. Wendel, Delbert L. Smee*

Texas A&M University - Corpus Christi, 6300 Ocean Drive, Corpus Christi, Texas 78412, USA

ABSTRACT: Organophosphate insecticides can compromise water quality and harm non-target species unintentionally. In this study, we examined the effects of a commonly used organophosphate insecticide, Malathion, on blue crabs *Callinectes sapidus*, which are an economically and ecologically important estuarine species. Adult and juvenile crabs were exposed to environmentally-occurring concentrations of Malathion at 2 salinity levels to determine how these factors would affect crab behavior and mortality. Initial Malathion concentrations of 1.0 ppb and 11.2 ppb caused a significant increase in mortality of juvenile blue crabs, and concentrations of 11.2 ppb caused a significant increase in mortality of adults, all within 36 h after exposure. Our results indicated that salinity did not affect blue crabs' susceptibility to Malathion at these concentrations. We measured the time needed for adult blue crabs to right themselves when placed on their backs before and after exposure to Malathion. After 1 h of exposure to Malathion concentrations of 11.2 ppb, adult crabs took significantly longer to right themselves, indicating that short-term exposure to Malathion at ecologically relevant concentrations can interfere with essential behaviors of blue crabs. Blue crab populations have been declining in recent years, and their decline has primarily been linked to overfishing. However, other mechanisms including pollution and disease may also contribute to this problem and require further investigation. Since blue crabs are important components of estuarine food webs, changes in blue crab mortality rates and behavior may have important consequences for entire estuarine systems.

KEY WORDS: *Callinectes sapidus* · Estuary · Malathion · Nonlethal effect · Pesticide · Salinity · Synergistic effects

INTRODUCTION

Blue crabs *Callinectes sapidus* are important components of estuarine systems (e.g. Virnstein 1977, Posey & Hines 1991, Eggleston et al. 1992, Micheli 1997). They are also economically important, as the annual commercial catch in 2007 generated $128.6 million in the United States (NMFS 2007). Despite their recognized importance both ecologically and economically, blue crab populations are declining in coastal areas throughout the US (Guillory et al. 1998, Hammerschmidt et al. 1998, NMFS 2007, Sutton & Wagner 2007, NOAA 2008). Although blue crab populations undergo seasonal fluctuations (Hines & Ruiz 1995), over the last 10 yr, there has been a general decrease in the total weight of blue crabs caught commercially in US estuaries, from 98 million kg in 1998 to 62 million kg in 2007 (NMFS 2007). These declines have often been associated with overfishing (Guillory et al. 1998, Hammerschmidt et al. 1998), but other factors, including environmental degradation and disease, may also contribute to the decline of blue crabs and require further investigation (Hines & Ruiz 1995, Guillory et al. 1998, Hammerschmidt et al. 1998, Sheppard et al. 2003, NMFS 2007, Sutton & Wagner 2007, NOAA 2008).

Pesticides (i.e. herbicides, insecticides) are used to eliminate unwanted pests but often affect non-target species unintentionally (Key et al. 1998, De Guise et al.

*Corresponding author. Email: lee.smee@tamucc.edu

2004, Relyea 2005a,b). Pesticides can enter estuarine systems through overspray or runoff and affect a variety of organisms including fish and invertebrates (Pedersen et al. 2006). Many pesticides are designed to kill terrestrial arthropods. As arthropods, blue crabs can be susceptible to these chemicals (Pearson & Olla 1980, Horst & Walker 1995, Kennish & Ruppel 1996, Rebach & French 1996, Whiting et al. 1996, Lee & Oshima 1998, Pierce et al. 2001). For example, Methoprene runoff damaged post-molt adult blue crabs, decreased the number of successful hatching zoeae, was toxic to megalopae by delaying the first molt, and caused 80% mortality of larvae after 10 d (Horst & Walker 1999). Such studies suggest that accidental release of pesticides into estuaries may have negative effects on blue crab survival.

Accumulation of pesticides within tissues of crabs and their effects on crab mortality have been well studied (McKenzie 1970, Petrocelli et al. 1974, Sheridan 1975, Costlow 1979, Schimmel et al. 1979, Bookhout et al. 1980, Johnston & Corbett 1985, 1986a,b), but how pesticides affect key behaviors such as foraging or predator avoidance has not been investigated. Since blue crabs are important components of estuarine food webs (Posey & Hines 1991, Eggleston et al. 1992, Silliman & Bertness 2002), changes in their ability to forage or avoid higher-order consumers may have large but currently unquantified effects on estuarine communities. Pesticides have been shown to affect foraging and predator avoidance in other crustaceans (e.g. crayfish, Wolf & Moore 2002). Wolf & Moore (2002) investigated the effects of environmentally relevant concentrations of the herbicide Metolachlor on the ability of crayfish to respond to 2 different chemical cues (food and injured conspecifics). Their findings revealed that minute doses of Metolachlor negatively affected the ability of crayfish to find food and respond to alarm cues.

Salinities above 34 psu are stressful to blue crabs and cause an increase in blue crab mortality (Tagatz 1969, Costlow 1979, Guillory et al. 2001). Higher salinities may also increase vulnerability to pesticides. For example, the organophosphate pesticide Fenitrothion was toxic to adult blue crabs at higher salinity (34 psu) and temperature (22°C) as compared to lower salinity (17 psu) and temperature (17°C) (Johnston & Corbett 1985, 1986a,b). This lower toxicity was related to the blue crabs' ability to detoxify the pesticide in lower salinity conditions, producing a less toxic metabolite that was easily released into the surrounding water (Johnston & Corbett 1986a,b). Another important factor was that at lower salinities, the oxidation of the pesticide Fenitrothion to its toxic metabolite Fenitrooxon does not occur as easily as it does at higher salinities (Johnston & Corbett 1986a,b).

