J. N. Am. Benthol. Soc., 2002, 21(3):457–467

Effects of the herbicide metolachlor on the perception of chemical stimuli by *Orconectes rusticus*

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Abstract. Previous research has suggested that certain environmental pollutants interfere with the perception of chemical stimuli. It is not clear if this interference comes from masking the odor, where the animal cannot detect the signal over the background noise, or if there is a physiological effect on the olfactory receptor cells or neuromusculature that influences the animal's behavior. We acutely exposed crayfish to the agricultural chemical metolachlor. We then tested the ability of crayfish to respond appropriately to 2 chemical stimuli important to their survival: 1) detection of damaged conspecifics, and 2) location of a food source in the absence of metolachlor. A damaged conspecific, commonly known as an alarm signal, produces a chemical signal composed of bodily fluids that could signify a predation event to the individual receiving the signal. Results indicated that crayfish were unable to successfully locate a food source after being exposed to metolachlor. In the presence of an alarm signal, animals exposed to metolachlor walked faster than nonexposed animals and tended to walk in the direction of the alarm signal instead of away, as in the control animals. Crayfish may perceive aspects of the chemical signal but make inappropriate decisions in response to the information received as a result of exposure to metolachlor.

Key words: herbicide contamination, metolachlor, crayfish, toxicology, food orientation, alarm signals, crushed conspecific.

Pesticides are used in many agricultural areas to control pests and to increase product yield. Some of the commonly used pesticides are S-triazine herbicides (atrazine, cyanazine, and simazine), chloroacetamide herbicides (metolachlor, alachlor, and acetochlor), and organophosphates (diazinon). These chemicals are found in the surface water of local streams, rivers, and lakes as a result of agricultural runoff. For example, northern Ohio rivers and streams have a yearly average of 5 ppb metolachlor with a maximum concentration of 80 ppb in the spring and summer (Battaglin et al. 2000, Frey 2001). The US Environmental Protection Agency (USEPA) monitors pesticides in surface waters for concentrations that are at or above human consumption levels and lethal concentration levels (LC50) for aquatic organisms (Ahrens 1994, USEPA 1995, Gilliom et al. 1999). The concentration of metolachlor routinely observed in surface waters falls well below the LC50 established for fish, which ranges from 2 mg/L (ppm) for rainbow trout to 15 mg/L (ppm) for bluegill sunfish (Ahrens 1994). Even though concentrations typically seen in surface waters are below the lethal level, they could have a detrimental effect on behaviors essential in the life strategies of aquatic organisms (Saucier et al. 1991, Klaprat et al. 1992, Moore and Waring 1996, Scholz et al. 2000).

Fish and aquatic invertebrates are exposed to various levels of contamination throughout the year, which can cause physical damage to different areas of the body. Because the olfactory organs of aquatic organisms are directly exposed to the environment, chemical pollutants may impair the ability to perceive and respond appropriately to chemical stimuli (Klaprat et al. 1992). Fish have the potential to recover from damage to the olfactory receptor cells because they have a high cellular turnover rate, whereas crayfish only replace the olfactory receptor cells during the molting process (Klaprat et al. 1992, Zeni et al. 1995). It is unclear if crayfish olfactory cells contain enzymes capable of breaking down toxic substances, but the enzyme P450 has been found in the crayfish hepatopancreas where breakdown of toxic substances is know to occur (James and Boyle 1998, Snyder 2000). This enzyme is also found in mammalian olfactory mucosa, and is believed to metabolize certain contaminants to which the animal has been exposed (Sivarajah et al. 1979, Ding et al. 1992).

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Recent studies showed that nominal (sublethal) concentrations of pollutants have a detrimental effect on several aspects of fish behavior (Saucier et al. 1991, Moore and Waring 1996, Saglio and Trijasse 1998, Scholz et al. 2000). Chinook salmon exposed to nominal levels of the pesticide diazinon exhibited decreased responses to predatory odors; however, they continued feeding in the presence of a predatory odor (Scholz et al. 2000). Diazinon, at several sublethal concentrations, also interfered in reproduction of Atlantic salmon by disrupting olfactory priming of the male by female urine (Moore and Waring 1996). Swimming patterns, orientation to food, and social interactions of goldfish were affected by short-term exposure to carbofuran, diuron, and atrazine (Saglio et al. 1996, Saglio and Trijasse 1998). Atrazine also altered the swimming behavior of zebrafish at a wide range of environmentally relevant concentrations (Steinberg et al. 1995).

Crayfish are ideal animals for studying the effect of pollutants on olfaction because information concerning many aspects of their environment is acquired through olfactory organs. Crayfish use chemical signals to search for food (Dunham et al. 1997, Moore and Grills 1999), mates (Dunham and Oh 1996, Geri and Dunham 2000), determine social status of conspecifics (Quinn and Graves 1998, Zulandt Schneider et al. 1999), and to locate and avoid potential predators (Hazlett 1985, 1990, 1994, Appelberg et al. 1993, Zulandt Schneider et al. 2000). The olfactory organs of crayfish are vulnerable to damage from environmental pollutants, so decisions crayfish must make based on chemical stimuli could be severely taxed (Steele et al. 1992, Hanazato 1999). Behavior of crayfish could be altered by contaminants masking odor signals, by inhibiting the ability of receptor cells to detect signals, or by altering an animal's behavioral response to chemical signals. Masking is a process by which the toxin influences the ability of the animal to distinguish signals from the background noise of the environment.

Metolachlor may interfere with normal olfactory behavior in crayfish. We examined the effects of acute exposure to metolachlor on the response to food and alarm signal odor cues by the freshwater crayfish *Orconectes rusticus*.

