

Dissolved organic matter from elevated-CO₂ detritus and its impact on the orientation of crayfish (*Orconectes virilis*) to a fish food source

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Abstract. Crayfish must locate food resources in a chemically complex environment in which chemicals from various sources interact and mix. We tested the hypothesis that an additional chemical food source of leachate from detritus (dissolved organic matter [DOM]) would affect crayfish orientation behavior toward a fish stimulus. In addition, we predicted that the effect of DOM would differ depending on whether the detritus was produced under ambient (AMB) or elevated (ELEV) CO₂ because of changes in foliar chemistry that accompany elevated CO₂ levels. DOM was collected from leaf litter derived from quaking aspen (*Populus tremuloides*) foliage that was produced at either AMB or ELEV CO₂ and was presented at 2 concentrations (3 mg/L, 6 mg/L). Stimulus treatments were: 1) CON (control; fish odor only), 2) AMB-low (fish + AMB DOM at 3 mg/L), 3) AMB-high (fish + AMB DOM at 6 mg/L), 4) ELEV-low (fish + ELEV DOM at 3 mg/L), and 5) ELEV-high (fish + ELEV DOM at 6 mg/L). Crayfish (*Orconectes virilis*) were observed in a recirculating flume in which the fish odor source was placed downstream of the DOM odor source. Behavioral responses measured were % success in locating the fish odor source, time to find source, walking speed, walking speed toward the source, turning angle, heading angle relative to the source, heading angle relative to upstream, and net-to-gross ratio (NGR). Crayfish in the AMB-high treatment were most successful in locating the source. Animals had higher turning angles in the ELEV-high treatment than in all treatments except the control and had increased heading angles relative to the source and decreased heading angles relative to upstream. No differences were found for walking speed or NGR. These results indicate that crayfish orientation to a fish odor source is affected by the presence of DOM from detritus, but only when it is presented at the high end of a natural range of DOM concentration. It also appears that the presence of ambient CO₂ detritus (with a lower concentration of secondary defensive compounds) increases success in finding the source, whereas the presence of elevated CO₂ detritus does not. Possibly, in an elevated-CO₂ future, behaviors associated with foraging and selection of food types by crayfish might be altered, and therefore affect the aquatic community. These effects might be seasonal and synchronized with the natural fluctuations of DOM found in lotic systems.

Key words: crayfish, *Orconectes virilis*, orientation, multiple chemical cues, complexity, elevated CO₂, *Populus tremuloides*, dissolved organic matter (DOM).

Many animals across taxa must identify and locate food that is patchily distributed in the environment (Dejean and Benhamou 1993, Moore and Grills 1999, Lohmann and Johnsen 2000, Sherman and Moore 2001). They find food by using highly developed sensory systems, including visual, auditory, chemo-

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receptive, and electroreceptive systems, to gather information (Dusenbery 1992). In particular, chemoreception plays an important role in the foraging behaviors of both terrestrial (Dethier 1976, Bell and Cardé 1984) and aquatic (Atema 1988, Zimmer and Butman 2000) organisms.

Organisms must extract directional information from the complex spatial and temporal aspects of an odor plume to locate a stimulus source. Distribution of odor molecules from a source results from both molecular diffusion and bulk flow of the medium (Dusenbery 1992). The odor plume is turbulent and filamentous for organisms >1 cm, with patches of high and low stimulus concentration (Atema 1988). As organisms approach an odor source, the odor filaments occur more often and are of higher concentration (Murlis and Jones 1981, Murlis et al. 1992). Therefore, odor distribution through space and time aids location of the odor source (Atema 1988, Vickers 2000). In addition to effects from turbulence, the chemical complexity of a fluid environment is increased by mixing of odors from different sources.

Animals use chemical information to identify and localize food sources (Croll and Chase 1980, Tierney and Atema 1988, Vickers and Baker 1992, Moore and Lepper 1997) and to identify potential mates (Sorenson and Scott 1994, Mafra-Neto and Cardé 1995, Zippel et al. 1997). Chemicals also are used in social situations to convey dominance status (Breithaupt and Atema 1993, Moore et al. 1995, Zulantz-Schneider et al. 1999, 2001) and alarm (Hazlett 1985, 1990, Chivers et al. 1996). In addition, chemicals released from predators can signal their presence to prey (Chivers and Smith 1993, Kiesecker et al. 1996). Animals might receive simultaneous chemical cues from many sources, such as various food types, multiple predators, and individual conspecifics. These cues might be in conflict (e.g., food odor and predator odor) or have additive effects (e.g., predator kairomone and conspecific alarm signals). When presented with these odors simultaneously, animals must make decisions about the relative importance of each and the appropriate behavioral response.