Unfortunately, average salinity levels are increasing in many estuaries as fresh water is diverted inland to meet demand and extended droughts have decreased available fresh water. More than 50% of fresh water is diverted inland by reservoirs, dams, or other removal processes (Postel et al. 1996, Powell et al. 2002). In coastal Texas, reduced freshwater inputs and extended droughts have elevated salinities in estuaries (Alber 2002, Montagna et al. 2002, Powell et al. 2002). The decrease in fresh water and subsequent increases in salinity affect numerous species, including blue crabs, that depend upon brackish waters with suitable habitats (Chambers 1993, Hammerschmidt et al. 1998).

We explored the effects of the insecticide Malathion and its interaction with salinity on blue crab survival and behavior. Malathion is an organophosphate insecticide that is widely used for mosquito control. In 2001, approximately 9.1 million kg of Malathion were used in the US (Donaldson et al. 2002, Alvarez & Fuiman 2006). Malathion is highly soluble, with a half-life of less than 7 d, and is often found in surface waters via runoff and overspray (Eto 1974, Wolfe et al. 1977, Rand 1995, EXTOXNET 1996, USEPA 2006). Malathion is toxic to larval amphibians at concentrations ranging from 1.25 to 5.9 mg l^{-1} (Relyea 2004) and was associated with large mortalities of American lobsters in Long Island Sound in 1999 (De Guise et al. 2004). Malathion has been found in Texas bays at concentrations ranging from 0.32 ppb to 1.0 ppb (Alvarez & Fuiman 2006) and in other estuaries at concentrations as high as 11.0 ppb (Bradley et al. 1997). Malathion residues before and after aerial application were measured in Hale County, Texas, in 1967, and after 4 h, the maximum concentration present in local waters was 0.5 ppm, but it degraded after 24 h (Guerrant et al. 1970). Because of its widespread use and its documented presence in estuarine systems, Malathion is an environmentally relevant pesticide for use in examining potential effects of pesticides on blue crab mortality and behavior.

Synergistic effects of simultaneous stressors can have significantly greater effects on organisms than when stressors are presented in isolation. For example, predator cues change the morphology of tadpoles and decrease their fitness (Relyea & Werner 1999, Relyea 2001, 2003a). The synergistic interactions of predator cues and pesticides caused almost 100% mortality of tadpoles within 72 h, even though these stressors were not lethal to amphibians when presented individually (Relyea & Mills 2001, Relyea 2003b, 2004). For blue crabs, increased temperature and salinity have been shown to magnify the harmful effects of Fenitrothion, another organophosphate insecticide (Johnston & Corbett 1985, 1986a,b). Mortality, degradation, and autotomy were examined by Johnston & Corbett (1985,

1986a,b), but behavioral changes related to exposure were not investigated.

The purpose of this research was to determine how Malathion concentration and salinity affect the mortality and behavior of blue crabs. We measured the effects of environmentally-occurring concentrations of Malathion on the mortality of juvenile and adult blue crabs in favorable and unfavorable salinities. We also examined sublethal effects of Malathion on the behavior of adult blue crabs. Limited data are available on how blue crabs are affected by pesticides or other contaminants in the Gulf of Mexico (Guillory et al. 2001), and changes in blue crab behavior resulting from Malathion exposure have not been reported. Our results suggest that unintentional Malathion exposure has lethal and harmful sublethal effects on both adult and juvenile blue crabs. If pesticides such as Malathion increase blue crab mortality or alter their behaviors, then these chemicals may have large but unquantified effects on blue crab populations and on estuarine ecosystems in general.

MATERIALS AND METHODS

Life stages. To better understand the potential effects of Malathion on blue crab populations, we elected to study the effects of Malathion on 2 life stages of blue crabs: adult (90 to 193 mm CW, measured along the largest lateral spine, tip to tip) and juvenile (8 to 35 mm CW). Earlier blue crab life stages have been studied extensively, and are more sensitive to pesticides and other contaminants than are adults (Sandoz & Rogers 1944, Lee & Noone 1995, Lee et al. 1996, Whiting et al. 1996, Lee & Oshima 1998, Horst & Walker 1999).

Exposure time. Estuarine organisms may be inundated with Malathion or other contaminants for extended periods in Texas bays and estuaries because of extended water residence times (Solis & Powell 1999, Engle et al. 2007). Residence time, defined as the time needed for water in a bay to be completely exchanged with new water from the Gulf of Mexico, was measured in several estuaries of coastal Texas and ranged from 9.4 to 360 d (Solis & Powell 1999). These residence times are all greater than the half-life of Malathion (<7 d), and thus, blue crabs likely experience continuous Malathion exposure in natural settings in Texas estuaries. In our experiment, we elected to use an initial exposure to Malathion and allow the crabs to remain in the container with the pesticide to mimic continuous exposure conditions likely experienced in Texas estuaries with little water exchange.

Malathion concentrations and introduction. Three concentrations of Malathion that had previously been measured in estuaries were used in these experiments: low (0.32 ppb), intermediate (1.0 ppb), and high (11.2 ppb) (Bradley et al. 1997, Alvarez & Fuiman 2006). Malathion has a short half-life and breaks down within 7 d depending on the abiotic conditions present (Eto 1974, Rand 1995, USEPA 2006). Thus, the concentrations used here may be conservative estimates of initial natural exposure experienced by blue crabs.