Methods

Crayfish

Male *O. rusticus* were obtained from the Portage River in Wood County, Ohio, during March and April 2000. Animals were housed in an environmental chamber on a reverse light/dark cycle (14:10 L:D) and at a temperature of 22.0 ± 0.2°C. The animals were maintained separately in physically isolated, 1.5-L translucent plastic flowerpot containers. Flowerpots were aerated and water was changed once a week. Animals were acclimated in isolation for 10 d before use in experiments. Animals were fed ~0.1 g of frozen haddock fillets 3 times a week. Crayfish were tested experimentally for their ability to respond appropriately to 2 odor cues: 1) food odor, and 2) alarm signal. Crayfish used in the food-orientation experiment were starved for 7 d prior to use, and animals used in the alarm signal response experiment were fed 24 h prior to use.

Odor preparation

The food odor was prepared fresh daily before the commencement of testing by homogenizing 5 to 6 g of frozen haddock fillet with 1 L of aged tank water. Alarm signal was prepared in the same manner as the food odor, except a 5 to 6 g crayfish was homogenized with 1 L of aged tank water (Hazlett 1985, 1990, 1994, Chivers and Smith 1994, Zulandt Schneider et al. 2000). All odor stimuli were strained through a USGS 60-μm mesh sieve to remove large pieces of fish fillet or crayfish tissue.

Chemical preparation and exposure

Crayfish were exposed to 3 environmentally relevant concentrations of metolachlor: 25, 50, and 75 ppb (Frey 2001). Metolachlor was purchased through the Supelco Chemical Company, Bellefonte, Pennsylvania (Lot # LA 237-91A). Stock solutions of metolachlor were made by weighing 23 mg of the chemical, dissolving it in 1 mL of acetone, and diluting it to 1 L with aged tank water. Stock solutions were stored in the dark at 4°C (Lin et al. 1999). Stock solutions were not used after 35 d; the half-life of this chemical in nature can range from 33 to 46 d (Hartgers et al. 1998, Graham et al. 1999). Controls using acetone alone (vehicle control) tested whether acetone affected behavior of the crayfish. Vehicle control stock solutions were prepared by diluting 1 mL of acetone to 1 L with aged tank water. Exposure treatments were as follows: 1) Treatment 1: 25 ppb, 1.09 mL of metolachlor stock solution : 1 L of aged tank water, 2) Treatment 2: 50 ppb, 2.17 mL of metolachlor stock solution : 1 L of aged tank water, 3) Treatment 3: 75 ppb, 3.26 mL of metolachlor stock solution : 1 L of aged tank water, 4) Vehicle control: 3.26 mL of acetone stock solution : 1 L of aged tank water, and 5) Control: aged tank water only.

The exposure treatments were conducted first, followed by control treatments the next day. The vehicle control was used in the food odor experiment only; acetone did not influence behavioral responses so this treatment was dropped from the alarm signal experiment. Each treatment consisted of 12 replicates for a total of 108 crayfish. For the food-orientation experiment, 12 crayfish (replicates) were used in each of 5 different treatments (see above). In the alarm signal experiment, 12 crayfish (replicates) were used in each of 4 different treatments. Crayfish were exposed to each treatment for 96 h (USEPA 1993). Exposure water was changed daily to ensure a relatively constant exposure concentration. After exposure, crayfish were rinsed with fresh tank water and acclimated to the flow tank for 25 min.

Flow tank and experimental setup

Trials were conducted in a plexiglass flow-through tank (20.6 × 42.7 × 17.0 cm). Fresh tank water used for odor experiments was held in a 10-L carboy located above the tank. Water was gravity fed from the carboy through Tygon tubing (# R-3603) into the flow tank, and was regulated using a Manostat Riteflow® flowmeter (size 4), set at a constant rate of 128.7 mL/min. Water entered and exited the tank through spouts drilled into the ends. The holes were located 5 cm from the bottom and 10.3 cm from the sides of the tank. Crayfish were allowed to acclimate in the flow tank for 20 min without flow, and 5 min with flow before odor was introduced. Odor stimulus was injected into the inflow tubing 15 cm prior to the tube attaching to the tank using a 50-mL syringe at a rate of 6 mL/min (Zulandt Schneider et al. 2000). Trials lasted for 2 min, with a total of 12 mL of odor stimulus injected. The flow tank was rinsed 3 times with dechlorinated tap water and refilled with fresh tank water between each trial. All trials were recorded using a Sony HI8 video camera (CCD-TR700) that was mounted above the flow tank.

Dye trials

Dye trials were conducted to determine the flow speed of the odor stimulus as it was injected into the flow tank. Twelve mL of a fluorescein dye solution were injected into the tank in the same fashion as the odor stimuli. Analysis of the dye trials indicated that the leading edge of the odor plume reached the end of the flow tank, on average, in 20.6 s and that the odor spread throughout the tank in 118 s. The flow speed of the odor was calculated to be 2.14 ± 0.21 cm/s.

Data analysis

Videotaped trials were digitized at 1 frame/s using Peak Motus Motion Analysis® to obtain X and Y spatial coordinates of the animals' movements. Spatial parameters were analyzed to determine changes in behavior, which included walking speed, walking speed toward the odor source, time to locate the source, % success in locating the source, and time spent motionless. Walking speed was calculated by dividing the distance traveled by the time spent to travel from one point to another for each frame of video analyzed. Thus, walking speed was calculated at each segment of the path. These numbers were then averaged for each trial giving a mean walking speed for each animal used. Walking speed is a measure of the walking speed anywhere within the tank, whereas walking speed toward the source is a measurement of movement toward the odor source. Walking speed toward the source can be a positive or negative vector (for details see Moore et al. 1991, Moore and Grills 1999).

In the food-orientation experiment, animals that came within 5 cm of the flow input were defined as successfully locating the odor source. The average time taken by control animals to locate the odor source was used in exposure trials where the crayfish did not locate the food odor. This analysis removed any possible time bias in those behavioral measures that were sensitive to total time. In the alarm signal response experiments, orientation to the odor source and the amount of time spent motionless during the trial were analyzed. In both alarm signal response and food-orientation experiments, walking speeds of the 2-min trials were normalized to correct for differences in activity before and after odor was introduced. Walking speeds were normalized by dividing the post-odor walking speed of each replicate by the average pre-odor walking speed, which yielded a ratio of the post-odor : pre-odor walking speed.