The interaction of chemicals from different sources influences behavior in a number of animals, especially in aquatic systems. For example, *Daphnia* exhibit vertical migration (a short-term/proximate antipredatory behavior) when presented with either fish kairomone or crushed conspecific alarm cue alone, but produce resting eggs (a long-term/ultimate antipredatory behavior) when these cues are presented simultaneously (Slusarczyk 1999). The snail, *Physella gyrina*, responds to fish predator odor by finding shelter and crayfish predator odor by using

surface waters, but shows an intermediate response when the stimuli are presented together (Turner et al. 2000). Crayfish reduce locomotory behavior associated with feeding when presented with food and predator (snapping turtle) odor (Hazlett 1999). Similarly, crayfish orienting to a food source (fish) in the presence of a predator odor along the midline of a flume moved along the sides of the flume and spent more time in shelters than foraging (Tomba et al. 2001). These interactions between chemical signals probably are important for organisms, such as crayfish, that rely heavily on chemoreception for many behaviors.

Crayfish, decapod crustaceans that inhabit freshwater lakes and rivers, are an excellent model for investigating the effect of odor interaction on chemoreceptive decisions because they depend on chemoreception for a variety of behaviors, ranging from foraging to recognition of dominance status in conspecifics (Moore and Grills 1999, Zulantz-Schneider et al. 2001). The chemical environment in which crayfish forage is complex (Moore and Grills 1999, Keller et al. 2001, Tomba et al. 2001, Wolf et al. 2004) and is composed of odors that directly pinpoint a particular source (food, conspecifics, mates, and predators) and ubiquitous background odors. These background odors consist largely of dissolved organic matter (DOM) that leaches from terrestrial leaf litter. At high concentrations, chemicals from DOM, such as condensed tannins and phenolics, might influence crayfish foraging through interference with chemoreception. Derby et al. (1984) found that chemoreceptors on the antennae and walking legs of lobsters were excited by tannic acid and that tannic acid behaviorally affected lobsters by inhibiting food ingestion. Tannins and other secondary defensive compounds from plants (lignoid and alkaloid compounds) have been implicated in the deterrence of herbivory (Lodge 1991, Bolser and Hay 1998, Bolser et al. 1998, Wilson et al. 1999, Kubanek et al. 2000, 2001). Given that tannic acid is detected by chemoreceptor cells of lobsters, which are closely related to crayfish, these defensive chemicals also might interfere with normal crayfish orientation to food.

The concentration of secondary defensive compounds in tissues of terrestrial tree species increases when trees are grown in the presence of elevated atmospheric CO₂ (Strain and Bazzaz 1983, Lindroth et al. 1993, see Peñuelas and Estiarte 1998, Coviella and Trumble 1999 for reviews). These changes in foliar chemistry are conserved in leaf litter that falls into streams (Rier et al. 2002) and in the DOM leaching from it (Rier et al. 2002, Adams et al. 2003). The increases in defensive compounds at the level of the

individual leaf potentially could result in a collective increase in concentration in the stream as a whole. The purpose of our study was to determine whether crayfish chemosensory orientation is affected differentially by leachate from detritus produced at the current or at elevated CO₂ concentration. We hypothesized that background DOM from elevated CO₂ detritus would decrease crayfish efficiency in finding food because of the higher concentration of secondary defensive chemicals present in the leachate.

Methods

Flume design

Orientation trials were conducted in a 3650-L recirculating flume (complete dimensions: 568 × 57 cm, working section: 244 × 57 × 61 cm; Fig. 1A). The flume was filled with dechlorinated water (average water temperature 20.1 ± 1.1°C) to an approximate depth of 20 ± 0.8 cm. The frame of the flume (working section and reservoir tanks) was constructed of stainless steel with glass as the sides and bottom of the working section. The bottom of the flume was lined with aquarium gravel (diameter 0.26 ± 0.03 cm, *n* = 50 measurements; depth 2.1 ± 0.2 cm). A sheet of polycarbonate core honeycomb (2.54-cm-diameter holes) and a sheet of fluorescent light grating (169-mm² holes) wrapped with fiberglass screen (1-mm² holes) were positioned upstream as collimators. A downstream baffle composed of fluorescent light grating wrapped with fiberglass screen prevented crayfish from escaping into the reservoir tank. Flow was regulated by a centrifugal pump (model 005180P3E184JM; WEG, Suwanee, Georgia) with an adjustable speed drive (model ID15H205-E, Baldor, Fort Smith, Arkansas). Average flow velocity for these experiments was ~4.96 ± 0.03 cm/s measured 5 cm above the substrate at 15 points along the center and each wall of the flume (Marsh-McBirney® model 2000 Portable Flow Meter; Marsh-McBirney, Frederick, Maryland). Background accumulation of odors was prevented with a Jacuzzi filter system (Landslide LS40, Little Rock, Arkansas) used between trials. The flume was drained and refilled approximately every 3 wk, or ~75 trials. An initial 3-dimensional flow profile measured with an Acoustic Doppler Velocimeter (Nortek® AS P107/01; Nortek USA, Annapolis, Maryland) showed an equilibrium boundary layer in the flume.

Animals

Male and female intermolt crayfish, *Orconectes virilis* (Hagen), were collected from the Department

of Natural Resources (DNR) Fisheries Research Station in Saline, Michigan, USA (lat 42°09'N, long 83°46'W) between April and July 2003. Animals (*n* = 349, with 260 males and 89 females; mean ± SE carapace length: 4.41 ± 0.04 cm, mass: 27.28 ± 0.72 g) were originally stored in population tanks and were fed rabbit food pellets 3×/wk. Animals were later visually and mechanically isolated and starved in individual pots (inner diameter: 16 cm, depth: 9 cm) stored in a holding tank for 72 h before use. The average water temperature of the holding tank was 22.8 ± 0.3°C. Both male and female crayfish were used for trials, and all crayfish used had a complete set of chemosensory appendages (1 pair 1st antennae, 2 pair 2nd antennae, 1 pair chelae, all walking legs). Each crayfish was used only once in an experiment.