Animal collection and care. Adult blue crabs (90 to 193 mm carapace width, CW) were collected from the upper Laguna Madre, Corpus Christi Bay, and Nueces Bay, Texas, using mesh crab traps (pots) baited with fresh fish. Juvenile blue crabs (8 to 35 mm CW) were collected from Corpus Christi Bay using a seine net. After collection, blue crabs were transported to the laboratory where CW and sex were recorded. Adult blue crabs were then placed individually into glass aquaria measuring (51 × 25 × 30 cm) containing 15 l of aerated artificial seawater made 1 of 2 salinities: favorable (17 to 21) or unfavorable (36 to 40). After collection, juvenile blue crabs were returned to the laboratory and placed into aquaria (51 × 25 × 30 cm, L × W × H) at either the favorable (17 to 21) or unfavorable (36 to 40) salinities. Juvenile blue crabs were numerous and were initially housed in glass aquaria at either favorable or unfavorable salinities in groups of ~15 before being transferred to smaller containers where they were housed individually for experimental assays. Crabs were maintained in these aquaria for a 2 d acclimation period prior to use in the experiment.

We were initially concerned that oxygen limitation might negatively affect the juvenile crabs in a small volume of water (0.125 l) used in this experiment. In preliminary assays, however, 95% of juvenile crabs tested survived in these small containers for 10 d (twice the time required for our experiment), at which time they were released back into the estuary unharmed. We therefore felt that oxygen limitation was not a factor in our experiments. Moreover, we saw only 2 crab deaths in these containers in control treatments during the assay, suggesting that container size had little effect on the outcome of our experiment.

All seawater used for these experiments was artificially made using tap water treated with Top Fin® tap water dechlorinator and Instant Ocean™ to create and maintain the desired salinity levels.

Effects of Malathion on blue crab mortality. ***Experimental design:*** After an acclimation period of 2 d, the juvenile blue crabs were transferred from their holding aquaria and placed individually into plastic containers (4.0 × 4.5 cm, height × radius) containing 0.125 l of water at 1 of 2 salinities and containing Malathion at low (0.32 ppb), intermediate (1.0 ppb), or high (11.2 ppb) concentrations or a control that did not contain Malathion. This created a 4 × 2 factorial design.

Crabs were randomly assigned to treatments, but we took care to ensure that equal numbers of male and female crabs were used in all treatments and that crab size did not bias our results. All treatments were interspersed.

Malathion solutions were made by adding a 50% Malathion solution into a 1.0 l flask using a micropipetter to achieve the desired concentration. The flask was swirled to mix the solution, and then the Malathion solution was added to each plastic container prior to introduction of the crab. Each plastic container was used only once to avoid any lingering effects of Malathion between treatments.

Adult blue crabs assays were identical to those performed on juveniles, except adult crabs were tested in the same aquaria in which they were acclimated. Using larger aquaria was necessary due to adult crab size, and because we needed a larger container to perform behavioral assays with adult crabs as described below. Each aquarium contained 15 l of aerated seawater at 1 of 2 salinities (17 to 21 or 36 to 40) and 1 of 4 Malathion concentrations: 0 ppb (control), 0.32 ppb, 1 ppb, or 11 ppb, creating a 4 × 2 factorial design. Crabs were randomly assigned to treatments, and all treatments were interspersed. We achieved these concentrations of Malathion by micropipetting a desired concentration of a 50% Malathion solution (or plain seawater control) into each aquarium and gently stirring with a glass stirring rod.

Data collection and analysis: Mortality of juvenile and adult blue crabs was measured in each treatment by recording the number of 12 h periods elapsed between Malathion introduction and crab death over a total of 5 d (max time periods elapsed = 10 or 120 h). Death was determined by lack of crab movement after gently prodding them with a glass rod; this standard has been used by other authors (Johnston & Corbett 1985). The 4 × 2 design created 8 treatments. Some of the juvenile crabs perished during the acclimation period, and we therefore had unequal sample sizes for the treatments. However, we did use at least 21 juvenile crabs in each treatment (177 total juveniles). Twelve adult blue crabs were tested in each treatment.

We used a 2-way analysis of variance (ANOVA) to compare the effects of salinity and pesticide concentration level on survival time, with salinity and Malathion concentration as fixed factors. Separate 2-way ANOVAs were used to compare the effects of Malathion on survival of juvenile and adult crabs. The percentage of time periods during which crabs were alive was arcsine transformed to meet ANOVA assumptions of normality. In both ANOVA models for juvenile and adult crabs, Malathion concentration had a significant effect on mortality, but salinity did not, nor was the interaction term significant. Therefore, we used a Tukey-Kramer post hoc test to test for pairwise differences among pesticide concentration effects on mortality (Sokal & Rohlf 1995) because the sample sizes were unequal (Day & Quinn 1989).

Effects of Malathion on blue crab behavior. ***Experimental design:*** Behavioral experiments were performed in conjunction with the adult blue crab mortality study, and crab housing methods were the same as described above. Using the established 4 × 2 design (2 salinities and 4 Malathion concentrations), behavioral changes caused by Malathion exposure were examined.

Description of behavior: Righting time behavior (the amount of time it takes a crab to resume its normal position after being placed on its back) was used as an indicator of the animals' overall ability to move after Malathion exposure. If blue crabs are unable to move or right themselves, they are likely less able to forage, find mates, avoid predators, and perform other vital activities. Malathion affects neurological function in other organisms, and righting time was used as a measurement to see if behaviors are affected by exposure. This technique has been successfully used by other authors to investigate the effects of toxins on crab behavior (Shirley & Stickle 1982, Zhou & Shirley 1995).