Orientation parameters were statistically analyzed using a MANOVA, and differences between groups were obtained using a LSD post-hoc comparison test (STATISTICA 5.1 97, StatSoft, Tulsa, Oklahoma). Percent success in locating the odor source was analyzed using a χ^2 test, where the expected value was defined as the success of the control animals in locating the odor source (Zar 1999). A χ^2 analysis was performed for all data points together to obtain an overall statistical measure of treatment effect. The differences between treatments were determined by separate χ^2 tests between each metolachlor treatment and the control treatment. Pre-odor walking speeds were analyzed using a 1-way ANOVA, and differences between treatment groups were analyzed using an LSD post-hoc comparison test.

Results

Pre-odor walking speed

Analysis indicated an overall significant effect of metolachlor treatment on pre-odor walking speeds (ANOVA; $F_{4,55} = 2.76$, $p < 0.05$). There was a significant decrease in pre-odor locomotory behavior in crayfish exposed to the highest concentration of metolachlor, 75 ppb (LSD post-hoc test, $p < 0.05$; Fig. 1).

Food-orientation experiment

Orientation pathways of control and exposed animals were compared to qualitatively measure the movements of animals toward the food odor (Fig. 2). Animals exposed to 50 and 75 ppb metolachlor were less likely to locate the food odor (Fig. 2D, E) compared to control animals (Fig. 2A, B). MANOVA results using all of the orientation parameters showed an overall treatment effect on the orientation behavior of crayfish toward food odor (Rao's $R_{32,178} = 2.08$, $p < 0.05$). LSD post-hoc comparisons for differences between treatments for the orientation parameters are addressed below.

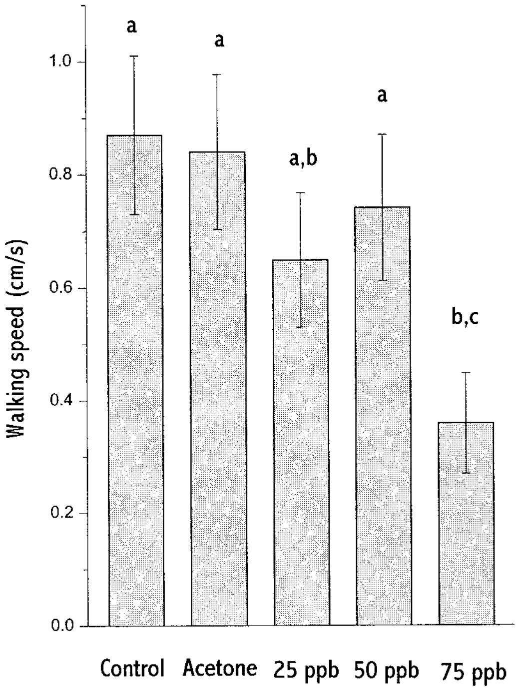

FIG. 1. Mean (± SEM) pre-odor walking speeds of crayfish during the acclimation period prior to introduction of odor stimulus into the flow tank. Columns with the same letter are not significantly different (LSD post-hoc test, $p > 0.05$).

Walking speed.—Analysis of the normalized walking speeds of crayfish presented with a food-odor stimulus showed there were no significant differences (LSD post-hoc test, $p > 0.05$) between animals treated with different concentrations of metolachlor and control treatments (Fig. 3).

Walking speed toward source.—Crayfish exposed to various concentrations of metolachlor exhibited diminished walking speeds toward the food odor (Fig. 4). Animals exposed to the 2 highest concentrations of metolachlor were the most affected. The animals in the 50 ppb treatment walked significantly slower toward the food odor (LSD post-hoc test, $p < 0.05$) than the control animals. Although the 75 ppb treatment was not significantly different from the control animals (LSD post-hoc test, $p = 0.053$), there

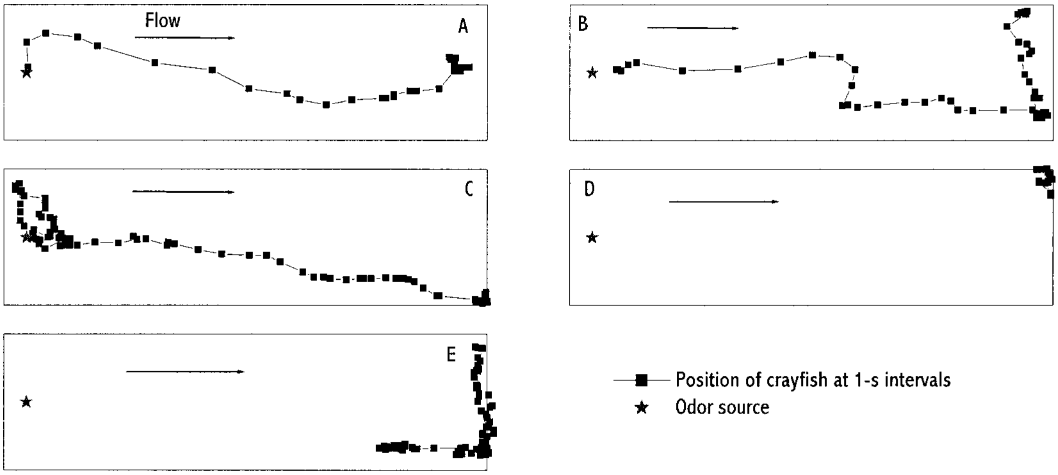

FIG. 2. Sample orientation pathways of animals presented with a food odor. Animals started the trial on the opposite side of the tank from the odor source. A.—Control. B.—Acetone. C–E.—Metolachlor at 25 (C), 50 (D), and 75 ppb (E) ppb.

was a strong indication that crayfish were walking much slower toward the food odor than the control animals.

Time to source.—Crayfish exposed to metolachlor took significantly longer to locate the food odor than crayfish from the control treatments (LSD post-hoc test, $p < 0.05$, Fig. 5). All crayfish exposed to metolachlor concentrations exhibited similar times to locate the food odor source.