Odor treatments, preparation, and delivery

Orientation to a fish odor source was examined in the presence of 1 of 2 different DOM odors at varying concentrations. DOM odors were collected from quaking aspen (*Populus tremuloides* Mischeaux) leaf litter that had been produced at either ambient (AMB, 360 mg/L) or elevated (ELEV, 720 mg/L) CO₂ conditions (for detailed methods of growing aspen trees under elevated CO₂ conditions, see Rier et al. 2002, Tuchman et al. 2003). Each type of DOM (AMB or ELEV) was introduced into the flume at 2 different concentrations: 3 mg/L and 6 mg/L (or 0 mg/L for the control treatment). These concentrations were selected because they represent natural concentrations of DOM from leaf litter found in streams (Fisher and Likens 1972). The 3-mg/L concentration represents a yearly average, and the 6-mg/L concentration represents a seasonally high concentration of DOM that occurs in autumn after leaf fall and early spring from snow melt (range of concentration from Fisher and Likens 1972 is 0.75–7 mg/L). The 5 DOM conditions tested were: 1) control, consisting of only flume water (CON), 2) AMB DOM at 3 mg/L (AMB-low), 3) AMB DOM at 6 mg/L (AMB-high), 4) ELEV DOM at 3 mg/L (ELEV-low), and 5) ELEV DOM at 6 mg/L (ELEV-high). The DOM odor was introduced through a separate odor source upstream of the fish odor; thus, the 2 odors were superimposed.

Two cylindrical, undergravel odor sources were constructed (diameter: 5 cm, depth: 0.5 cm) with Plexiglas® tops and bottoms connected to a small polyvinyl chloride (PVC) rim with PVC cement (Fig. 1B). Holes (1-mm diameter) were evenly spaced on the top of the odor source to allow diffusion of the stimulus through the gravel and into flow. Stimulus flowed from glass odor-delivery jars (one for each