Data collection and analysis: Righting time was measured by recording the elapsed time to the nearest tenth of a second from placing the crab on its back to when the crab reached half-way (90°) to its normal position (Shirley & Stickle 1982, Zhou & Shirley 1995). The required time for the crabs to right was measured twice: after the 2 d acclimation period (immediately before the introduction of the pesticide) and 1 h after pesticide exposure. The experiments included 16 treatments (4 pesticide levels × 2 salinity levels). We subtracted the initial righting time before Malathion exposure from the righting time after 1 h of Malathion exposure and compared this value using a 2-way ANOVA with salinity and Malathion concentration as fixed factors. Malathion concentration had a significant effect on mortality but salinity did not, nor was the interaction term significant. Therefore, we used a Tukey-Kramer post hoc test to test for pairwise differences in righting time of crabs exposed to different concentrations of Malathion (Day & Quinn 1989, Sokal & Rohlf 1995).

RESULTS

Juvenile mortality

Malathion concentration had significant effects on juvenile blue crab mortality ($F_{7, 169} = 68.05$, $p << 0.001$, Fig. 1), but salinity did not ($F_{7, 169} = 0.35$, $p = 0.55$). The

interaction term was also not significant ($F_{7, 169}$ = 1.56, p = 0.21). Post hoc analysis revealed significantly higher mortality among juvenile blue crabs at the intermediate and high Malathion concentrations as compared to controls, and the high Malathion concentration caused significantly greater mortality among juvenile blue crabs than did the intermediate concentration. Ninety percent (40 out of 44) of the juvenile crabs exposed to the high Malathion concentration and 30% (14 out of 46) of the juvenile crabs exposed to the intermediate Malathion concentration died. Of 131 juvenile crabs exposed to Malathion, 61 died, and 53 of those that died succumbed to the pesticide within 36 h after exposure. In contrast, 46 juvenile crabs were used as controls, and only 2 perished during the experiment.

Adult mortality

As with the juvenile crabs, Malathion had a significant effect on adult crab mortality ($F_{7, 87}$ = 3.04, p = 0.03) but the salinity factor ($F_{7, 87}$ = 0.68, p = 0.41) and interaction term ($F_{7, 87}$ = 1.76, p = 0.16) were not significant. Post hoc analysis revealed a significant increase in mortality in the high Malathion concentrations as compared to all other treatments. We did not detect a significant difference in mortality between the no-pesticide control, low, and intermediate Malathion concentrations, although the intermediate concentration did have higher mortality than the no-pesticide and low pesticide treatments (Fig. 2). Adult crabs were less likely to die after Malathion exposure than juveniles (Fig. 3). Of 71 adult crabs exposed to Malathion, 11 died, while 24 were used as controls and only 1 died during the experiment. Of the 11 that died after Malathion exposure, 6 were in the high Malathion concentration and 4 were in the intermediate concentration. In total, 25% (6 out of 24) adult crabs died after exposure to the high concentration of Malathion, and 17% (4 out of 24) died after exposure to the intermediate concentration.

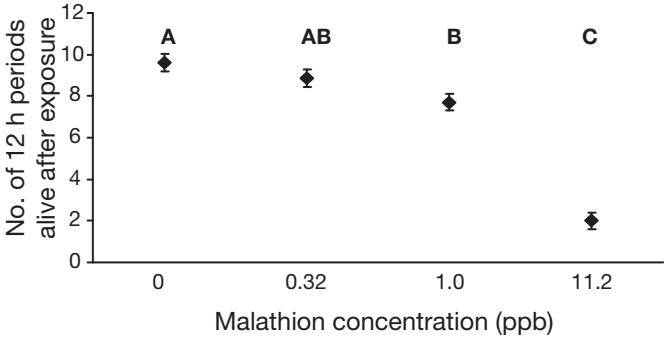

Fig. 1. *Callinectes sapidus.* Mean (±SE) survival time of juvenile blue crabs in controls and 1 of 3 Malathion concentrations. Survival time ranged from 12 h (1 period) to 120 h (10 periods), which was the duration of the experiment and maximum possible survival time. ANOVA revealed that Malathion concentrations had a significant effect on crab survival time (p << 0.01). Letters denote significant pairwise differences in mean survival time of juvenile blue crabs based upon a Tukey-Kramer post hoc test. Intermediate (1.0 ppb) and high (11.2 ppb) Malathion concentrations caused significantly higher mortality than controls. Sample sizes for each treatment were 46, 43, 44, and 44 for no-pesticide controls, and low (0.32 ppb), intermediate, and high concentrations, respectively

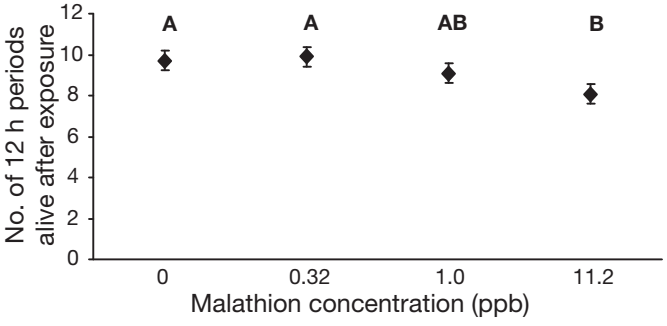

Fig. 2. *Callinectes sapidus.* Mean (±SE) survival time of adult blue crabs in controls and 1 of 3 Malathion concentrations. Survival time ranged from 12 h (1 period) to 120 h (10 periods), which was the duration of the experiment and maximum possible survival time. ANOVA revealed that Malathion concentrations had a significant effect on crab survival time (p << 0.01). Letters denote significant pairwise differences in mean survival time of adult blue crabs based upon a Tukey-Kramer post hoc test. Sample sizes for each treatment were 24 for the no-pesticide controls, intermediate (1.0 ppb), and high (11.2 ppb) concentrations, and 23 for the low (0.32 ppb) concentration