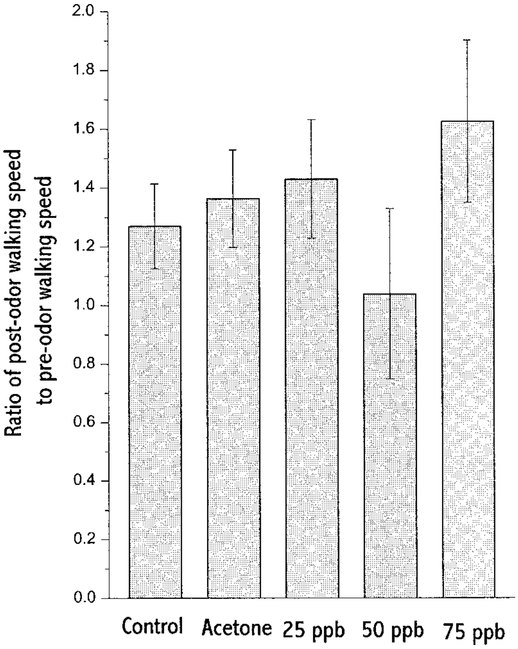

FIG. 3. Mean (± SEM) normalized walking speeds of animals presented with food odor. There were no significant differences (LSD post-hoc test, $p > 0.05$).

Success in locating odor source.—χ^2 analysis indicated an overall effect of the exposure treatments on the ability of crayfish to locate a food odor ($\chi^2_7 = 62.2$, $p < 0.05$, Fig. 6). There were significant differences between the control animals and each metolachlor exposure group: 25 ppb ($\chi^2_1 = 4.36$, $p < 0.05$), 50 ppb ($\chi^2_1 = 39.3$, $p < 0.05$), and 75 ppb ($\chi^2_1 = 17.5$, $p < 0.05$).

Alarm signal response experiment

Orientation pathways of control and exposed animals were compared to qualitatively measure the movements of animals in response to an alarm signal (Fig. 7). The paths of animals demonstrated the tendency to stay to the sides of the tank, which is the area of least concentration of alarm signal as shown by the dye trials. MANOVA results using all of the orientation parameters showed that there was a significant overall effect of the treatments on behavior of crayfish towards an alarm signal (Rao's $R_{24,107} = 2.66$, $p < 0.05$). LSD post-hoc comparisons for differences between treatments for the orientation parameters are addressed below.

Walking speed.—Walking speed of crayfish showed that there was a dose-related response

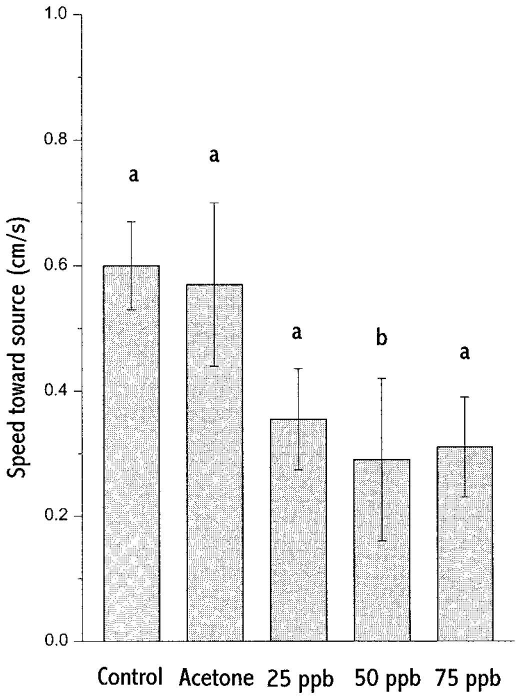

FIG. 4. Mean (± SEM) walking speeds of animals toward the food odor source. Columns with the same letter are not significantly different (LSD post-hoc test, $p > 0.05$).

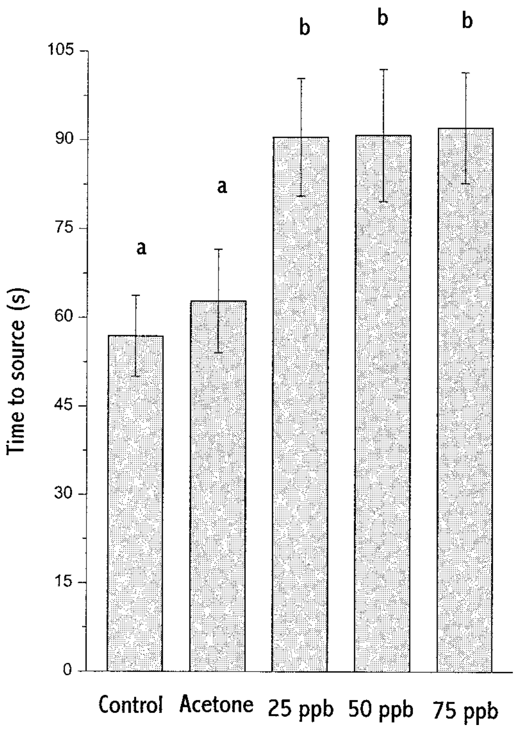

FIG. 5. Mean (± SEM) time taken for crayfish to locate the food odor. Columns with the same letter are not significantly different (LSD post-hoc test, $p > 0.05$).

as a result of metolachlor exposure (Fig. 8). The animals exposed to 75 ppb walked significantly faster in the presence of an alarm signal than all other treatments (LSD post-hoc test, $p < 0.05$). All metolachlor-exposed crayfish increased their walking speeds when compared to control animals.

Walking speed toward source.—There were no significant differences in crayfish walking speeds toward the source in the presence of an alarm signal (LSD post-hoc test, $p > 0.05$), but some trends were evident (Fig. 9). Control animals showed negative walking speeds toward the source, which is expected when an alarm signal is present. However, the animals exposed to metolachlor exhibited positive walking speeds toward the aversive signal.