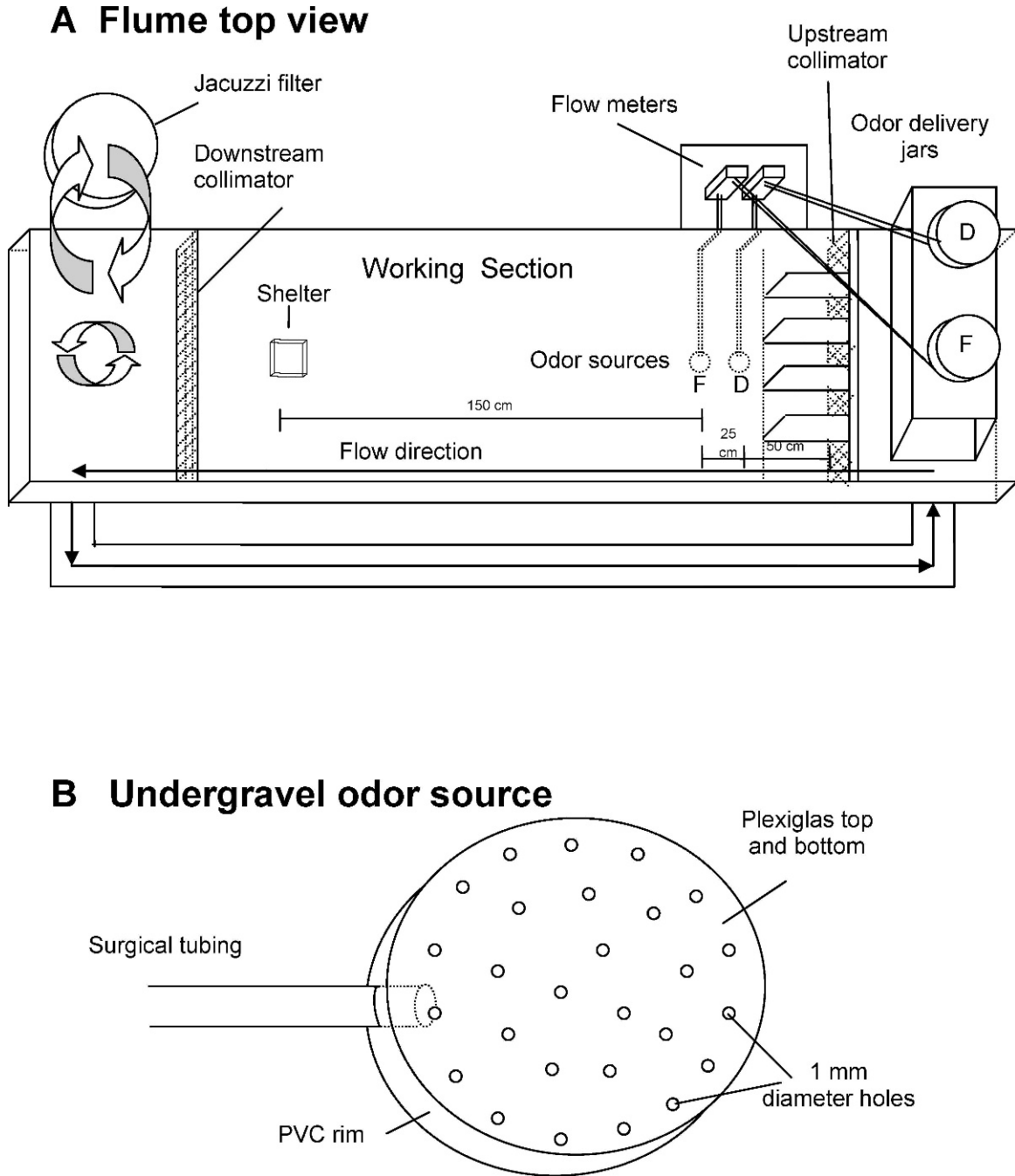


FIG. 1. A.—Top view of the 3650-L recirculating flume used for experiments (complete dimensions: 568 × 57 cm, working section: 244 × 57 × 61 cm). Water flow (5 cm/s) was from right to left in the diagram. Collimators were placed upstream and downstream of the working section. Two different odors flowed from the appropriate odor delivery jar, through a flow meter, to an undergravel odor source (D = DOM odor, F = fish odor). Crayfish were acclimated in a shelter for 20 min (18 min without odor + 2 min with odor). A Jacuzzi filter was used between trials to clear the water of odors from the previous trial. B.—The undergravel odor source used to deliver odor into the flume was constructed from a plexiglas top and bottom connected to a small polyvinyl chloride (PVC) ring with PVC cement (diameter: 5 cm, depth: 0.5 cm, holes: 1-mm diameter).

odor source) through surgical tubing (inner diameter: 0.64 cm, outer diameter: 1.6 cm) to a flow meter (size 2 steel ball; Manostat, New York, New York) that regulated the amount of odor delivered through

surgical tubing to the undergravel source. The 2 odor sources were placed in series along the bulk-flow axis equidistant from both walls of the flume (Fig. 1A), with the 1st source 50 cm and the 2nd source 75 cm

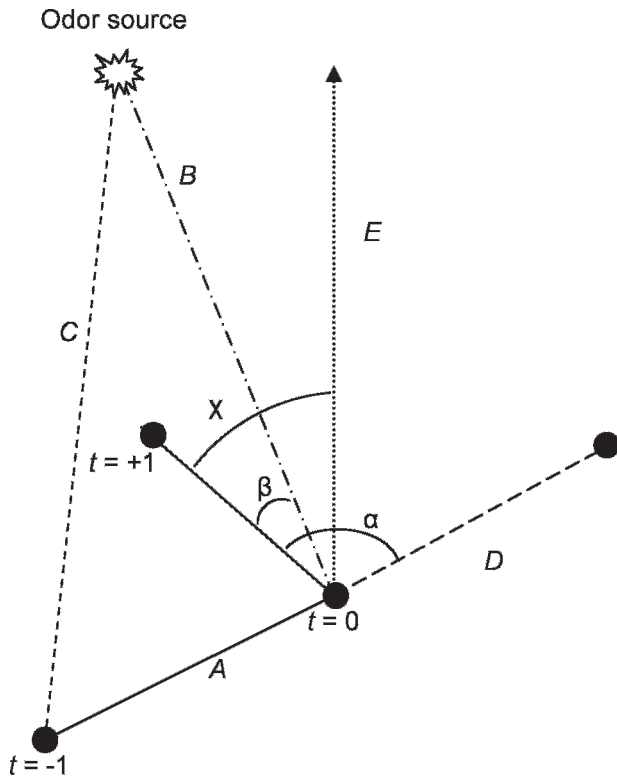


FIG. 2. Vector diagram illustrating orientation variables. Dots represent successive crayfish positions ($t = -1$, 0 , and $+1$) on a hypothetical orientation path. The line \bar{A} represents distance traveled over the time period $t = -1$ to $t = 0$ (walking speed). The difference between distance to the odor source at time $t = -1$ (line \bar{C}) and distance to the odor source at time $t = 0$ (line \bar{B}) is the walking speed toward the source. The dashed line represents the crayfish's projected path (had it continued on line \bar{A}). The angle between the projected path and the line connecting positions $t = 0$ and $t = +1$ is the turn angle (α). Line \bar{B} represents the shortest distance to the odor source (star), and the angular deviation from this path is the heading angle relative to the odor source (β). The angle between the arrow E (straight upstream) and the line connecting positions $t = 0$ and $t = +1$ is the heading angle relative to upstream (γ). Redrawn with permission from Moore and Grills (1999).

downstream of the honeycomb collimator (sources were 25 cm apart directly downstream). DOM odor was introduced through the upstream odor source and fish odor was introduced through the downstream odor source. Locations of the odor sources were marked with a circle of black gravel for later visualization during analysis.

