

The Effects of the Herbicide Metolachlor on Agonistic Behavior in the Crayfish, *Orconectes rusticus*

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Abstract Previous research suggests that agricultural herbicides interfere with olfactory-mediated behavior, such as responses to alarm signals and the ability to locate food, in aquatic organisms. In crayfish, aggressive interactions are also mediated by chemical signals. These social signals are important in establishing dominance, which in turn has an impact on an individual's ability to find and use mates, food, and habitat space. In this study, we investigated the impact of exposure to sublethal levels of the herbicide metolachlor on the ability of crayfish to respond to olfactory signals used in agonistic behaviors. Crayfish were exposed to three different environmentally relevant concentrations (60 ppb, 70 ppb, and 80 ppb) of metolachlor for 96 hours. Each exposed crayfish was then placed in a fight arena and was allowed to interact with a naïve, untreated crayfish for 15 minutes. We analyzed several characteristics of fighting behavior, including initial aggressiveness, time to fight, intensity levels, duration, number of encounters, and the winner and loser of each fight. Crayfish exposed to 80 ppb metolachlor were less likely to initiate and win encounters against naïve conspecifics than any other treatment group. Analysis of fight dynamics shows that metolachlor does not alter the temporal fighting dynamics within crayfish aggression. We conclude that high sublethal concentrations of metolachlor may be interfering with the ability of crayfish to receive or respond to social signals and thus affect certain agonistic behaviors.

Introduction

Insecticides and herbicides are used regularly to rid agricultural areas of pests and to increase crop yield. In the Midwestern United States, herbicides are the most abundant group of pesticides employed, and are used extensively on corn and soybean fields (Gilliom et al. 1999; Battaglin et al. 2000; Frey 2001). Although there are many examples of commonly used herbicides, including chloroacetamides (alachlor, acetochlor, metolachlor, and propachlor) and S-triazines (atrazine, cyanazine, and simazine), atrazine, and metolachlor are among the most heavily applied to crops in the Lake Erie Basin (Frey 2001).

Following application to crops, pesticides can enter into aquatic environments from agricultural runoff, either through irrigation or precipitation (Gilliom et al. 1999). Since precipitation varies seasonally, the amount of pesticides found within aquatic environments can also vary. Metolachlor concentrations in Ohio rivers have a yearly average of 5 µg/L (ppb), but during the spring and summer concentrations can reach 80 µg/L shortly after rainfalls (Battaglin et al. 2000; Frey 2001). The yearly averages of metolachlor in aquatic systems are typically well below the established lethal levels for aquatic organisms, which range from 3.9 mg/L (ppm) for rainbow trout (*Oncorhynchus mykiss*) to 25.1 mg/L (ppm) for *Daphnia* (Ahrens 1994). These concentrations of contaminants, although below the established lethal limits, may still have sublethal detrimental effects on aquatic organisms (Saglio and Trijasse 1998; Scholz et al. 2000, Kashian and Dodson 2002; Teh et al. 2005; Bowen et al. 2006; Sandahl et al. 2006; Tierney et al. 2006; Sandahl et al. 2007; Tierney et al. 2007b).

Sublethal levels of pesticides may be high enough to impair the physiology and/or the behavior of aquatic