Time spent motionless.—Exposed crayfish tended to exhibit more activity than the control crayfish, although the difference was not significant (LSD post-hoc test, $p > 0.05$, Fig. 10). Crayfish exposed to metolachlor spent ⅓ of the trial motionless, whereas the control animals remained motionless for nearly ½ the trial.

Discussion

Metolachlor alters crayfish behavior

Metolachlor significantly altered the chemosensory behavior of crayfish. Crayfish exposed to metolachlor exhibited impaired responses toward food odor. This result was especially evident at the 2 highest exposures, 50 and 75 ppb. Crayfish at these concentrations took significantly longer to locate the food odor than control groups. In previous studies, crayfish exposed to sublethal levels of metals exhibited altered responses to food odors (Steele et al. 1992, Shebra et al. 2000). In the alarm signal response experiments, crayfish also exhibited altered responses to the stimulus presented. The normal response of crayfish to an alarm signal is either to cease movement to become less conspicuous or to seek shelter (Hazlett 1990). Fish exposed to a conspecific alarm signal or predator odor stop foraging, decrease swimming speed, and avoid the area where the odor is being emitted

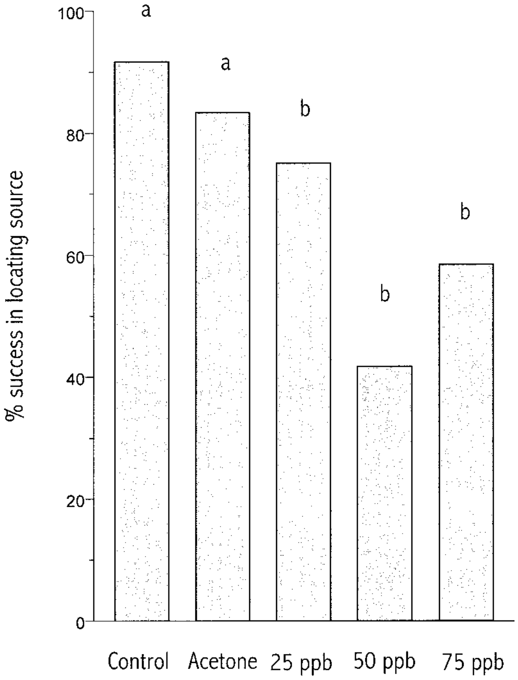

FIG. 6. Percent success in locating the food odor. Columns with the same letter are not significantly different (χ^2 test, $p > 0.05$).

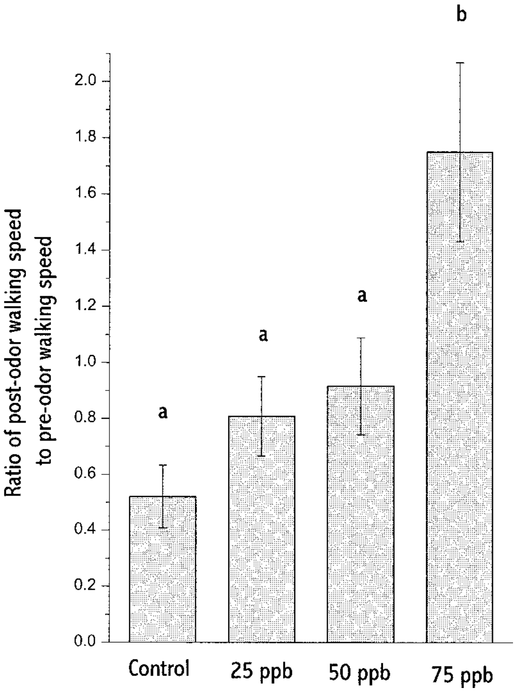

FIG. 8. Mean (± SEM) normalized walking speeds of animals presented with an alarm signal. Columns with the same letter are not significantly different (LSD post-hoc test, $p > 0.05$).

(Chivers et al. 1995, Brown and Smith 1998). We observed that exposed crayfish walked faster, displayed a tendency to move toward the aversive stimulus, and showed increased activity levels not usually associated with an alarm signal response.

Our results show that the ability of crayfish to respond appropriately to chemical stimuli is impaired when exposed to metolachlor. There are 3 potential explanations for the changed be-

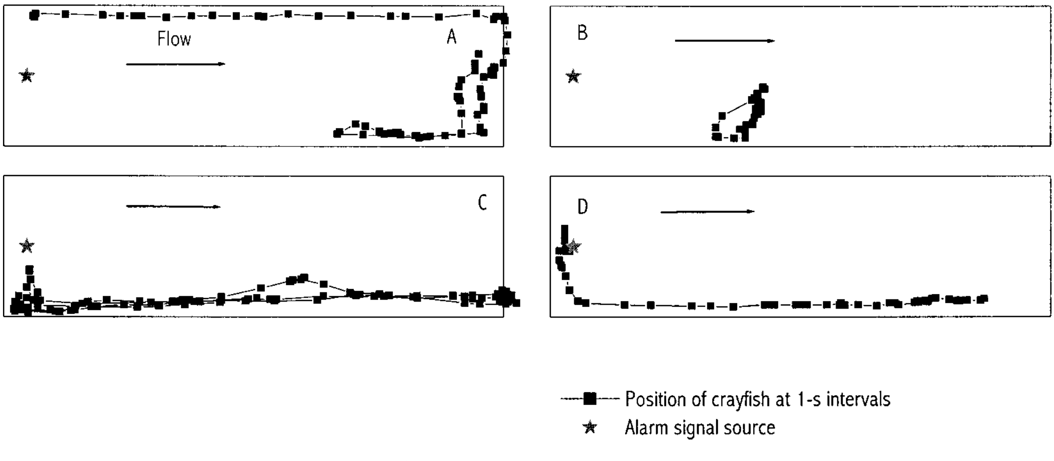

FIG. 7. Sample orientation pathways of animals presented with an alarm signal. Animals started the trial on the opposite side of the tank from the alarm signal source. A.—Control. B–D.—Metolachlor at 25 (B), 50 (C), and 75 (D) ppb.