Treatment preparation

Fish stimulus.—Liquid fish stimulus was prepared by homogenizing ~ 11 g (10.96 ± 0.11 g) of previously frozen cod with 1 L of flume water in a blender for

4 min. Similar concentrations of odorant have been used successfully in previous orientation trials with crayfish (Wolf and Moore 2002, Kozłowski et al. 2003). Homogenate was then passed twice through a 60- μ m-mesh sieve to remove large particles of fish flesh.

DOM odors.—Concentrated stock solutions of AMB or ELEV DOM were made by placing 25 g of dried AMB or ELEV detritus into a plastic container with 1.25 L of dechlorinated water and agitating them on a magnetic stir plate for 24 h. Detrital material was filtered from the leachate with a sieve (60- μ m mesh). Concentrated leachate was stored in 0.5-L plastic Nalgene containers in the freezer at -20°C until use. Leachate was thawed as needed and was never stored for >1 wk. The concentration of the stock solution was determined from estimates of dry mass lost from leaching of *P. tremuloides* leaf litter (Rier et al. 2002). Approximately 30% of leaf litter dry mass is lost as DOM after 24 h of leaching. Therefore, the concentration of stock solution was ~ 6 g DOM/L (30% of 25 g leaves/1.25 L water = 6 g/L). Background odor was diluted to the appropriate concentration by adding a particular volume of stock solution (3 mg/L = 0.5 mL of stock solution, 6 mg/L = 1 mL of stock solution) and filling to 1 L with flume water. The treatment concentration of DOM odor was the concentration released at the odor source and not after dilution in the flume. The actual concentrations in the flume might have been less than the yearly background average calculated from Fisher and Likens (1972), but were still representative of natural concentrations experienced by crayfish. This design was instead a more realistic mimic of the heterogeneous chemical conditions crayfish probably experience in proximity to leaf packs or individual detrital leaves.

Experimental protocol

Crayfish were acclimated before trials for 18 min to temperature and flow in a cubical shelter. The shelter was composed of 2 alternated layers of fluorescent light grating (shelter dimensions: $12.7 \times 12.7 \times 12.7$ cm, hole dimensions: 1.0×0.5 cm) and was placed 150 cm downstream of the fish odor source. Odor flow was initiated at a rate of 15 mL/min for 2 min. After the 2-min acclimation to odor (for a total of 20 min of acclimation), the crayfish was released and allowed to explore the flume until it either found or walked past the source or 10 min had elapsed. A trial was deemed successful only if the animal did not touch the wall within 75% of the distance between the shelter and the food source (112.5 cm downstream of the food odor source). If crayfish walked directly up the side of the flume (touching the wall), they were

TABLE 1. Orientation variables measured during crayfish orientation trials (See Fig. 2 for further explanation of variables). t = time point.

Variable	Definition
Time to find source (s)	Time from shelter removal to touching the source with ≥ 1 chela
Time spent not moving (s)	Sum of all times when crayfish walking speed was < 0.2 cm/s (accounting for human error in digitization)
Walking speed (cm/s)	Distance traveled per unit time
Walking speed toward the source (cm/s)	A vector measuring the change in distance from the source per unit time
Walking speed while moving (cm/s)	Average walking speed after removal of time blocks when crayfish were stationary (walking speed < 0.2 cm/s)
Turning angle (α)	Angle between the path connecting the previous ($t = -1$) crayfish position to the present ($t = 0$) position and the path connecting the present ($t = 0$) position and the next ($t = +1$) position (Fig. 2)
Heading angle relative to the source (β)	Angle between the path connecting the source to the present ($t = 0$) crayfish position and the path connecting the present ($t = 0$) position and the next ($t = +1$) position with an angle of 0 pointing directly at the source (Moore et al. 1991)
Heading angle relative to upstream (χ)	Similar to heading angle relative to the source, except that the source was replaced with a point directly upstream
Net-to-gross ratio (NGR)	A ratio of the straight line distance from start to end of path divided by the total path length

netted and returned to the starting position for a 2nd chance. Animals that walked along the wall for the length of the trial (for both the 1st and 2nd chance) were deemed unsuccessful and were eliminated from analyses because they might have been using cues other than chemical information, e.g., mechanical stimulation from the walls of the flume, to move upstream. Trials were videotaped from above for later video analysis using a Canon XL-1 digital camcorder (Canon, Tokyo, Japan) and were displayed on a Sony PVM-1351G monitor (Sony USA, New York, New York) during the experiment. Preliminary analysis indicated that 20 replicates were adequate to test for differences among treatments. It was necessary to run 66 trials to reach 20 successful trials for the CON treatment, so 66 trials were run in all other treatments as well. Percent success calculations were based on the number of successful trials of 66, and all other variables were measured only from successful trials.

Data and statistical analysis

Videotapes were analyzed using Peak Motus Motion Analysis[®] software (Vicon, Centennial, Colorado) to digitize the x and y coordinates of the crayfish. The x and y coordinates of the crayfish rostrum were digitized once every second for the total period of the trial. Preliminary calculations showed no differences in walking speed or time to locate source between males and females (1-way multivariate analysis of variance [MANOVA], $F_{0.05,1,28} = 0.243$, $p > 0.05$), so data from both sexes were pooled for subsequent analyses. In each treatment, all trials (of the 66 trials performed) in which animals

successfully oriented to the odor source were digitized. Final sample sizes (number of digitized trials) for each treatment were: CON: $n = 20$, AMB-low: $n = 19$, AMB-high: $n = 27$, ELEV-low: $n = 19$, ELEV-high: $n = 17$.