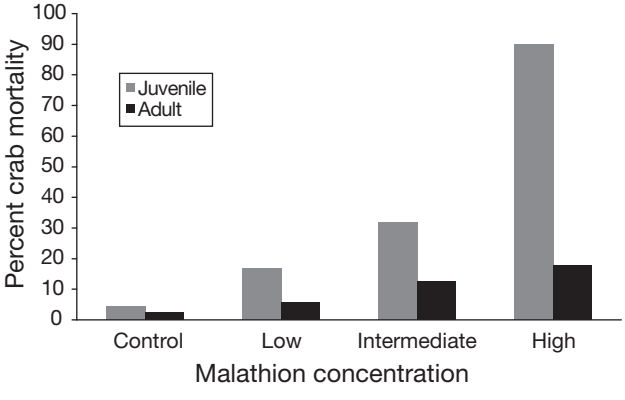

Fig. 3. *Callinectes sapidus.* Comparison of the percentages of juvenile and adult crabs that died in pesticide-free controls and in low (0.32 ppb), intermediate (1.0 ppb), and high (11.2 ppb) Malathion concentrations

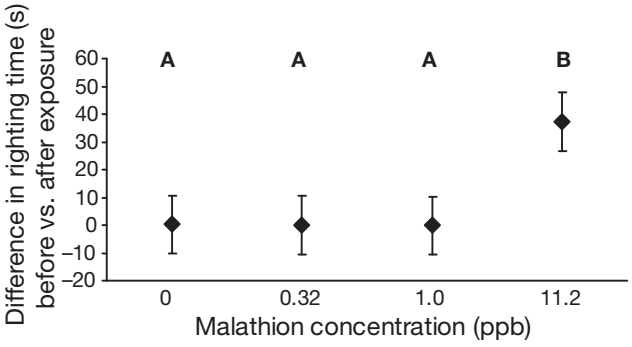

Fig. 4. *Callinectes sapidus.* Mean (±SE) difference in righting time (s) of adult blue crabs after Malathion exposure. Negative difference values indicate that it took less time to right after exposure, while positive values indicate an increase in righting time. ANOVA revealed that Malathion concentrations had a significant effect on the difference in righting time ($p < 0.05$). Letters denote significant pairwise differences in mean survival time of adult blue crabs based upon a Tukey-Kramer post hoc test. Blue crabs took significantly longer to right themselves in the high (11.2 ppb) Malathion concentration. Sample sizes for each treatment were 24 for the no-pesticide controls, intermediate (1.0 ppb), and high concentrations, and 23 for the low (0.32 ppb) concentration

Adult righting time

Malathion exposure caused a significant increase in blue crab righting time ($F_{7, 87} = 3.123$, $p = 0.03$, Fig. 4), but salinity did not ($F_{7, 87} = 0.340$, $p = 0.56$), and the interaction term was also not significant ($F_{7, 87} = 1.37$, $p = 0.26$). Post hoc analysis revealed that in the highest Malathion concentration of 11.2 ppb, blue crabs took significantly longer to right themselves, but the low and intermediate concentrations were not statistically distinguishable from the control.

DISCUSSION

Malathion has been found in low concentrations in estuarine systems (Bradley et al. 1997, Alvarez & Fuiman 2006), and our results indicate that at these ambient concentrations Malathion increases blue crab mortality and alters their behavior. Malathion is an organophosphate with a relatively short half-life of ~7 d (Eto 1974, Wolfe et al. 1977, Rand 1995, EXTOXNET 1996, USEPA 2006), so the concentrations found in nature and used in this study are likely to be lower than initial concentrations that enter estuaries. Exposure to Malathion at seemingly low, but environmentally-occurring, concentrations increased the mortality of juvenile and adult blue crabs and significantly increased the length of time adult blue crabs needed to right themselves when placed on their backs. The increased righting time suggests that Malathion interferes with crab physiological processes and likely makes them more vulnerable to predators and less able to carry out critical functions such as foraging.

We elected to expose blue crabs to Malathion continuously to mimic natural conditions within local estuaries. Due to the low tidal exchange in Texas estuaries and the potential volume of water entering the estuaries containing pesticides, it is unlikely that pesticide-free water entering from the Gulf of Mexico would be exchanged with pesticide-laden water before the complete degradation of Malathion occurs (Eto 1974, Solis & Powell 1999, Engle et al. 2007). Although we did not test how brief exposures of Malathion would affect blue crabs, we did note a significant alteration of behavior after only 1 h of Malathion exposure, indicating that short-term exposures can have negative effects on crabs.

The toxicity of Malathion results from the inhibition of acetylcholinesterase, which interferes with nervous system functions, resulting in paralysis and death (Eto 1974, Smith 1992). Death in arthropods exposed to Malathion and other organophosphate chemicals occurs via respiratory paralysis (Murphy 1975). Of the crabs that died after Malathion exposure, there were unique behaviors observed that suggested the crabs died from asphyxiation. In particular, the scathognathites of these crabs were expanded open, and both the juvenile and adult crabs were found upside down when deceased. These observations suggest that Malathion has a negative effect on neurological functions, in particular a reduction in the crab's ability to acquire oxygen, which causes death.

The lethal effects of Malathion on blue crabs may result from the pesticide itself, from a toxic intermediate formed as Malathion breaks down, or from a combination of both. The chemical structure of Malathion includes a carboxy ester group, and this group is susceptible to hydrolysis (Eto 1974). During Malathion breakdown via hydrolysis, the carboxy ester group produces the metabolic intermediate Malaoxon (Eto 1974, Smith 1992). Similar to the organophosphate pesticide Fenitrothion, and its degradation product Fenitrooxon, the Malaoxon intermediate metabolite is actually more toxic than the parent compound Malathion (Johnston & Corbett 1985, 1986a,b, Smith 1992). The intermediates Fenitrooxon and Malaoxon are not very stable, and they break down and degrade rapidly (von Rumker et al. 1974). Thus, both Malathion and Malaoxon may have contributed to blue crab mortality, particularly at the high concentrations.

