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Effects of CO_2-altered detritus on growth and chemically mediated decisions in crayfish (*Procambarus clarkii*)

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Abstract. We determined whether chemosensory selection of detritus by crayfish is affected by past experience and is related to the nutritional quality of the food and subsequent growth on that food. These questions were addressed with a long-term growth assay and with short-term behavioral assays of chemotaxis and chemical preference. A 10-mo growth experiment was conducted in which crayfish (*Procambarus clarkii*) were reared under different growth conditions, each consisting of a different diet. Crayfish were fed 1 of 3 types of detritus: leaf litter from *Populus tremuloides* grown under ambient (AMB)- or elevated (ELEV)-CO_2 conditions (which altered the nutritional quality and defensive chemistry of leaf litter) or fish (FISH). Carapace length (CL), mass, molt frequency, and % mortality were recorded. Crayfish reared on the AMB diet had decreased relative growth rate (RGR) and molt frequency and increased % mortality compared to those reared on the ELEV diet, whereas crayfish reared on the FISH diet had higher RGR and molt frequency than those reared on either type of litter diet. C, N, and macromolecular composition of crayfish tissues were determined after all growth assays were complete. Percent C, % N, % protein, and % lipids were lower, and % carbohydrates was higher for crayfish reared on the ELEV diet than for crayfish reared on the AMB or FISH diets, corroborating the results of the growth experiment. Before tissue analysis, crayfish from each growth condition were tested in a Y-maze to determine whether the diet experienced during the growth experiment affected foraging decisions. Crayfish from each growth condition were offered AMB or ELEV detritus prepared as: 1) fresh leaf litter, 2) leaf litter leached in water for 24 h, and 3) leachate from the litter in the form of gelatin cubes. Within each type of detritus preparation, the preferences of crayfish were tested for pairwise combinations of AMB detritus, ELEV detritus, and a no-stimulus control (CON). Preferences were linked to growth variables. Crayfish reared on AMB and ELEV detritus preferred AMB detritus, but those reared on FISH showed no preference for either AMB or ELEV detritus relative to CON. Detrital diets during rearing did not affect food preferences, suggesting that nutritional quality may influence detritus selection and that selective feeding may carry a growth benefit.

Key words: foraging decisions, growth, elevated CO_2, detritus, chemoreception, crayfish, *Procambarus clarkii.*

Animals make foraging decisions that should optimize nutrient intake, growth, and fitness (Stephens and Krebs 1986). Nutritional value of the food, handling time, and prey density affect prey choice (Charnov 1976, Stephens and Krebs 1986). In addition, purely behavioral factors such as previous diet experience and learning associated with particular prey types can affect dietary choices (Dethier 1988). Theories related

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to optimal foraging assume that information about the prey item, its abundance, or its nutritional value is *known* to the forager or that the forager can acquire such information through its sensory systems.

Foragers in terrestrial (Blaney 1981, Bell and Cardé 1984) and aquatic habitats (Hay et al. 1998) rely heavily on chemoreception when making foraging decisions. The tobacco hornworm *Manduca sexta* (Linnaeus) discriminates between odors of potential food and host plants (see Schoonhoven 1968 for review). Lizards use tongue-flicking behavior to distinguish between chemical mixtures in the environment and to select food sources (Garrett and Card 1993, Cooper 2000). Olfactory discrimination also occurs in some aquatic species, including crustaceans (Carter and Steele 1982) and mollusks (Chase 1982). The ability to discriminate between food items probably confers some advantage to the organism (Stephens and Krebs 1986).

Crayfish are decapod crustaceans that make extensive use of chemoreception to identify and locate food resources (Tierney and Atema 1988, Moore and Grills 1999, Keller et al. 2001), and they make olfactory decisions while foraging for leaf detritus (Adams et al. 2003). Crayfish discriminated between detritus produced under ambient-CO_2 and elevated-CO_2 conditions, and they preferred leaf detritus from trees grown at ambient CO_2 (Adams et al. 2003). This preference was based on chemicals that leached into the water from the detritus. However, Adams et al. (2003) did not answer whether the ambient-CO_2 detritus was preferred because it differed from elevated-CO_2 detritus nutritionally or simply because crayfish were familiar with ambient-CO_2 detritus (i.e., it was the normal condition in their environment).