Behavioral variables measured for each treatment were the proportion of animals that successfully located the source (% success) and the average time to locate the source (s). Orientation variables calculated for each treatment from (x , y) coordinates included time spent not moving (s), walking speed (cm/s), walking speed toward the source (cm/s), walking speed while moving (cm/s), turning angle, heading angle toward the source, heading angle upstream, and net-to-gross ratio (or straightness of path, NGR). All variables are defined in Table 1 and Fig. 2. In previous orientation studies of crayfish, these variables accurately measured the spatial and temporal characteristics of orientation (Moore et al. 1991, Moore and Grills 1999, Keller et al. 2001, Kraus-Epley and Moore 2002). The absolute value of all angle measures was used in subsequent data analysis; thus linear statistics were used to analyze angle distributions, which ranged in value from 0 to 180°. Average values of all variables were calculated for individual crayfish from the digitized paths. These measures of individual behavior were averaged for each treatment, and the averages were used for statistical analyses.

A contingency table for the multiple comparison of proportions (Z_C) was used to test for differences in % success. A 2-way factorial MANOVA with least significant difference (LSD) post hoc tests (Statistica 6.0; Statsoft, Tulsa, Oklahoma) was used to test for differences in variables (time to find the source, time

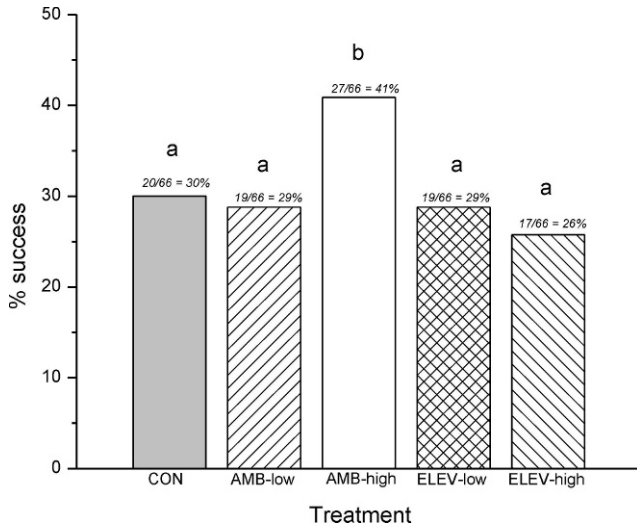


FIG. 3. Percent success in finding the fish odor source by crayfish in the presence of a dissolved organic matter (DOM) odor source derived from leaf litter from aspen leaves grown in ambient (AMB) or elevated (ELEV) CO₂. Stimulus treatments were: 1) CON (control; fish odor only), 2) AMB-low (fish + AMB DOM at 3 mg/L), 3) AMB-high (fish + AMB DOM at 6 mg/L), 4) ELEV-low (fish + ELEV DOM at 3 mg/L), and 5) ELEV-high (fish + ELEV DOM at 6 mg/L). Numbers above bars show the number of successful trials of a total of 66 in each treatment and the percentage successful trials. Bars with different letters are significantly different (contingency table, $p \leq 0.05$).

spent not moving, average walking speed, walking speed toward the source, walking speed while moving, turning angle, heading angle toward the source, heading angle upstream, NGR) with DOM odor type (AMB vs ELEV) and DOM concentration (low vs high) as the 2 factors. Significance was assessed at $p \leq 0.05$.

Results

Behavioral variables

Percent success was higher in the AMB-high treatment than in any other treatment (contingency

table, $Q_{0.05,inf,0.5} = 3.86$, $Q_{AMB-high \text{ vs } CON} = 4.44$, $Q_{AMB-high \text{ vs } ELEV-high} = 5.18$, $Q_{AMB-high \text{ vs } AMB-low} = 4.10$, $Q_{AMB-high \text{ vs } ELEV-low} = 4.10$; Fig. 3, Table 2). Time to locate source and time spent not moving did not differ significantly among treatments (1-way MANOVA, $F_{0.05,9,70} = 0.983$, $p > 0.05$; Table 2).

Orientation variables

The DOM odor \times DOM concentration interaction term of the MANOVA was statistically significant ($F_{0.05,9,70} = 1.553$, $p > 0.05$). DOM odor significantly affected crayfish orientation variables (1-way MANOVA, $F_{0.05,9,70} = 1.919$, $p < 0.004$; Table 2). LSD post hoc tests showed no differences in walking speed, walking speed toward the source, walking speed while moving, and NGR among treatments. Turning angle did not differ between the CON and ELEV-high treatments (LSD, $p = 0.07$, $df = 78$; Fig. 4A) but was significantly higher in the ELEV-high than in the AMB-low (LSD, $p < 0.004$, $df = 78$), AMB-high (LSD, $p < 0.02$, $df = 78$), and ELEV-low (LSD, $p < 0.04$, $df = 78$) treatments. Heading angle toward the source was significantly higher in the ELEV-high than in any other treatment (LSD, CON: $p < 0.005$, $df = 78$; AMB-low: $p < 0.002$, $df = 78$; AMB-high: $p < 0.007$, $df = 78$; ELEV-low: $p < 0.005$, $df = 78$; Fig. 4B). In contrast, heading angle upstream was significantly lower in the ELEV-high treatment than in any other treatment (LSD, CON: $p < 0.002$, $df = 78$; AMB-low: $p < 0.006$, $df = 78$; AMB-high: $p < 0.002$, $df = 78$; ELEV-low, $p < 0.002$, $df = 78$; Fig. 4C).