Juvenile blue crabs are more sensitive to contaminants than are adults (Lee & Noone 1995, Lee et al. 1996, Lee & Oshima 1998, Horst & Walker 1999), and in this study juvenile crabs suffered much higher mortal-

ity than adults when exposed to the same concentrations of Malathion (Fig. 3). Juvenile blue crabs molt more frequently than adults, sometimes in as little as 2 wk (Rebach & French 1996), and frequent molting may increase sensitivity to toxins. In this study, juveniles molted in all treatments. Three juveniles molted in the no-pesticide control and all survived, whereas the 8 that molted in treatments containing Malathion died (3 in low, 4 in intermediate, and 1 in high). Thus, in natural systems, even at concentrations below 1.0 ppb, Malathion may increase juvenile crab mortality if they are exposed during or soon after molting. Only 1 adult crab molted. It was in the control and survived the duration of the experiment.

Salinity did not increase the effects of Malathion on blue crabs, which was surprising considering that increasing salinity from 17 to 34 significantly increased blue crab mortality caused by the organophosphate pesticide Fenitrothion (Johnston & Corbett 1985, 1986a,b). Higher salinities in general can be stressful to crabs (Costlow 1979, Guillory et al. 2001), and Johnston & Corbett (1985) noted that Fenitrothion breaks down more rapidly at higher salinities, producing a higher concentration of its toxic intermediate form. Blue crabs were also less able to detoxify Fenitrothion at higher salinities (Johnston & Corbett 1986a,b). One possible explanation for the absence of a salinity effect in this study may be that we used an almost 50% lower initial concentration of Malathion (highest was 11.2 ppb) than did Johnston & Corbett (1985) when investigating the effects of Fenitrothion (initial concentration 20 ppb). Further studies are needed to more carefully examine the relationship between salinity and Malathion concentration to determine if the different effects of these pesticides on blue crabs at different salinities are related to chemical differences between Malathion and Fenitrothion, initial concentration differences, or other factors. Regardless, our results along with those of Johnston & Corbett (1985, 1986a,b) indicate that organophosphate pesticides can increase blue crab mortality at ecologically relevant concentrations.

Malathion, at environmentally-occurring concentrations, was found to cause increased mortality of both juvenile and adult blue crabs. Lethal effects of pesticides and other contaminants are important to identify, but the sublethal effects experienced by these organisms *in situ* may not be as evident, but may have significant effects that require further investigation. To date, mass blue crab mortalities have not been associated with Malathion. However, unlike mass die-off of lobsters, increased mortality of juvenile blue crabs from a Malathion exposure may go unnoticed, as these crabs would be consumed by higher trophic levels. Sublethal effects associated with Malathion may have considerable effects in estuarine ecosystems by modifying critically important activities such as foraging, mate location, and predator avoidance of blue crabs. Our results suggest pesticide exposure should be considered as a contributing factor in the decline of blue crab populations.

Acknowledgements. This project was supported by the Texas Research Development Fund, a Texas A&M – Corpus Christi University Faculty Enhancement Grant, Texas A&M University – Corpus Christi Start-UP Funds, and the National Science Foundation BIO-OCE (Grant no. 0648433) issued to D.L.S. M. Nipper, T. Shirley, and G. Stunz provided important comments that improved the submission. Cos-Way Bait & Tackle helped with the collection of blue crabs. The TAMU-CC Marine Ecology Lab, especially P. Horsman and J. Reuther, provided extensive help with crab collection and with animal care.

LITERATURE CITED

Alber M (2002) A conceptual model of estuarine freshwater inflow management. Estuaries 25:1246–1261

Alvarez MC, Fuiman L (2006) Ecological performance of red drum (*Sciaenops ocellatus*) larvae exposed to environmental levels of the insecticide Malathion. Environ Toxicol Chem 25:1426–1432

Bookhout CG, Costlow JD, Monroe R (1980) Kepone effects on larval development of mud crab and blue crab. Water Air Soil Pollut 13:57–77

Bradley A, Woolford P, Gallavan R, Lee P, Troiano J (1997) Environmental monitoring results of the Mediterranean fruit fly eradication program, Ventura County, 1994-1995. EH 97-05. Final Report. State of California Environmental Protection Agency, Department of Pesticide Regulation, Sacramento, CA

Chambers JR (1993) The need for strong habitat protection in rebuilding U.S. marine fisheries. Draft Report. National Marine Fisheries Service, Silver Spring, MD

Costlow JD (1979) Effect of Dimilin on development of larvae of the stone crab *Menippe mercenaria,* and the blue crab *Callinectes sapidus*. In: Vernberg WB, Calabrese A, Thurburg FP, Vernberg FJ (eds) Marine pollution: functional responses. Academic Press, New York, p 355–363

Day RW, Quinn GP (1989) Comparisons of treatments after an analysis of variance in ecology. Ecol Monogr 59:433–463

De Guise S, Maratea J, Perkins C (2004) Malathion immunotoxicity in the American lobster (*Homarus americanus*) upon experimental exposure. Aquat Toxicol 66:419–425

Donaldson D, Kiely T, Grube A (2002) Pesticides, industry scales and usage: 1998 and 1999 market estimates. USEPA Rep No. 733-R-02-001, USEPA, Washington, DC

Eggleston DB, Lipcius RN, Hines AH (1992) Density-dependent predation by blue crabs upon infaunal clam species with contrasting distribution and abundance patterns. Mar Ecol Prog Ser 85:55–68