Elevation of atmospheric CO_2 increases rates of C fixation in C_3 plants, and leads to higher plant growth rates and higher C:N ratios in leaf tissues (see Peñuelas and Estiarte 1998 for review). Plants grown under elevated-CO_2 conditions tend to produce relatively higher amounts of structural and nonstructural carbohydrates and C-based plant defense compounds than plants grown under ambient-CO_2 conditions (see Coviella and Trumble 1999 for review). Thus, elevated-CO_2 leaf material has lower nutritional quality than ambient-CO_2 leaf material for herbivores (Körner and Arnone 1992, Lavola and Julkunen-Tiitto 1994). In aquatic ecosystems where terrestrial plant detritus, mainly deciduous leaf litter, forms the C and energy foundation of the food web, alteration of leaf nutritional quality by atmospheric CO_2 concentration could negatively affect aquatic decomposers and invertebrate detritivores.

Growth and survival of detritivores in stream ecosystems are compromised by diets of elevated-CO_2 leaf litter. Cranefly larvae (Tuchman et al. 2002) and 4 species of mosquito larvae (Tuchman et al. 2003) fed elevated-CO_2 leaf detritus consumed less leaf material, had lower assimilation rates, and grew slower than larvae reared on ambient-CO_2 leaf detritus. The same trends have been found in studies with isopods (*Asellus* sp.), amphipods (*Gammarus* sp.), and blackfly larvae (*Simulium* sp.) (NCT, unpublished data), supporting the hypothesis that detritus produced by plants grown at elevated CO_2 is less nutritious than detritus grown at ambient CO_2 and negatively affects the growth of aquatic detritivores. However, behavioral measurements, in combination with growth data, would help to predict more accurately the effects of elevated-CO_2 on the production of stream invertebrates.

The goal of our study was to determine whether crayfish foraging decisions made in response to types of detritus were related to crayfish growth on different types of detritus. The crustacean chemoreceptive system has the ability to discriminate many chemicals and chemical mixtures in the environment (Ache et al. 1976, Carr and Derby 1986). A relationship between foraging decisions that use chemosensory information and the growth or survival of crayfish fed diets of different types of detritus would support an inference of a physiological benefit to maintaining a specific chemosensory system that can identify food and assess its quality. In addition, understanding the way in which experience with particular food types influences chemosensory foraging decisions of crayfish may help investigators make better predictions of the potential effects of elevated CO_2 on aquatic ecosystems through crayfish–leaf litter interactions.

Methods

Growing aspen on elevated atmospheric CO_2

Quaking aspen (*Populus tremuloides* Micheaux) trees were grown under both ambient CO_2 (360 ppm, [AMB]) and double-ambient CO_2 (720 ppm, [ELEV]) at the Elevated CO_2 Research Facility at the University of Michigan Biological Station (UMBS) in Pellston, Michigan. For detailed methods of growing aspen trees under elevated-CO_2 conditions, see Tuchman et al. (2002). Leaves were collected after natural senescence and abscission from the trees in late November 1999 and dried at room temperature.

Previous work details alterations in foliar chemistry of aspen detritus collected from these AMB- and ELEV-CO_2 growth chambers (see Adams et al. 2003). In brief, ELEV detritus has a higher % C per area of leaf tissue than AMB detritus because ELEV detritus has higher concentrations of C-containing structural carbohydrates (lignin) and secondary defensive chemicals (phenolics) than AMB detritus. This increase in % C, coupled with a decrease in % N caused by down-regulation of ribulose bisphosphate carboxylase/oxygenase (Rubisco), leads to a higher C:N ratio in ELEV detritus than in AMB detritus. C:N ratios are inversely related to nutritional quality. Animal flesh has a lower C:N ratio than plant tissue because it contains fewer C-containing structural carbohydrates and more protein than plant tissue and, therefore, is more nutritious than either AMB or ELEV detritus.