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animals (Scott and Sloman 2004). Possible sublethal effects of pesticides include nervous system alteration, biochemical changes, impact on reproduction, and possible chronic effects, such as a reduction in biomass and dry weight of adults (Sanchez et al. 2000; Schulz and Liess 2000; Kashian and Dodson 2002; Beketov and Liess 2005; Rakotondravelo et al. 2006). It has been hypothesized that exposure to sublethal concentrations of toxicants may be contributing to the worldwide decline of amphibians (Verrell 2000; Hayes et al. 2002; Rohr et al. 2003). Exposure to sublethal concentrations of atrazine had an impact on sexual development in frogs (*Xenopus laevis*), in that exposure caused the demasculinization of the male larynx and hermaphroditism (Hayes et al. 2002). In salamanders (*Ambystoma macrodactylum*), sublethal exposure to methoxychlor not only caused deleterious effects on larval survival, but also impacted predator-prey relationships (Verrell 2000; Ingermann et al. 2002). The behavior of lobsters and crayfish is affected by exposure to contaminants, in addition to that of amphibians (Abgrall et al. 2000; Sherba et al. 2000; Wolf and Moore 2002). Sublethal exposure to copper and metolachlor had an impact on the ability of *Cambarus bartonii* and *Orconectes rusticus* to successfully locate a food source (Sherba et al. 2000; Wolf and Moore 2002). Juvenile American lobsters (*Homarus americanus*) exposed to azalmethiphos vacated their shelters in order to avoid exposure to the pesticide. Juvenile lobsters spend most of their time in shelters to avoid predation (Abgrall et al. 2000).

In cyprinids and salmonids, sublethal levels of pesticides can affect a number of different behaviors (Scott and Sloman 2004). Exposure to sublethal concentrations of carbofuran, atrazine, and diuron, a phenylurea herbicide, had an impact on swimming patterns, social behavior, and orientation in goldfish (*Carassius auratus*, Saglio et al. 1996; Saglio and Trijasse 1998). Chinook salmon (*Oncorhynchus tshawytscha*) demonstrate a reduced antipredator response when exposed to diazinon, an organophosphate insecticide (Scholz et al. 2000). In addition, diazinon disrupted the reproductive capabilities of male Atlantic salmon parr (*Salmo salar*, Moore and Waring 1996). Since some of these behaviors are regulated by olfaction, it is possible that the behavioral detriments result from an impacted olfactory pathway (see Hara 1992).

The olfactory system is thought to be especially susceptible to impairment from pesticides because olfactory receptors are in direct and constant contact with the environment (Saucier et al. 1991; Moore and Waring 1996; Saglio et al. 1996; Scholz et al. 2000; McPherson et al. 2004; Carreau and Pyle 2005; Tierney et al. 2006; Tierney et al. 2007a; Tierney et al. 2007b). Long-term exposure to sublethal concentrations of copper impaired the olfactory discrimination ability of young rainbow trout, in that

exposed animals showed no preference between own rearing water and either well water or heterospecific water (Saucier et al. 1991). Work undertaken by Sandahl and colleagues has shown that sublethal exposure to copper alters the sensory physiology and predator avoidance behavior in juvenile coho salmon (*Oncorhynchus kisutch*, Baldwin et al. 2003; Sandahl et al. 2004; Sandahl et al. 2007). In addition, copper is broadly toxic to the olfactory nervous system in coho salmon indicating that many olfactory system-mediated behaviors could be affected (Baldwin et al. 2003; Sandahl et al. 2004). Reproduction is one of the olfactory-mediated behaviors that can be altered by sublethal exposure to copper. Belanger et al. (2006) showed that formation of a lesion with copper sulfate in olfactory sensory neurons of the round goby (*Neogobius melanostomus*) affects the responses of male gobies to putative female pheromones. Also, adult male Atlantic salmon parr have reduced olfactory responses to prostaglandins $F_{2\alpha}$ (PGF $_{2\alpha}$) following short-term sublethal exposures to diazinon, carbofuran, and atrazine (Moore and Waring 1996; Waring and Moore 1997; Moore and Waring 1998). Diazinon affected olfactory-mediated alarm-signal responses and homing ability in chinook salmon (Scholz et al. 2000). Salmon failed to reduce their swimming and feeding activities in response to alarm signals in comparison to controls. Also, fewer salmon exposed to diazinon returned to their natal stream to spawn, indicating that their homing ability was impaired (Scholz et al. 2000). Also, it is interesting to mention the work from Tierney et al. (2006) in coho salmon parr showing that both behavioral and physiological aspects of the olfactory-mediated alarm reaction can be altered by environmentally realistic concentrations of the carbamate fungicide IPBC. Although most of this work has focused on fish, chemoreception is important for foraging, mating, and other behaviors in aquatic organisms other than fish (Atema 1980; Ward et al. 1992; Snell and Morris 1993; Estebenet 1995; Snell 1998).