Discussion

Our experiment tested whether the foraging behavior of crayfish could be affected by the presence of DOM from detritus produced at elevated CO₂ conditions. Concentrations of phenolics and tannins are higher in DOM from detritus produced at elevated CO₂ than in DOM from detritus produced under ambient CO₂ concentrations, so increased

TABLE 2. Mean (\pm SE) values of behavioral and temporal orientation variables for crayfish seeking a fish odor source in the presence of a dissolved organic matter (DOM) odor source derived from leaf litter from aspen leaves grown in ambient (AMB) or elevated (ELEV) CO₂ at low (3 mg/L) or high (6 mg/L) concentrations. CON = fish odor only, WS = walking speed, NGR = net-to-gross ratio (see Table 1 for explanation).

Treatment	% success	Time to source (s)	Proportion of time not moving (s)	WS (cm/s)	WS to source (cm/s)	WS moving (cm/s)	NGR
CON	30	124 \pm 18	0.02 \pm 0.01	4.0 \pm 0.2	3.2 \pm 0.2	4.0 \pm 0.2	0.81 \pm 0.02
AMB-low	29	128 \pm 26	0.03 \pm 0.01	4.2 \pm 0.3	3.4 \pm 0.3	4.3 \pm 0.3	0.83 \pm 0.03
AMB-high	41	103 \pm 10	0.02 \pm 0.01	4.4 \pm 0.2	3.5 \pm 0.3	4.5 \pm 0.2	0.81 \pm 0.02
ELEV-low	29	100 \pm 8	0.02 \pm 0.01	4.1 \pm 0.3	3.4 \pm 0.4	4.3 \pm 0.3	0.84 \pm 0.03
ELEV-high	26	147 \pm 29	0.02 \pm 0.01	3.6 \pm 0.3	2.8 \pm 0.3	4.0 \pm 0.3	0.74 \pm 0.03

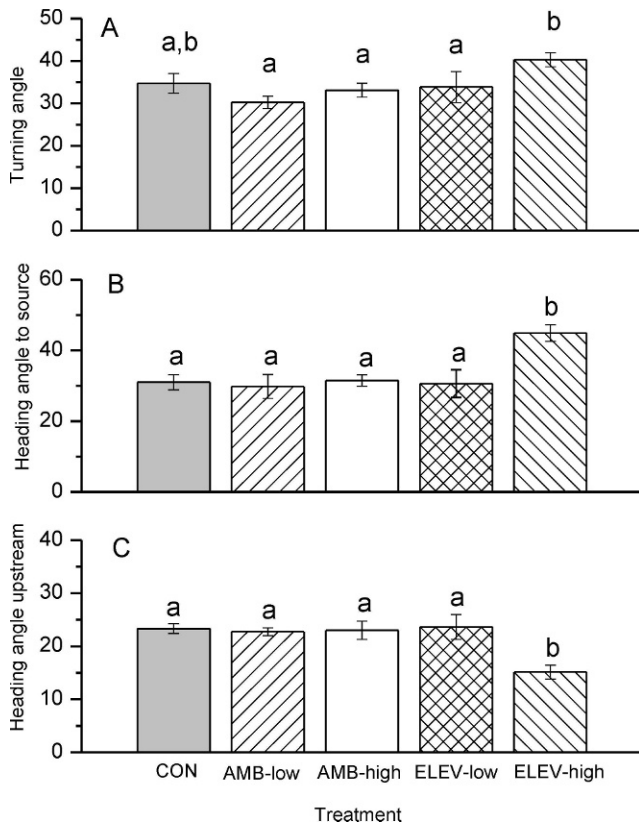


FIG. 4. Mean (± 1 SE) turning angle (A), heading angle relative to source (B), and heading angle relative to upstream (C) calculated for crayfish in each treatment (see Fig. 2 for explanation of angles and Fig. 3 for treatment abbreviations). The number of trials digitized for each treatment was $n = 20$ (control), $n = 19$ (AMB-low), $n = 27$ (AMB-high), $n = 19$ (ELEV-low), and $n = 17$ (ELEV-high). Bars with different letters are significantly different (least significant difference test, $p \leq 0.05$).

background levels of these compounds in streams (collectively from all leaf litter) might interfere with crayfish chemoreception and, therefore, foraging behavior. Overall, our study demonstrated that the presence of DOM from detritus does affect crayfish orientation to food when DOM is at the high end of concentrations found in natural streams. Temporal variables of orientation (time to find source, time spent not moving, walking speed, walking speed toward the source, walking speed while moving) were not altered, but % success and orientation variables (turning angle, heading angle to source, and heading angle upstream) differed significantly among treatments. Thus, we conclude that DOM from detritus produced at elevated CO₂ might decrease the orientation efficiency of crayfish toward flesh-based food sources.