Experimental animals

Adult crayfish (*Procambarus clarkii* Girard) were obtained from a commercial supplier (Atchafalaya Biological Supply Company, Racelan, Louisiana). Crayfish were allowed to mate in the laboratory, and gravid females were isolated in 32.5-L aquaria. After hatching, juveniles were placed in individual 1.5-L pots (17.4 cm inner diameter [ID]) filled with 0.75 L dechlorinated water. Pots were placed randomly by growth condition (see below) within an environmental chamber (23°C, 14 h:10 h light:dark cycle). Water was changed every other day.

Crayfish were reared for 10 mo (September 2001–July 2002) in 1 of 3 growth conditions defined by the diet on which the crayfish were reared: AMB detritus, ELEV detritus, or fish (FISH). Ten crayfish from each of 5 mothers were pseudorandomly assigned to a growth condition ($n = 50$/growth condition). The pseudorandom design minimized the influence of maternal effects on experimental results. Placement of an individual from a particular mother into a given growth condition was random among siblings, but each mother was equally represented in all treatments. Initial carapace length (CL) and mass were measured and did not differ between growth conditions (1-way analysis of variance [ANOVA], CL: $F = 0.051$, $p = 0.901$; mass: $F = 0.004$, $p = 0.952$).

Crayfish growth experiment and tissue analysis

Feeding protocol.—Detritus was prepared by cutting disks (diameter = 5 mm) from aspen leaves collected after leaf abscission. The masses of AMB and ELEV detritus disks were 3.2 ± 0.4 mg and 3.3 ± 0.5 mg, respectively (dry mass; $n = 100$ for each type). Disks were soaked in water for 20 min prior to feeding to prevent them from floating. Crayfish raised in the AMB or ELEV growth condition were fed the appropriate detritus ad libitum (~9 mg/d for months 0–3, 18 mg/d for months 3–6, 35 mg/d for months 6–10). Pots were checked daily throughout the growth period to ensure that food was always available, and additional disks were added as needed (~1/d). Crayfish raised in the FISH growth condition were fed small portions of cod (50 ± 10 mg) each day. Uneaten cod was removed before feeding the next day (amount consumed: 9.8 ± 0.4 mg/d for months 0–3, 10.3 ± 0.6 mg/d for months 3–6, 39.3 ± 3.2 mg/d for months 6–10; $n = 50$ measurements for each age group made on random occasions in each time period). Crayfish also were fed one commercial rabbit food pellet (122.4 ± 3.5 mg, $n = 100$) biweekly to supplement their diet and reduce the likelihood of nutritional deficit with only detritus as a food source.

Crayfish CL and mass were measured weekly during the first 4 mo and monthly during the last 6 mo of the growth experiment. Digital pictures were taken of each crayfish next to a reference length (a ruler), and the pictures were analyzed with an imaging software package (Corel Draw 7.0, Corel, Ottawa, Ontario). Mass (mg) was measured with an analytical balance after crayfish had been towel-dried. The number of deaths and molts were recorded daily. Only crayfish that survived the entire growth experiment were used for behavioral studies and tissue analyses (AMB, $n = 38$; ELEV, $n = 30$; FISH, $n = 36$).