Crayfish, in addition to fish, are ideal animals for the investigation of the effects of toxicants on olfaction. These animals use olfaction to detect and avoid predators (Hazlett 1985; Hazlett 1990), locate food sources (Dunham et al. 1997; Moore and Grills 1999), find mates (Dunham and Oh 1996; Giri and Dunham 2000; Belanger and Moore 2006), and recognize social status among conspecifics (Zulandt Schneider et al. 1999; Bergman et al. 2003). Wolf and Moore (2002) have studied the effects of sublethal exposure to metolachlor on the ability of crayfish to perceive chemical stimuli by testing the ability of exposed crayfish to locate food and respond to alarm signals. This study concluded that metolachlor interferes with olfactory-mediated behavior in the crayfish, *Orconectes rusticus*. After sublethal exposure, crayfish could not successfully locate a food odor and walked towards alarm cues, which is not a typical alarm response in these animals (see Hazlett 1990).

Crayfish are benthic macroinvertebrates that are ecologically important and are found in a variety of aquatic habitats including lakes, rivers, and streams. They are omnivores, consuming invertebrates, algae, detritus, and macrophytes, and therefore they have the ability to influence many different trophic levels in their environment (Lodge et al. 1994; Usio 2000). They are also important for carbon cycling in streams, by shredding detritus and releasing energy for other organisms to use (Usio 2000). As a result of their ability to influence many trophic levels and their shredding capabilities, crayfish have a disproportionately large effect on their communities and have thus been referred to as keystone species (Helms and Creed 2005). Therefore, crayfish have the potential to be used as indicators of stream health during times of high agricultural run-off.

For crayfish, chemoreception is integral not only to finding food and avoiding predators, but also to success in social situations (Zulandt Schneider et al. 1999; Bergman et al. 2003). Agonistic encounters, or aggressive interactions, are important for establishing dominance, which in turn may determine reproductive success, affect food and shelter acquisition, and influence population dynamics (Hazlett et al. 1975; Zulandt Schneider et al. 2001; Bergman et al. 2003; Bergman and Moore 2003; Fero et al. 2007). Typical agonistic encounters begin with a threat display and escalate in intensity until one animal retreats. This establishes a dominance relationship, which is reinforced during subsequent encounters (Daws et al. 2002). The encounter plays a pivotal role in determining the relative fitness of each crayfish. The purpose of this experiment was to determine whether sublethal exposure to metolachlor alters the fighting abilities of crayfish (*Orconectes rusticus*). We hypothesize that crayfish exposed to metolachlor will be unable to successfully participate in agonistic encounters against unexposed conspecifics, perhaps due to the inability to receive or respond to chemical signals used in agonistic encounters or due to a change in internal aggressive state. These social impacts, such as an impaired ability to form dominance hierarchies, may subsequently affect fitness and population structure of crayfish exposed to sublethal metolachlor levels in natural aquatic habitats.

Materials and Methods

Animals

Male crayfish, *Orconectes rusticus*, were collected from the Portage River near Bowling Green, OH. Crayfish were housed in an environmental chamber at a constant temperature and light/dark cycle (23°C, 14 hours light:10 hours dark). Intermolt form I male crayfish [mean \pm standard error (SE); carapace, 3.4 ± 0.1 cm; chelae length,

3.4 ± 0.1 cm; and weight, 14.4 ± 0.5 g], with fully intact sensory appendages, were kept socially and physically isolated in a flow-through holding tank. Crayfish had no social experience for at least one week prior to experimentation, to negate the influence of any previous social effects on experimental outcomes (Zulandt Schneider et al. 2001). All crayfish were fed one rabbit pellet three times per week. Crayfish were used only once in these trials.