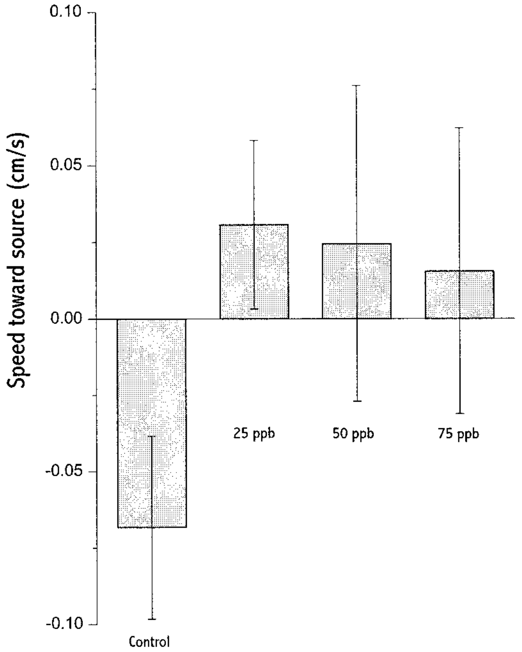

FIG. 9. Mean (± SEM) walking speeds of animals toward the alarm signal source. There were no significant differences (LSD post-hoc test, $p > 0.05$).

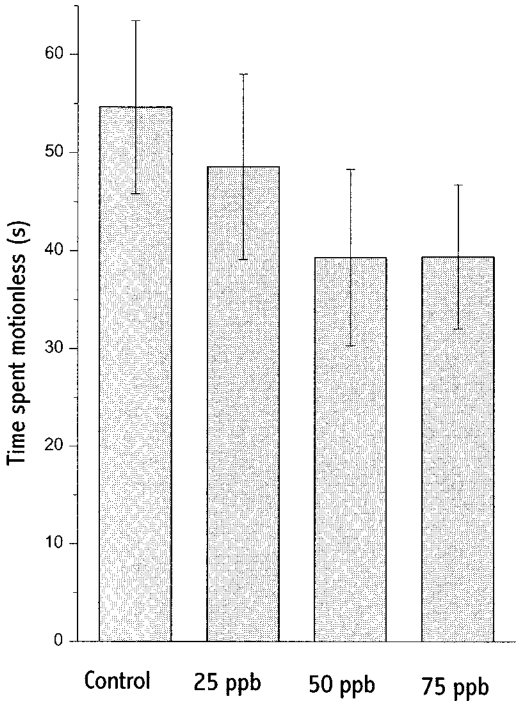

FIG. 10. Mean (± SEM) time spent motionless by animals presented with an alarm signal. There were no significant differences (LSD post-hoc test, $p > 0.05$).

havior after exposure: 1) a masking of the odor by the chemical, 2) impairment to the external chemosensory receptors, or 3) physiological impairment that could affect the animal's locomotory abilities. We were able to rule out number 1 because the animals were not exposed to contamination during stimulation.

Possible neurological effects

Results of our study suggest impairment to both neuromuscular control and the chemosensory ability to respond to particular stimuli. Previous research on sublethal exposure to pesticides has suggested that sensory impairment can occur as a result of inhibition of neurotransmitter receptors necessary for the processing of information (Moore and Waring 1996, Hanazato 1999, Scholz et al. 2000). A neurosensory impairment could affect systems used to detect potential threats and for foraging and feeding. Tomba et al. (2001) demonstrated that crayfish can distinguish between different types of odors when presented simultaneously and make decisions based on available olfactory information. There must be specific components or amino acids that code for the different stimuli to differentiate between an alarm signal and a food odor.

Studies with lobsters and catfish have illustrated that different olfactory receptors are sensitive to different amino acids (Caprio et al. 1993, Michel et al. 1993, Cromarty and Derby 1997, Steullet and Derby 1997). A combination of electrophysiological and behavioral studies demonstrated that lobsters can process a complex mixture of odors. This processing can allow for behavioral discrimination of these signals (Steullet and Derby 1997, Derby 2000). Contaminants can serve as inhibitors to olfactory neural pathways by interfering with communication between sensory organs and the processing center (Steele et al. 1992, Moore and Waring 1996, Saglio et al. 1996, Hebel et al. 1997, Carlson et al. 1998, Hanazato 1999, Scholz et al. 2000).

Possible physiological effects

Metolachlor may alter basic movement patterns in crayfish. Animals in our study exposed to the highest concentration displayed significantly slower walking speeds compared to the other treatment groups. Behavioral studies on fish and invertebrates demonstrated that sublethal exposure to contaminants causes changes in locomotory patterns (Steinberg et al. 1995, Hebel et al. 1997, Rice et al. 1997, Preston et al. 1999). Acute exposure of Japanese medaka to organic chemicals and 5 types of pesticides resulted in changes in swimming behavior, startle response, and equilibrium (Rice et al. 1997, Carlson et al. 1998). Preston et al. (1999) showed that the rotifer *Branchionus calciflorus* exhibited hyperactive swimming in the presence of a predator after pesticide exposure.

The mode of action of metolachlor is not well established. Metolachlor may inhibit the synthesis and metabolism of lipids and fatty acids in plants (Wu et al. 2000), but even less is known about its effects on aquatic organisms. Because pesticides cause inhibition to the nervous system (Moore and Waring 1996, Carlson et al. 1998), further study is necessary to establish the neuroanatomical sites of activity for metolachlor.

Implications for crayfish

Our study shows that, even though metolachlor is occurring at sublethal concentrations, biological effects that LC50 values do not address still exist (Shebra et al. 2000). Exposure to pesticides such as metolachlor may disable appropriate behavioral responses to food, mates, social status determination, and predator avoidance. Crayfish are an integral part of stream and lake ecosystems. Changing the feeding, social interactions, and avoidance behaviors of these animals can have wide-ranging implications for these systems.