Tissue analyses.—Crayfish that survived the

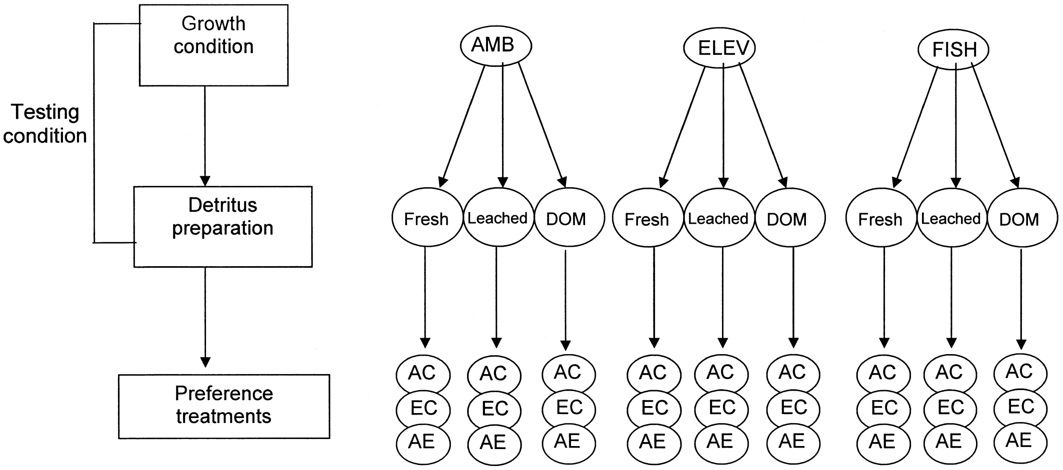

FIG. 1. Flow chart describing the 3 × 3 factorial design of preference tests. Crayfish were raised in 3 growth conditions, based on the type of food available: ambient-CO_2 (AMB) leaf litter detritus, double-ambient-CO_2 (ELEV) detritus, or fish (FISH). Leaf litter was offered as 3 detritus preparations: fresh, leached, or dissolved organic matter (DOM) from litter leachate made into gelatin cubes. Within each growth condition–detritus preparation combination (testing condition), pairwise stimuli were offered in 3 preference treatments: AMB vs no-stimulus control (CON) (AC), ELEV vs CON (EC), and AMB vs ELEV (AE). The order in which crayfish were exposed to preference treatments was random.

growth experiment were tested for feeding preferences (see below) and then sacrificed and frozen in liquid N. These samples were freeze-dried in a lyophilizer and ground to a fine powder with a tissue-grinding mill.

Percent C and % N (and C:N ratio) were measured using a Carlo–Erba Elemental Analyzer. Percent protein was calculated as % N × 6.25 (estimated N actually in proteins, Gnaiger and Bitterlich 1984). Percent lipids were measured using a 2:1 chloroform:methanol extraction. The extracted liquid was evaporated and lipids remaining were measured. Percent mineral ash (inorganic material) was determined from separate tissue samples of known mass. Samples were combusted at 550°C in a muffle furnace and weighed. Percent carbohydrates were calculated as the remainder of tissue dry mass after lipids, proteins, and ash had been removed. Thus, % values for individual tissue components were not independent of values of other components in the same tissue, and a bias or error in one measurement may have systematically presented itself throughout the other variables. This codependence is a weakness of this part of the study, but the results can be used to supplement the other results of the growth experiment.

Data and statistical analysis.—Relative growth rate (RGR) was calculated for each individual by subtracting initial mass (or CL) from final mass (or CL) and dividing by initial mass (or CL) ([Final − Initial]/Initial). Differences in growth variables (RGR[by CL], RGR[by mass], molt frequency) among growth conditions were tested using a multiple analysis of variance (MANOVA) with least significant difference (LSD) post-hoc tests to identify means that differed. Differences in % mortality were analyzed using a multiple comparisons contingency table for proportions (Zar 1999). C:N ratio, % C, % N, % lipids, % proteins, % carbohydrates, and % mineral ash were arcsine transformed, and differences in these variables among growth conditions were analyzed using MANOVA with LSD post-hoc tests.

Tests of detritus preference

Crayfish preferences for different types of detritus were tested using a Y-maze. This experiment had a 3 × 3 factorial design with growth condition, detritus preparation, and preference treatment as the 3 factors (Fig. 1). Crayfish from each growth condition (AMB, ELEV, FISH) were presented with 3 detritus preparations designed to simulate forms of leaf litter that crayfish might experience in a natural system: 1) fresh detritus (fresh), 2) detritus leached in water for 24 h (leached), or 3) dissolved organic matter (DOM) prepared from leachate collected for 24 h from detritus and made into slow-diffusing blocks of gelatin. Thus, 9 testing conditions (testing condition = growth condition–detritus preparation combination) were used. Within each of the 9 testing conditions, pairwise combinations of detritus stimuli (AMB, ELEV, no-chemical stimulus [CON]) were offered to crayfish. Preference treatment combinations were AMB vs ELEV (AE), AMB vs CON (AC), and ELEV vs CON (EC). Mortality differed among growth conditions, so only 30 of the 50 animals initially placed in each growth condition were used for preference testing. Thus, representation among broods may have been uneven.