Percent success in locating the source was higher in the AMB-high treatment than in any other treatment. This result suggests that, for this experimental setup, high concentrations of DOM from detritus produced at ambient CO₂ concentration might enhance the ability of a crayfish to orient. Detritus is an important food source for crayfish (Huryn and Wallace 1987, Momot 1995), and crayfish respond to chemicals found in the leachate from detritus (Adams et al. 2003). The DOM source in our experiment was basically another point source upstream of the food source. Thus, crayfish could have perceived 2 food sources (fish and detritus) and been strongly attracted to the general location of the odor sources. This attraction would have led to a higher probability of locating the fish odor source than the detritus because the DOM source was upstream of the fish source.

In contrast, ELEV-high DOM in the background increased heading angle toward the source and decreased heading angle relative to upstream. Turning angle also was higher in the ELEV-high treatment than in all treatments except CON. These 3 variables indicate that in the ELEV-high treatment, crayfish were, on average, heading more directly upstream (not necessarily toward the source or up the center of the flume) before making larger turns to find the source, with the result that their path to the food was less direct and their orientation less efficient than in other treatments. Crayfish tended to take more time to locate the source in the ELEV-high treatment than in the AMB-high treatment, but this difference was not statistically significant ($p = 0.07$). Nevertheless, these results suggest that some component of the chemical mixture in the ELEV DOM might have impaired their ability to make decisions about the spatial location of the odor source.

Possible explanations for differences in orientation variables

The chemicals present in food items can either initiate or deter crayfish feeding behavior and, therefore, influence orientation. Crayfish are deterred from feeding by certain plant defense compounds found in macrophytes, including lignoid compounds, alkaloids, and tannins or tannic acid (Lodge 1991, Bolser and Hay 1998, Bolser et al. 1998, Cronin et al. 2002). The concentration of tannins and other secondary defensive compounds is higher in detritus from *P. tremuloides* grown in elevated CO₂ than from *P. tremuloides* grown in ambient CO₂ (Rier et al. 2002, Adams et al. 2003, Tuchman et al. 2003). Crayfish are attracted to detritus produced at ambient CO₂ but not at elevated CO₂ (Adams et al. 2003, 2005). These 2 studies suggested that differences in preference might

have been caused by high concentrations of plant defensive compounds in the elevated CO₂ detritus, which contains more lignin and tannins than does ambient CO₂ detritus (Tuchman et al. 2003). Both compounds are repellents to crayfish feeding on plant material and might mix with chemicals from the fish source to influence crayfish orientation efficiency.

Temporal measures of orientation did not differ among treatments, despite difference in spatial orientation variables among treatments. Time spent searching for the source and walking speed did not differ among treatments. Thus, crayfish were more likely to find the source in the AMB-high treatment, but otherwise showed no real differences in orienting accurately to the source. These animals probably have the sensory capabilities and decision mechanisms to cope with a chemically complex environment, and actually might forage more efficiently with increased complexity of chemical signals (Tomba et al. 2001, Wolf et al. 2004). A similar phenomenon might occur when crayfish forage in an environment that has a ubiquitous background of DOM from leaf litter. Orientation efficiency was not necessarily increased by DOM in our study, but crayfish might simply cope with DOM odor in the background and orient normally because of adaptation to a chemically complex habitat.

Ecological implications

The influence of crayfish on the stream food web is a direct result of their behavior and choice of food resources. Crayfish are omnivores that interact with multiple trophic levels in aquatic systems and can serve as important detritivores in leaf litter-based food webs (Lodge et al. 1994, Momot 1995, Nyström et al. 1996). They might function as keystone species in the systems they inhabit (Brönmark et al. 1992, Hill and Lodge 1995, Charlebois and Lamberti 1996). Therefore, factors that influence crayfish survival or behavior could have consequences for the entire aquatic community. If foraging efficiency (i.e., orientation efficiency) is decreased by the presence of background chemicals, such as those from elevated CO₂ detritus, in the stream, then these chemicals also might affect movement patterns and survival of crayfish, thereby increasing their conspicuousness to predators or altering food choices.

Spatial, but not temporal, variables of orientation in crayfish were altered by DOM odor type and concentration. Crayfish did not spend statistically more time searching for the food source in the ELEV-high than in other treatments. Presumably, in natural conditions, crayfish exposed to high concentrations of

elevated CO₂ DOM would not be at increased predation risk because of higher conspicuousness. Moreover, crayfish orientation variables differed only at concentrations of DOM at the high end of the natural concentrations that would occur only in autumn, so the potential effects might be seasonal. DOM from ambient CO₂ detritus (with a lower concentration of secondary defensive compounds) increased crayfish success in finding the source, whereas DOM from elevated CO₂ detritus did not. Thus, in an elevated-CO₂ future, behaviors associated with foraging and selection of food types by crayfish might be altered, with potential consequences for the aquatic community.

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