Engle VD, Kurtz JC, Smith LM, Chaney C, Bourgeois P (2007) A classification of U.S. estuaries based on physical and hydrological attributes. Environ Monit Assess 129: 397–412

Eto M (1974) Organophosphate pesticides: organic and biological chemistry. CRC Press, Cleveland, OH

EXTOXNET (Extension Toxicology Network) (1996) Pesticide information profiles: Malathion. Available at http://extoxnet.orst.edu/pips/malathio.htm

Guerrant GO, Fetzer LE Jr, Miles JW (1970) Pesticide residues in Hale County, Texas, before and after ultra-low volume aerial application of Malathion. Pestic Monit J 4: 14–20

Guillory V, Perry H, Wagner T, Hammerschmidt P, Heath S, Moss C (1998) The Gulf of Mexico blue crab fishery: historical trends, status, management, and recommendations. J Shellfish Res 17:395–403

Guillory V, Perry H, VanderKooy S (eds) (2001) The blue crab fishery of the Gulf of Mexico, United States: a regional management plan. Gulf States Marine Fisheries Commission, No. 96, National Oceanic and Atmospheric Administration Award Number NA56FI0085

Hammerschmidt P, Wagner T, Lewis G (1998) Status and trends in the Texas blue crab (*Callinectes sapidus*) fishery. J Shellfish Res 17:405–412

Hines AH, Ruiz GM (1995) Temporal variation in juvenile blue crab mortality: nearshore shallows and cannibalism in Chesapeake Bay. Bull Mar Sci 57:884–901

Horst MN, Walker AN (1995) Biochemical effects on diflubenzuron on chitin synthesis in the postmolt blue crab *Callinectes sapidus.* J Crustac Biol 15:401–408

Horst MN, Walker AN (1999) Effects of the pesticide Methoprene on morphogenesis and shell formation in the blue crab *Callinectes sapidus.* J Crustac Biol 19:699–707

Johnston JJ, Corbett MD (1985) The effects of temperature, salinity and a simulated tidal cycle on the toxicity of Fenitrothion to *Callinectes sapidus.* Comp Biochem Physiol Part C Toxicol Pharmacol 80:145–149

Johnston JJ, Corbett MD (1986a) The effects of salinity and temperature on the *in vitro* metabolism of the organophosphorous insecticide Fenitrothion by the blue crab, *Callinectes sapidus.* Pestic Biochem Physiol 26:193–201

Johnston JJ, Corbett MD (1986b) The uptake and *in vivo* metabolism of the organophosphate insecticide fenitrothion by the blue crab, *Callinectes sapidus.* Toxicol Appl Pharmacol 85:181–188

Kennish MJ, Ruppel BE (1996) DDT contamination in selected estuarine and coastal marine finfish and shellfish of New Jersey. Arch Environ Contam Toxicol 31:256–262

Key PB, Fulton MH, Scott GI, Layman SL, Wirth EF (1998) Lethal and sublethal effects of Malathion on three life stages of the grass shrimp, *Palaemonetes pugio.* Aquat Toxicol 40:311–322

Lee RF, Noone T (1995) Effects of reproductive toxicants on lipovitellin in female blue crabs, *Callinectes sapidus.* Mar Environ Res 39:151–154

Lee RF, Oshima Y (1998) Effects of selected pesticides, metals, and organometallics on development of blue crab embryos. Mar Environ Res 46:479–482

Lee RF, O'Malley K, Oshima Y (1996) Effects of toxicants on developing oocytes and embryos of the blue crab, *Callinectes sapidus.* Mar Environ Res 42:125–128

McKenzie MD Jr (1970) Fluctuations in abundance of blue crab and factors affecting mortalities. Tech Rep 1, South Carolina Resources Department Marine Resources, Charleston, SC

Micheli F (1997) Effects of predator foraging behavior on patterns of prey mortality in marine soft bottoms. Ecol Monogr 67:203–224

Montagna P, Alber M, Doering P, Conner M (2002) Freshwater inflow: science, policy, management. Estuaries 25: 1243–1245

Murphy SD (1975) Pesticides. In: Casarett LJ, Doull J (eds) Toxicology, the basic science of poisons. MacMillan Publishing, New York, p 408–453

NMFS (National Marine Fisheries Service) (2007) Annual commercial landing statistics. Fisheries Statistics Division, Department of Commerce, National Oceanographic and Atmospheric Administration. Available at: www.st.nmfs.noaa.gov/st1/commercial/landings/annual_landings.html

NOAA (National Oceanographic and Atmospheric Administration) (2008) Number of blue crabs in bay remains below long-term average. Blue Crab Advisory Report News from NOAA 28 July 2008. Department of Commerce. Available at: http://chesapeakebay.noaa.gov

Pearson WH, Olla BL (1980) Threshold for detection of naphthalene and other behavioral responses by the blue crab, *Callinectes sapidus.* Estuaries 3:224–229

Pedersen JA, Yeager M, Suffet IH (2006) Organophosphorous insecticides in agricultural and residential runoff: field observations and implications for total maximum daily load development. Environ Sci Technol 40:2120–2127

Petrocelli SR, Anderson JW, Hanks AR (1974) DDT and dieldrin residues in selected biota from San Antonio Bay, Texas–1972. Pestic Monit J 8:167–171

Pierce DC, Butler KD, Roer RD (2001) Effects of exogenous N-acetylhexosaminidase on the structure and mineralization of the post-ecdysial exoskeleton of the blue crab, *Callinectes sapidus.* Comp Biochem Physiol B Biochem Mol Biol 128:691–700