Ten crayfish were assigned to each testing condition. Individual crayfish within each testing condition experienced all 3 preference treatments (n = 10 for each preference treatment). The order in which the preference treatments were done was random for each crayfish, and ≥1 wk passed between successive trials to reduce the effect of experience with the bioassay on food choice. In the week between trials, crayfish were fed 2 rabbit food pellets several days apart to decrease the risk of starvation. All crayfish received the same type of pellets, so any effect of the pellet on trials should have been uniform across testing conditions and preference treatments. Randomization of the order of trials ensured that any effects caused by crayfish learning the bioassay also would be random throughout the 3 preference treatments within each testing condition. Error caused by learning the bioassay would have increased statistical noise in the data and decreased the likelihood of finding a significant difference among treatments. Therefore, a significant result returned from a statistical test in this experiment indicated enough difference between measures to overcome the possible statistical noise imposed by learning or feeding regimen experienced between trials.

Detritus preparations for preference testing

Fresh detritus.—Fresh leaf detritus was prepared from intact freshly abscised leaves. Mesh bags (1-mm^2 aperture, 10.0 × 8.5 cm) were filled with 0.07 ± 0.01 g of either AMB or ELEV detritus. Each trial was run with new bags of fresh detritus. The CON stimulus was an empty mesh bag.

Leached detritus.—Leached leaf detritus was produced as needed, and all experiments were conducted within 1 wk of leaching. A total of 3 batches of leached detritus were prepared during the experiment. For each batch, ~20 g of fresh leaf detritus (AMB or ELEV) were soaked in 1 L of unfiltered Douglas Lake (Pellston, Michigan, lat 45°33′N, long 84°40′W) water. Detritus was agitated with a magnetic stir bar as it soaked for 24 h. Unfiltered lake water was chosen to mimic the chemically complex background of a natural habitat in which crayfish must make olfactory choices. Detritus was removed by hand after 24 h and stored at 4°C in a Ziploc® bag until experimentation. Leachate was set aside in plastic Nalgene® bottles and stored at 4°C until later use (see below). For each preference treatment, ~0.24 ± 0.004 g towel-dried leached ELEV or AMB detritus (corresponding to 0.07 g dry mass) were placed in a mesh bags as described above. The CON stimulus was an empty mesh bag.

DOM.—Leachate (from preparation of AMB or ELEV leached detritus, see above) was used to prepare gelatin squares, which provided a solid matrix from which DOM could diffuse. During experiments, gelatin blocks were placed in a mesh bag. All experiments were completed within 2 wk of making gelatin. For detailed preparation of DOM gelatin, see Adams et al. (2003). Two batches of DOM gelatin were prepared during the experiment. Squares were wrapped individually in cellophane and refrigerated at 4°C until experimentation. A gelatin control was not performed because previous work showed that crayfish do not perceive gelatin as a stimulus (Moore and Grills 1999).

Y-maze design and protocol

A flow-through Y-maze constructed of black plexiglas was used to test crayfish responses to different chemical stimuli (tank = 77.5 × 42 × 18 cm, arm = 56 × 21 × 18 cm, 56.4 L; Fig. 2). The bottom of the Y-maze was lined with black gravel that was cleaned between trials. Water was pumped from Douglas Lake into 2 reservoir tanks (24 × 13 × 14 cm, 4.2 L) above the maze. Water flowed from reservoir tanks into