Acknowledgements

We thank Rebecca Zulandt Schneider, Robert W. S. Schneider, Marilyn Sherman, Julie Adams, and Kim Kraus-Epley for their review of the manuscript and for their suggestions for this project. We also thank the members of Robert Huber's laboratory for their helpful suggestions, especially Alisdair Daws and Robert Huber for their help with the statistical analysis. This project was funded by NSF Grant numbers IBN 9514492 and 9874608.

Literature Cited

AHRENS, W. H. (EDITOR). 1994. Herbicide handbook. 7th edition. Weed Science Society of America, Champaign, Illinois.

APPELBERG, M., B. SÖDERBÄCK, AND T. ODELSTRÖM. 1993. Predator detection and perception of predation risk in the crayfish *Astacus astacus* L. Nordic Journal of Freshwater Research 68:55–62.

BATTAGLIN, W. A., E. T. FURLONG, M. R. BURKHARDT, AND C. J. PETER. 2000. Occurrence of sulfonylurea, sulfonamide, imidazolinone, and other herbicides in rivers, reservoirs and ground water in the Midwestern United States. Science of the Total Environment 248:123–133.

BROWN, G. E., AND R. J. F. SMITH. 1998. Acquired predator recognition in juvenile rainbow trout (*Oncorhynchus mykiss*): conditioning hatchery-reared fish to recognize chemical cues of a predator. Canadian Journal of Fisheries and Aquatic Sciences 55:611–617.

CAPRIO, J., J. G. BRAND, J. H. TEETER, T. VALENTINCIC, D. L. KALINOSKI, J. KOHBARA, T. KUMAZAWA, AND S. WEGERT. 1993. The taste system of the channel catfish: from biophysics to behavior. Trends in Neurosciences 16:192–197.

CARLSON, R. W., S. P. BRADBURY, R. A. DRUMMOND, AND D. E. HAMMERMEISTER. 1998. Neurological effects on the startle response and escape from predation by medaka exposed to organic chemicals. Aquatic Toxicology 43:51–68.

CHIVERS, D. P., AND R. J. F. SMITH. 1994. The role of experience and chemical alarm signaling in predator recognition by fathead minnows, *Pimphales promelus*. Journal of Fish Biology 44:273–285.

CHIVERS, D. P., B. D. WISENDEN, AND R. J. F. SMITH. 1995. The role of experience in the response of fathead minnows (*Pimephales promelus*) to skin extract of Iowa darters (*Etheostoma exile*). Behaviour 132:665–674.

CROMARTY, S. I., AND C. D. DERBY. 1997. Multiple excitatory receptor types on individual olfactory neurons: implications for coding of mixtures in the spiny lobster. Journal of Comparative Physiology A 180:481–491.

DERBY, C. D. 2000. Learning from spiny lobsters about chemosensory coding of mixtures. Physiology and Behavior 69:203–209.

DING, X., H. PENG, AND M. J. COON. 1992. Cytochromes P450 Nma, NMb (2G1), and LM4 (1A2) are differentially expressed during development in rabbit olfactory mucosa and liver. Molecular Pharmacology 42:1027–1032.

DUNHAM, D. W., K. A. CIRUNA, AND H. H. HARVEY. 1997. Chemosensory role of antennules in the behavioral integration of feeding by the crayfish *Cambarus bartonii*. Journal of Crustacean Biology 17:27–32.

Dunham, D. W., and J. W. Oh. 1996. Sex discrimination by female *Procambarus clarkii* (Girard, 1852) (Decapoda, Cambaridae): use of chemical and visual stimuli. Crustaceana 69:534–542.

Frey, J. W. 2001. Occurence, distribution, and loads of selected pesticides in streams in the Lake Erie-St. Clair basin, 1996–1998. USGS Water-Resource Investigations Report 00–4169. National Water Quality Assessment Program, Branch of Information Services, US Geological Survey, Denver, Colorado.

Geri, V. S., and D. W. Dunham. 2000. Female crayfish (*Procambarus clarkii*) (Girard, 1852) use both antennular rami in the localization of male odour. Crustaceana 73:447–458.

Gilliom, R. J., J. E. Barbash, D. W. Kolpin, and S. J. Larson. 1999. Testing water quality for pesticide pollution. Environmental Science and Technology 33:164A–169A.

Graham, W. H., D. W. Graham, F. Denoyelles, V. H. Smith, C. K. Larive, and E. M. Thurman. 1999. Metolachlor and alachlor breakdown product formation patterns in aquatic field mesocosms. Environmental Science and Technology 33:4471–4476.

Hanazato, T. 1999. Anthropogenic chemicals (insecticides) disturb natural organic chemical communication in the plankton community. Environmental Pollution 105:137–142.

Hartgers, E. A., G. H. Aalderink, P. J. Van Den Brink, R. Gylstra, J. Wiegman, F. Wilfred, and T. C. M. Brock. 1998. Ecotoxicological threshold levels of a mixture of herbicides (atrazine, diuron, and metolachlor) in freshwater microcosms. Aquatic Ecology 32:135–152.

Hazlett, B. A. 1985. Disturbance pheromones in the crayfish *Orconectes virilis*. Journal of Chemical Ecology 12:1695–1711.

Hazlett, B. A. 1990. Source and nature of disturbance-chemical system in crayfish. Journal of Chemical Ecology 16:2263–2275.

Hazlett, B. A. 1994. Alarm responses in the crayfish *Orconectes virilis* and *Orconectes propinquus*. Journal of Chemical Ecology 20:1525–1535.

Hebel, D. K., M. B. Jones, and M. H. Depledge. 1997. Responses of crustaceans to contaminant exposure: a holistic approach. Estuarine, Coastal and Shelf Science 44:177–184.

James, M. O., and S. M. Boyle. 1998. Cytochromes P450 in crustacea. Comparative Biochemistry and Physiology C 121:157–172.

Klaprat, D. A., R. E. Evans, and T. J. Hara. 1992. Environmental contaminants and chemoreception in fishes. Pages 321–341 in T. J. Hara (editor). Fish chemoreception. Chapman and Hall, New York.