Chemical Preparation and Exposure Protocol

Crayfish were exposed to three environmentally relevant concentrations of metolachlor: 60 ppb, 70 ppb, and 80 ppb (Frey 2001). Concentrations were chosen based on results from Wolf and Moore (2002), which showed that lower concentrations of metolachlor (25 ppb and 50 ppb) did not significantly impair crayfish foraging behavior. Metolachlor was purchased from the Supelco Chemical Company, Bellefonte, PA (Lot # LB274–68B, 96.1% purity). Stock solutions (17 mg/L metolachlor) were kept in the dark at 4°C and were not used after 30 days (Hartgers et al. 1998; Graham et al. 1999; Lin et al. 1999).

Exposure treatments were as follows:

- Treatment 1: 80 ppb (4.71 mL metolachlor stock: 1 L of dechlorinated tank water)
- Treatment 2: 70 ppb (4.12 mL metolachlor stock: 1 L of dechlorinated tank water)
- Treatment 3: 60 ppb (3.53 mL metolachlor stock: 1 L of dechlorinated tank water)
- Treatment 4: dechlorinated tank water only (control treatment)

Crayfish were exposed to each treatment for 96 hours before engaging in an agonistic encounter (USEPA 1993; Wolf and Moore 2002). Crayfish were exposed to metolachlor in 1500 mL pots with the water and metolachlor being changed each day to ensure a constant concentration. This temporal regime of water and metolachlor should ensure that exposure concentrations remained constant, as significant volatilization of metolachlor occurs at temperatures above 30°C (Lau et al. 1995; Rice et al. 2004). Crayfish were kept at 23°C during exposure. A sample size of $n = 15$ was used for each treatment, and a total of 120 crayfish were used for this experiment (one exposed and one tester crayfish for each bout).

Fight Protocol

Two crayfish (exposed versus naïve tester crayfish or control versus tester) were used in each agonistic encounter. For identification purposes, one crayfish was picked at

random to be marked with correction fluid (Liquid Paper®) on the back of the carapace, which does not alter fight dynamics (Bergman et al. 2003). An opaque plexiglas fight arena (40 × 40 × 14 cm) with four removable dividers separated the tank into four equal sections, in order to run two trials at once. The fight arena was filled with 15 liters of dechlorinated tank water. The crayfish were placed into separate sections and allowed to acclimate for 20 minutes. Therefore, exposed crayfish had 20 minutes to recover from their exposure to metolachlor. The divider was then removed and the crayfish were allowed to interact for 15 minutes. After 15 minutes, all crayfish had either won or lost the fight. All trials were recorded from a camera (Panasonic WV-CL350) mounted one meter above the fight arena onto a video recorder (Panasonic AG-1980) and displayed on a monitor (Sony PVM-1351G).

Data Analysis and Fight Evaluation

A blind analysis was performed on all videotaped fight trials. Crayfish agonistic interactions were analyzed using an ethogram, adapted from Bruski and Dunham (1987), as shown in Table 1. Fights were analyzed for duration of the first fight and number of different encounters over the 15-minute trial period. An encounter is defined when the distance between two crayfish is less than one body length, and separate encounters are defined when that distance is greater than one body length or no interaction occurs for 10 seconds (Bergman et al. 2003). The temporal mechanics of fight intensity (i.e., the escalation of the intensity of a fight) were also recorded, along with the initiator and winner of each fight. Winners were defined when opponents (losers) retreated or tail-flipped away. Initiation and winning were analyzed using a contingency table for multiple comparisons of proportions analysis analogous to Student–Neuman–Keuls or Tukey test ($q_{0.05, \infty, 4} = 3.633$) (Zar 1999). Duration of the initial fight, number of encounters, and times of escalation to different fight intensity levels were analyzed using a one-way multivariate analysis of variance (MANOVA) with Tukey honestly significant differences (HSD) post hoc analysis. All data

were analyzed using Statistica 6.0 (one-way MANOVA, StatSoft, Tulsa, OK) and Microsoft Excel (contingency table), with significance set at *p*-values less than 0.05.