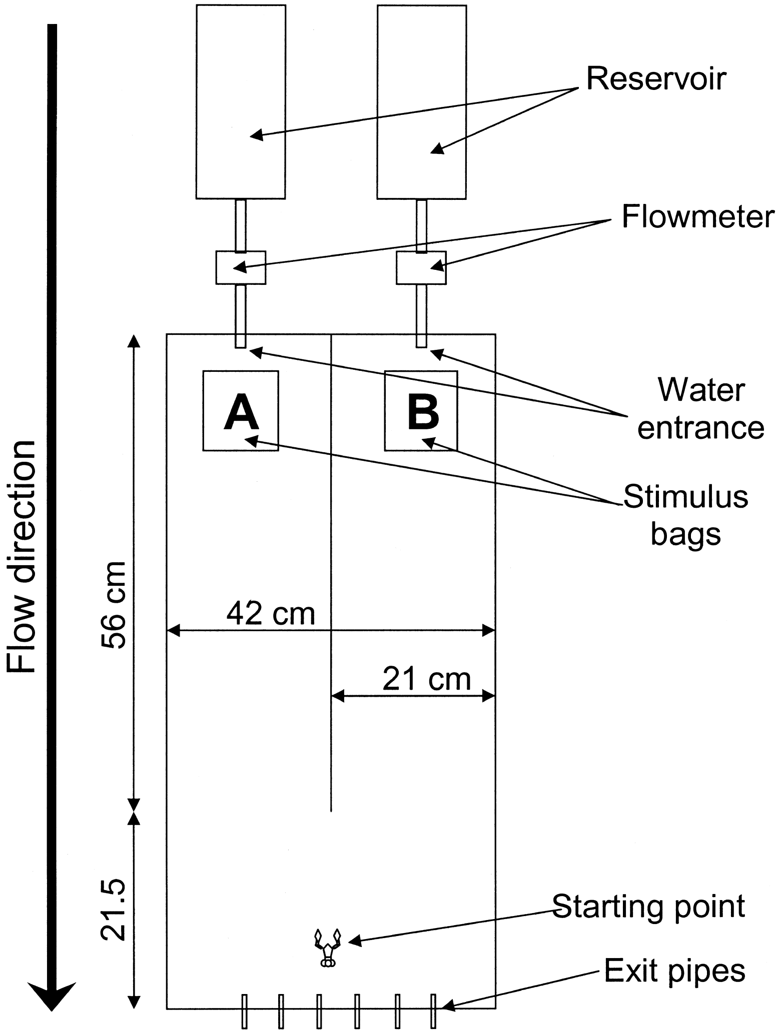

FIG. 2. Y-maze design. Flow is from top to bottom in the diagram. Water flowed from the reservoirs, through flowmeters, and into either arm A or B. Water exited through 6 holes at the base of the Y-maze. Crayfish began the experiment in the downstream portion of the Y-maze. Crayfish is drawn to scale.

each arm of the maze through 1.0-cm (ID) Nalgene® tubing. Two inline flowmeters (Manostat Riteflow #3, Manostat, Peaquannock, New Jersey) controlled flow rate (20 ± 0.5 mL/min). The water flowed over mesh bags or gelatin squares containing stimuli that had been placed at the upstream end of the Y-maze. Dye trials using commercial food coloring were run to ensure that flow from each holding tank was separate and equal when traveling through arms of the Y-maze. Water exited the tank through 6 outflow pipes (5 cm above substrate) controlled by clamps. The temperature of both tank and stimulus water were the same to ensure that the vertical position of the odor plume was conserved along the entire arm of the tank.

A sample size of $n = 10$ crayfish was used for each preference treatment within each testing condition. For preference tests, a small spot of white correction fluid was painted on each crayfish carapace for later visualization during video analysis. The crayfish was allowed to explore the test arena with no stimulus present for 20 min prior to each trial. Crayfish were stimulated to move to the back of the arena by physically disrupting the water in front of them without physical contact with the animals, and mesh bags containing stimuli were randomly assigned to an arm by flipping a coin. The bags were placed at the upstream end of each arm immediately downstream of the water nozzle. Crayfish began each trial at the starting point and were allowed to explore the Y-maze for 10 min while being video-taped from above (Sony Hi-8 Handycam, Model # CCD-TR700). Trials in which the experimental animal did not move, escaped from the maze, or appeared visibly disturbed by researchers were removed from analyses (20 of a total 290 trials, <7%).