Lin, Y., M. Karuppiah, A. Shaw, and G. Gupta. 1999. Effect of simulated sunlight on atrazine and metolachlor toxicity of surface waters. Ecotoxicology and Environmental Safety 43:35–37.

Michel, W. C., H. G. Trapido-Rosenthal, E. T. Chao, and M. Wachowiak. 1993. Stereoselective detection of amino acids by lobster olfactory receptor neurons. Journal of Comparative Physiology A 171:705–712.

Moore, A., and C. P. Waring. 1996. Sublethal effects of the pesticide diazinon on olfactory function in mature male Atlantic salmon parr. Journal of Fish Biology 48:758–775.

Moore, P. A., and J. L. Grills. 1999. Chemical orientation to food by the crayfish *Orconectes rusticus*: influence of hydrodynamics. Animal Behaviour 58:953–963.

Moore, P. A., N. Scholz, and J. Atema. 1991. Chemical orientation of lobsters, *Homarus americanus*, in turbulent odor plumes. Journal of Chemical Ecology 17:1293–1307.

Preston, B. J., G. Cecchine, and T. W. Snell. 1999. Effects of petachlorophenol on predator avoidance behavior of the rotifer *Brachionus calyciflorus*. Aquatic Toxicology 44:201–212.

Quinn, V. S., and B. M. Graves. 1998. Responses to the odors of conspecifics by the crayfish *Orconectes virilis*. Crustaceana 71:856–861.

Rice, P. J., C. D. Drewes, T. M. Klubertanz, S. P. Bradbury, and J. R. Coats. 1997. Acute toxicity and behavioral effects of chlorpyrifos, permethrin, phenol, strychnine and 2,4-dinitrophenol to 30-day-old Japanese medaka (*Oryzias latipes*). Environmental Toxicology and Chemistry 16:696–704.

Saglio, P., and S. Trijasse. 1998. Behavioral responses to atrazine and diuron in goldfish. Archives of Environmental Contamination and Toxicology 35: 484–491.

Saglio, P., S. Trijasse, and D. Azam. 1996. Behavioral effects of waterborne carbofuran in goldfish. Archives of Environmental Contamination and Toxicology 31:232–238.

Saucier, D., L. Astic, and P. Rioux. 1991. The effects of early chronic exposure to sublethal copper on the olfactory discrimination of rainbow trout, *Oncorhynchus mykiss*. Environmental Biology of Fishes 30:345–351.

Scholz, N. L., N. K. Truelove, B. L. French, B. A. Berejikian, T. P. Quinn, E. Casillas, and T. K. Collier. 2000. Diazinon disrupts antipredator and homing behaviors in Chinook salmon (*Oncorhynchus tshawytscha*). Canadian Journal of Fisheries and Aquatic Sciences 57:1911–1918.

Shebra, M., D. W. Dunham, and H. H. Harvey. 2000. Sublethal copper toxicity and food response in the freshwater crayfish *Cambarus bartonii* (Cambaridae, Decapoda, Crustacea). Ecotoxicology and Environmental Safety 46:329–333.

Sivarajah, K., C. S. Franklin, and W. P. Williams.

1979. Some studies of the hepatic microsomal enzyme activities and steroid hormone levels in carp, *Cyprinus carpio* exposed for six months. Journal of Fish Biology 15:249–253.

SNYDER, M. J. 2000. Cytochrome P450 enzymes in aquatic invertebrates: recent advances and future directions. Aquatic Toxicology 48:529–547.

STEELE, C. W., S. STRICKLER-SHAW, AND D. H. TAYLOR. 1992. Attraction of crayfishes *Procambarus clarkii*, *Orconectes rusticus* and *Cambarus bartonii* to a feeding stimulant and its suppression by a blend of metals. Environmental Toxicology and Chemistry 11:1323–1329.

STEINBERG, C. E. W., R. LORENZ, AND O. H. SPIESER. 1995. Effects of atrazine on swimming behavior of zebrafish, *Brachydanio rerio*. Water Research 29: 981–985.

STEULLET, P., AND C. D. DERBY. 1997. Coding of blend ratios of binary mixtures by olfactory neurons in the Florida spiny lobster, *Panulirus argus*. Journal of Comparative Physiology A 180:123–135.

TOMBA, A. M., T. A. KELLER, AND P. A. MOORE. 2001. Foraging in complex odor landscapes: chemical strategies during stimulation by conflicting chemical cues. Journal of the North American Benthological Society 20:211–222.

USEPA (US Environmental Protection Agency). 1993. Methods for measuring the acute toxicology of effluents and receiving waters to freshwater and marine organisms. EPA/600/4–90/027F. 4th edition. Office of Research and Development, US Environmental Protection Agency, Washington DC.

USEPA (US Environmental Protection Agency). 1995. R. E. D. facts metolachlor. EPA-738-F-95-007. Office of Pesticide Programs, US Environmental Protection Agency, Washington, DC.

WU, J., I. HWANG, AND K. K. HATZIOS. 2000. Effects of chloroacetanilide herbicides on membrane fatty acid desaturation and lipid composition in rice, maize and sorghum. Pesticide Biochemistry and Physiology 66:161–169.

ZAR, J. H. 1999. Biostatistical analysis. 4th edition. Prentice-Hall, Upper Saddle River, New Jersey.

ZENI, C., A. STAGNI, AND M. R. BOVOLENTA. 1995. Damage and recovery of *Ictalurus* sp. barbel taste buds exposed to sublethal concentrations of an anionic detergent. Aquatic Toxicology 31:113–123.

ZULANDT SCHNEIDER, R. A., R. W. S. SCHNEIDER, AND P. A. MOORE. 1999. Recognition of dominance status by chemoreception in the red swamp crayfish, *Procambarus clarkii*. Journal of Chemical Ecology 25:781–794.

ZULANDT SCHNEIDER, R. A., R. W. S. SCHNEIDER, AND P. A. MOORE. 2000. Urine as a source of conspecific disturbance signals in the crayfish *Procambarus clarkii*. Journal of Experimental Biology 203: 765–771.

Received: 3 December 2001
Accepted: 29 April 2002