Results

Initiating and Winning

Crayfish exposed to the highest concentration of metolachlor (80 ppb) were significantly less likely to initiate an encounter with an opponent than all other treatment groups (Fig. 1, $p < 0.05$). Crayfish in this group initiated fights only 20% of the time. None of the other treatment groups were significantly different from each other or the control group. Crayfish exposed to both the control treatment and 70 ppb metolachlor initiated fights 47% of the time, and crayfish exposed to 60 ppb metolachlor initiated 53% of the time.

Similarly, crayfish exposed to 80 ppb won significantly fewer fights (20%) than all other treatment groups (Fig. 2, $p < 0.05$). Control crayfish won 47% of fights, crayfish exposed to 60 ppb metolachlor won 67% of fights, and crayfish exposed to 70 ppb metolachlor won 53% of fights.

Time to Different Intensities, Duration, Number of Encounters

There were no significant differences in time take to reach different intensities, duration, and number of encounters (one-way MANOVA, Figs. 3, 4, and 5, $p > 0.05$). Regarding time to different intensities, for intensity levels two, three, and four, crayfish exposed to 80 ppb metolachlor averaged 5.3 ± 1.6 s, 14.3 ± 6.1 s, and 60.6 ± 17.0 s, respectively (Fig. 3, $p > 0.05$). For the duration of the first encounter, the results were not significant. Crayfish exposed to 80 ppb averaged 281.3 ± 83.6 s for the first encounter (Fig. 4, $p > 0.05$). Finally, with respect to the number of different encounters within a fight, the results were not significant. Crayfish exposed to 80 ppb averaged 8.8 ± 1.0 encounters (Fig. 5, $p > 0.05$).

Table 1 Crayfish ethogram codes (fight intensity levels)

Intensity level	Behavior
−2	Tail-flip away from opponent or a fast retreat
−1	Retreat – slowly back away from opponent
0	No response or threat display
1	Approach without a threat display
2	Approach with threat display – antennal whip, meral spread, or raised posture
3	Initial claw use – boxing, pushing, or touching with closed claws
4	Active claw use – grab opponent with claws or hold other crayfish with claw

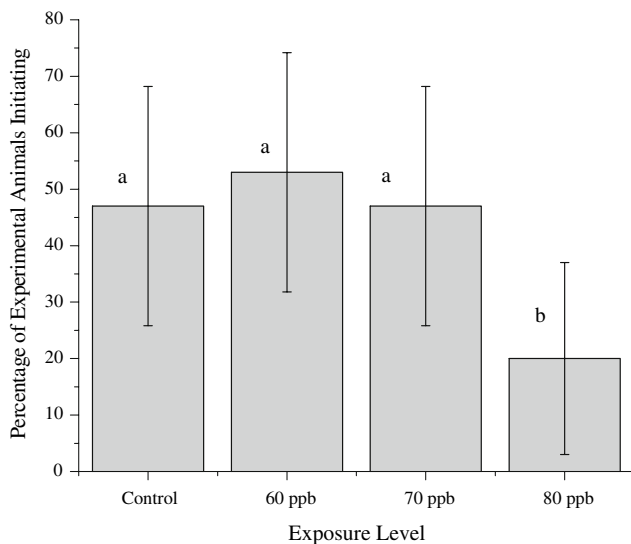


Fig. 1 Percentage of experimental animals initiating the agonistic encounter (\pm 90% confidence intervals). Data were analyzed using the contingency table for comparisons of proportions. Eighty ppb was significantly different from the control, 60 ppb, and 70 ppb ($n = 15$, $q_{0.05, \infty, 4} > 3.633$, $p < 0.05$). Letters indicate significant differences