Data and statistical analysis

Video tapes were analyzed using Peak Motus 8 Motion Analysis® (Peak Performance, Centennial, Colorado) software to digitize the spatial locations of the crayfish within the maze over time. The (x,y) coordinates of the spot on the crayfish carapace were digitized once every second for the total length of the trial. All behavioral measures were analyzed and calculated using computers, removing observer bias.

Behavioral variables obtained from computer analysis included initial arm choice, time spent in each arm, and time spent at each source. Initial arm choice was defined as the first arm the crayfish entered. A normal approximation of the χ^2 for proportions (Z_C) ($n = 10$ for all treatments) was used to determine whether crayfish demonstrated differences in initial arm choices in each preference treatment (Zar 1999). The total amount of time spent in each arm was the sum of all individual times when a crayfish was within a particular arm (including time spent at the source). Time at the source was defined as the total amount of time a crayfish spent touching the source with at least one chela. All times were converted to proportions of the 10 min total and were arcsine transformed. Two-tailed paired *t*-tests were used to test for significant differences in the proportions of time spent within each arm and at each source.

Results

Growth experiment and tissue analysis

RGR(by CL), RGR(by mass), and molt frequency differed significantly among growth conditions (MANOVA: $R_{6,194,0.05} = 14.86$, $p < 0.001$). RGR(by CL) (Fig. 3A) and RGR(by mass) (Fig. 3B) were highest in the FISH condition, and they were lower in the ELEV condition than in the AMB ($p < 0.040$) or FISH ($p < 0.001$) conditions. Molt frequency (Fig. 3C) was lower in the ELEV condition than the AMB ($p < 0.001$) and FISH ($p < 0.001$) conditions, indicating delayed development. Percent mortality (Fig. 3D) was significantly higher in the ELEV condition than in AMB ($Q = 6.97$, $p < 0.001$) and FISH ($Q = 6.34$, $p < 0.001$) conditions (AMB = 26%, ELEV = 46%, and FISH = 28% mortality). Percent mortality did not differ between the AMB and FISH conditions ($Q = 0.62$, $p = 0.92$).

Chemical variables of tissues differed significantly among growth conditions ($R_{14,170,0.05} = 10.10$, $p < 0.001$). Percent C (Fig. 4A) and % N (Fig. 4B) differed among growth conditions, but C:N ratio (Fig. 4C) did not. Crayfish grown in the FISH condition had higher % C ($p < 0.001$) and % N ($p < 0.009$) than crayfish grown in the AMB condition, and crayfish grown in the AMB condition had higher % C ($p < 0.006$) and % N ($p < 0.011$) than crayfish grown in the ELEV condition Percent composition of tissue macromolecules differed significantly among growth conditions (Fig. 5). Crayfish grown in the FISH condition had the highest % protein (FISH vs AMB, $p < 0.009$; FISH vs ELEV, $p < 0.001$) and % lipids (FISH vs AMB, $p < 0.028$; FISH vs ELEV, $p < 0.001$). Crayfish grown in the AMB condition had higher % protein ($p < 0.010$) and % lipids ($p < 0.001$) than crayfish grown in ELEV conditions. Percent carbohydrates did not differ between the ELEV and FISH growth conditions ($p = 0.328$), but were lower in the AMB condition than in either FISH or ELEV conditions (AMB vs ELEV, $p < 0.004$; AMB vs FISH, $p < 0.035$).