Posey MH, Hines AH (1991) Complex predator-prey interactions within an estuarine benthic community. Ecology 72:2155–2169

Postel SL, Daily GC, Ehrlich PR (1996) Human appropriation of renewable freshwater. Science 271:785–788

Powell G, Matsumoto J, Brock D (2002) Methods for determining minimum freshwater inflow needs of Texas bays and estuaries. Estuaries 25:1262–1274

Rand GM (ed) (1995) Fundamentals of aquatic toxicology: effects, environmental fate and risk assessment, 2nd edn. CRC Press, New York

Rebach S, French D (1996) Effects of dimilin on the blue crab, *Callinectes sapidus*, in shallow-water habitats. Estuaries 19:279–287

Relyea RA (2001) The relationship between predation risk and antipredator responses in larval anurans. Ecology 82:541–554

Relyea RA (2003a) How prey respond to combined predators: a review and an empirical test. Ecology 84:1827–1839

Relyea RA (2003b) Predator cues and pesticides: a double dose of danger for amphibians. Ecol Appl 13:1515–1521

Relyea RA (2004) Synergistic impacts of Malathion and predatory stress on six species of North American tadpoles. Environ Toxicol Chem 23:1080–1084

Relyea RA (2005a) The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. Ecol Appl 15:618–627

Relyea RA (2005b) The lethal impacts of Roundup® and predatory stress on six species of North American tadpoles. Arch Environ Contam Toxicol 48:351–357

Relyea RA, Mills N (2001) Predator-induced stress makes the pesticide Carbaryl more deadly to grey treefrog tadpoles (*Hyla versicolor*). Proc Natl Acad Sci USA 98:2491–2496

Relyea RA, Werner EE (1999) Quantifying the relation between predator-induced behavior and growth performance in larval anurans. Ecology 80:2117–2124

Sandoz MD, Rogers R (1944) The effect of environmental factors on hatching, molting, and survival of zoea larvae of the blue crab *Callinectes sapidus* Rathbun. Ecology 25:216–228

Schimmel SC, Patrick JM Jr, Faas LF, Oglesby JL, Wilson AJ Jr (1979) Kepone: toxicity and bioaccumulation in blue crabs. Estuar Coasts 2:9–15

Sheppard M, Walker A, Frischer ME, Lee RF (2003) Histopathology and prevalence of the parasitic dinoflagellate *Hematodinium* sp. in crabs (*Callinectes sapidus, Callinectes similis, Neopanope sayi, Libinia emarginata, Menippe mercenaria*) from a Georgia estuary. J Shellfish Res 22:873–880

Sheridan PF (1975) Uptake, metabolism, and distribution of DDT in organs of the blue crabs, *Callinectes sapidus.* Chesapeake Sci 16:20–26

Shirley TC, Stickle WB (1982) Responses of *Leptasterias hexactis* (Echinodermata: Asteroidea) to low salinity. I. survival, activity, feeding, growth and absorption efficiency. Mar Biol 69:147–154

Silliman BR, Bertness MD (2002) A trophic cascade regulates salt marsh primary production. Proc Natl Acad Sci USA 99:10500–10505

Smith G (1992) Toxicology and pesticide use in relation to wildlife: organophosphorous and carbamate compounds. Patuxent Wildlife Research Center, US Department of the Interior, USFWS, Springfield, VA

Sokal RR, Rohlf FJ (1995) Biometry: the principles and practice of statistics in biological research, 3rd edn. WH Freeman, New York

Solis RS, Powell GL (1999) Hydrography, mixing characteristics, and residence times of Gulf of Mexico estuaries. In: Bianchi TS, Pennock JR, Twilley RR (eds) Biogeochemistry of Gulf of Mexico estuaries. John Wiley & Sons, New York, p 29–62

Sutton G, Wagner T (2007) Stock assessment of blue crab (*Callinectes sapidus*) in Texas coastal waters. Texas Parks and Wildlife, Coastal Fisheries Division Management Data Ser 249, Austin, TX

Tagatz ME (1969) Some relations of temperature acclimation and salinity to thermal tolerance of the blue crab, *Callinectes sapidus.* Trans Am Fish Soc 98:713–716

USEPA (United States Environmental Protection Agency) (2006) Reregistration Eligibility Decision (RED) for Malathion. EPA-738-R-06-030, USEPA, Washington, DC

Virnstein R (1977) The importance of predation by crabs and fishes on benthic infauna in Chesapeake Bay. Ecology 58:1199–1217

von Rumker RE, Lawless EW, Meiners AE, Lawrence KA, Kelso GL (1974) Production, distribution, use, and environmental impact potential of selected pesticides. Office of Pesticide Programs, United States Environmental Protection Agency, Washington, DC

Whiting VK, Cripe GM, Lepo JE (1996) Effects of anionic surfactant, sodium dodecyl sulfate, on newly-hatched blue crabs, *Callinectes sapidus*, and other routinely tested estuarine crustaceans. Arch Environ Contam Toxicol 31: 293–295

Wolf M, Moore P (2002) Effects of the herbicide Metolachlor on the perception of chemical stimuli by *Orconectes rusticus.* J North Am Benthol Soc 21:457–467

Wolfe NL, Zepp RE, Gordon JA, Baughman GL, Cline DM (1977) Kinetics of chemical degradation of Malathion in water. Environ Sci Technol 11:88–93

Zhou S, Shirley T (1995) Effects of handling on feeding, activity and survival of red king crabs, *Paralithodes camtschaticus* (Tilesius, 1815). J Shellfish Res 14:173–177

Editorial responsibility: Kenneth Heck, Dauphin Island, Alabama, USA

Submitted: February 23, 2009; Accepted: July 7, 2009
Proofs received from author(s): September 21, 2009