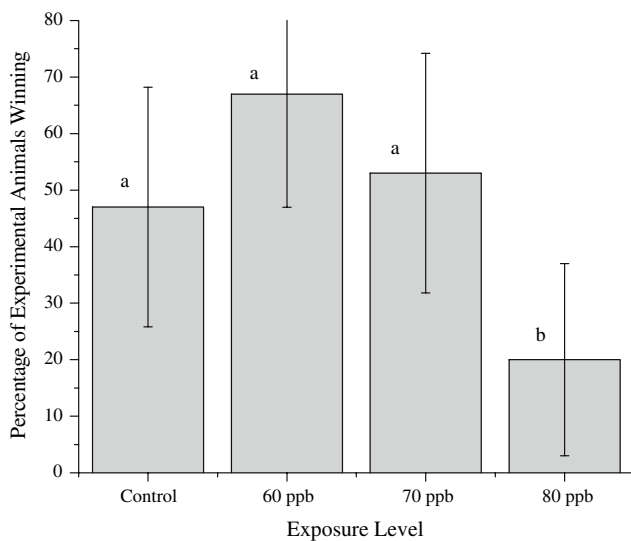


Fig. 2 Percentage of experimental animals winning (\pm 90% confidence intervals). Letters indicate significant differences. Data were analyzed using the contingency table for comparisons of proportions. Animals exposed to 80 ppb were significantly different from the animals exposed to aged tank water, 60 ppb, and 70 ppb ($n = 15$, $q_{0.05, \infty, 4} > 3.633$, $p < 0.05$)

Discussion

Possible Mechanisms for Results

Sublethal concentrations of metolachlor altered only the two endpoints of aggressive interaction (i.e., the beginning and the final outcome of a fight; Figs. 1 and 2), and only at

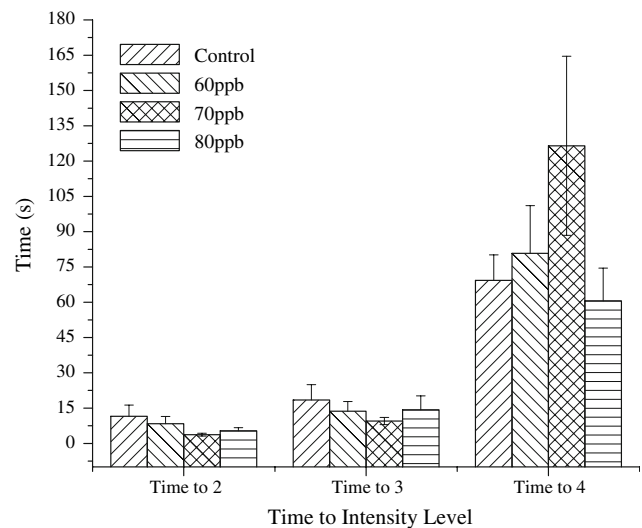


Fig. 3 Time [\pm standard error of the mean (SEM)] to different intensities. Data were analyzed using a one-way MANOVA followed by a Tukey HSD post hoc analysis. There were no significant differences for the time to different intensities ($n = 15$, $F = 1.22$, $p > 0.05$)

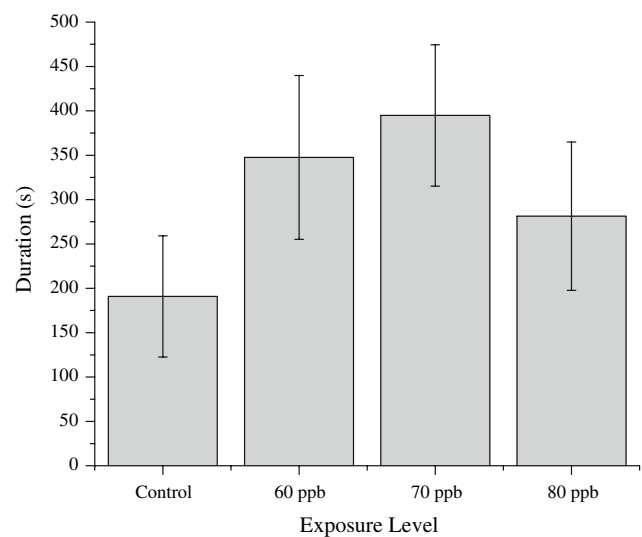


Fig. 4 Fight duration [\pm standard error of the mean (SEM)] of the first encounter. Data were analyzed using a one-way MANOVA followed by a Tukey HSD post hoc analysis. There were no significant differences ($n = 15$, $F = 1.18$, $p > 0.05$)

the highest concentration tested (80 ppb). It appears that exposure to metolachlor may be altering the underlying sensory or neural mechanism that dictates the animal's decision to either initiate an encounter or to win the encounter, compared to other fight behaviors. In crayfish, initiating an encounter indicates an individual's initial aggressive state and winning an encounter establishes an individual's ability to become dominant within a population (Daws et al. 2002; Bergman et al. 2003). Therefore,

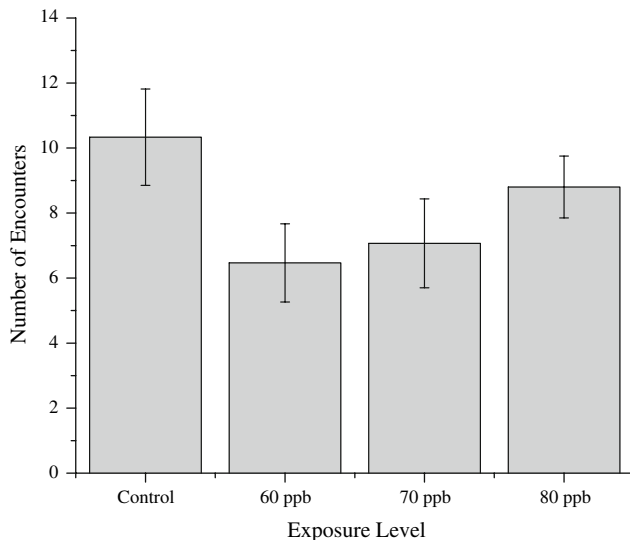


Fig. 5 Number of encounters during the entire trial time [$T = 15$ minutes, \pm standard error of the mean (SEM)]. Data were analyzed using a one-way MANOVA followed by a Tukey HSD post hoc analysis. There were no significant differences ($n = 15$, $F = 1.91$, $p > 0.05$)

animals exposed to 80 ppb metolachlor were less aggressive initially and less dominant than conspecifics exposed to weaker concentrations of metolachlor and the control crayfish. It is important to note that less aggression and less dominant are not necessarily equivalent (Francis 1988; Drews 1993), and thus there is potential for two separate effects of exposure. One effect could be altering the initial levels of aggression (Fig. 1), while a separate effect could be altering the potential for a crayfish to become dominant (Fig. 2).

In previous research on crayfish, Wolf and Moore (2002) showed that sublethal exposure to metolachlor altered the sensory ability of crayfish to locate food and respond to alarm signals; both of these behaviors are mediated by the ability of crayfish to detect and respond to chemical signals in the environment. Not only are chemical signals needed for detection of food and for avoidance of predators, but they are also used in agonistic encounters (Zulandt Schneider et al. 2001; Bergman et al. 2003). Fight dynamics have been altered by removing the ability of crayfish to receive and respond to chemical signals used in agonistic encounters (Zulandt Schneider et al. 2001; Bergman et al. 2003). For example, in agonistic bouts where chemical communication was blocked, either by destruction of the chemoreceptors or by blocking urine release, encounters lasted longer and reached higher intensity levels (lobsters: Karavanich and Atema 1998; crayfish: Zulandt Schneider et al. 2001; Bergman et al. 2003). Furthermore, in experiments where sensory intact crayfish were given a winning experience and then paired with an opponent without sensory abilities, the winner

effect was absent (Bergman et al. 2003). Bergman et al. (2003) concluded that the changes observed in fight dynamics were due to the inability of crayfish to perceive chemical stimuli rather than changes in aggressive state alone. Although we did not observe any variations among treatment groups for fight dynamics, we did observe changes in initiating and winning (Figs. 1 and 2) due to metolachlor exposure. While chemosensory abilities may have been affected, exposure to metolachlor may also have resulted in internal changes in aggressive state or baseline motor activity.

Alternatively, metolachlor may alter fight dynamics by affecting the neurochemistry that regulates the internal aggressive state of the crayfish or may alter baseline motor activity. Biogenic amines, such as serotonin, octopamine, and dopamine help to regulate aggression in decapod crustaceans (Kravitz 1988; Huber and Delago 1998; Huber et al. 2001). Furthermore, serotonin confers a heightened aggressive state on crayfish (Huber et al. 2001). Crayfish exposed to metolachlor were less aggressive than unexposed conspecifics, and it is possible that metolachlor may interfere with crayfish neurochemistry responsible for regulating aggression. In addition, metolachlor may have an effect on baseline motor activity at the highest dose, so crayfish may have been too lethargic to initiate and win agonistic encounters. Our results are likely to be the product of the inability to perceive and respond to chemical signals, and from changes in the state of aggression, as some aspects of aggression were affected while others were not.

Possible Consequences on Social Hierarchies

Aggressive interactions between individuals have the potential to result in a dominant relationship when one individual emerges as the winner and the other as the loser, and as these roles are reinforced, social hierarchies form. Since exposure to metolachlor alters the probability of winning, it may also impact the formation of social hierarchies in crayfish, just as some pollutants affect hierarchy formation in social groups of fish (Henry and Atchinson 1979; Henry and Atchinson 1986; Sloman et al. 2003). Henry and Atchinson (1986) found that sublethal concentrations of copper affected social hierarchy formation in bluegills (*Lepomis macrochirus*), particularly in relation to an individual's rank. Sloman et al. (2003) concluded that any contaminants in salmonid communities have a potential threat to populations, because salmonids, like crayfish, form social dominance hierarchies. Changes in these hierarchies, induced by sublethal concentrations of metals and herbicides, may have the ability to influence population dynamics. If exposed crayfish are no longer able to win

encounters, and become subordinate, they may have reduced access to resources, such as habitat space, mates, and food. Consequently, an individual's fitness within a population may be reduced.

In aquatic environments, it is likely that both combatants would be exposed to metolachlor. Fight dynamics between two exposed individuals may differ from fight dynamics between two healthy crayfish. This could affect the temporal dynamics of the formation of social dominance hierarchies. In normal populations of crayfish, social hierarchies become stable after 96 hours (Fero et al. 2007). Metolachlor could interfere with the length of the time period over which dominance hierarchies are established, as is the case with rainbow trout exposed to cadmium (Sloman et al. 2003). Populations of rainbow trout exposed to cadmium form dominance hierarchies at a faster rate than populations with control fish (Sloman et al. 2003). Changes in formation and maintenance of social hierarchies could contribute to the overall productivity of the population, such as the ability of dominant crayfish to maintain their position and retain resources.

Conclusion and Future Research

Crayfish are proposed to be keystone species in their environments, which makes them ideal indicators of water quality as they have a disproportional effect on their community (Helms and Creed 2005). We have shown that sublethal concentrations of metolachlor influence the fighting ability of crayfish, making them less aggressive and less dominant. In this way, metolachlor has the potential to decrease the health of individuals within the population. If this effect is severe enough, the population may be impacted, which could be used as an indicator of relative stream health. Future studies should be conducted to investigate the effects of pulsed herbicide exposures, as well as recovery rates. Crayfish may be most susceptible to metolachlor impairment at times when agricultural runoff is high and pulse contamination may occur in streams. However, runoff events are likely to be shorter than 96 hours, so future studies should investigate the effects of using pulsed exposures in the order of 6 hours or less. In aquatic systems, metolachlor may occur with other pesticides. Studies are needed to explore the synergistic effects of metolachlor mixed with other herbicides on animal behavior. Furthermore, additional work should be completed to link changes in an individual's fitness within a hierarchy to population models and the formation of social hierarchies in crayfish.

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