Detritus preference testing

Consistency among batches of leached detritus and DOM.—Trials within each testing condition were combined, and a 1-way MANOVA with leached detritus batch number as the independent variable and proportions of time spent in one of the arms and at one of the sources (transformed data) as the dependent variables was conducted for each preference treatment (AC, EC, and AE). Batch number did not affect crayfish preference (AC, $R_{4,52} = 1.56$, $p = 0.198$; EC, $R_{4,52} = 1.211$, $p = 0.317$; AE, $R_{4,52} = 0.735$, $p =$

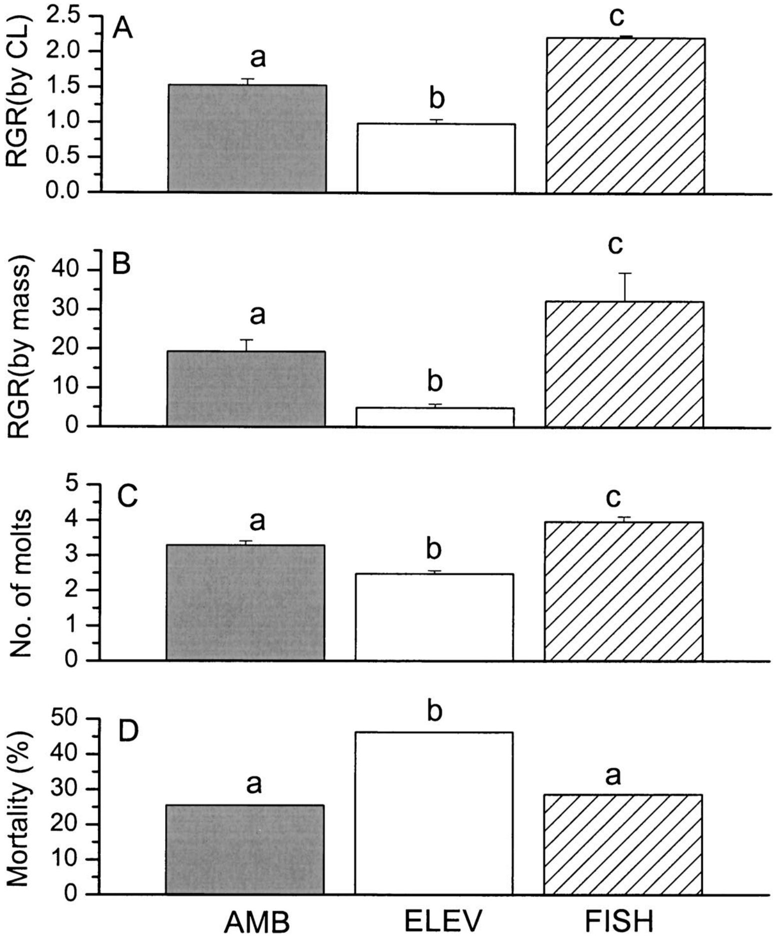

FIG. 3. Mean (+1 SE) relative growth rate (RGR) calculated using carapace length (CL) (A), RGR calculated using mass (B), molt frequency (C), and mortality (D) after 10 mo of growth for crayfish reared in each growth condition. Growth condition abbreviations are as in Fig. 1. Bars with the same letter are not significantly different.

0.573). Trials within each testing condition were combined, and a 1-way MANOVA with DOM gelatin batch number as the independent variable and proportion of time spent in one of the arms and at one of the sources (transformed data) as dependent variables was conducted for each preference treatment (AC, EC, and AE). Batch number did not affect crayfish preference (AC, $R_{2,27} = 1.05$, $p = 0.362$; EC, $R_{2,27} = 0.321$, $p = 0.728$; AE, $R_{2,27} = 0.984$, $p = 0.387$).

AMB growth condition.—When offered fresh detritus or DOM, crayfish reared in the AMB growth condition preferred AMB detritus to both ELEV detritus and CON. This preference was shown by initial arm choice (Table 1) and time spent in each arm (Fig. 6A) and at each source (Fig. 6B) in the AC or AE treatments. Crayfish did not demonstrate a preference in the EC treatment. Crayfish offered leached detritus showed no differences in initial arm choice (Table 1) or in time in each arm or at each source for any treatments (Fig. 6A, B).

ELEV growth condition.—When offered fresh detritus or DOM, crayfish reared in ELEV growth conditions preferred AMB detritus to both ELEV detritus and CON. This preference was shown by initial arm choice (Table 1) and time spent in each arm (Fig. 7A) and at each

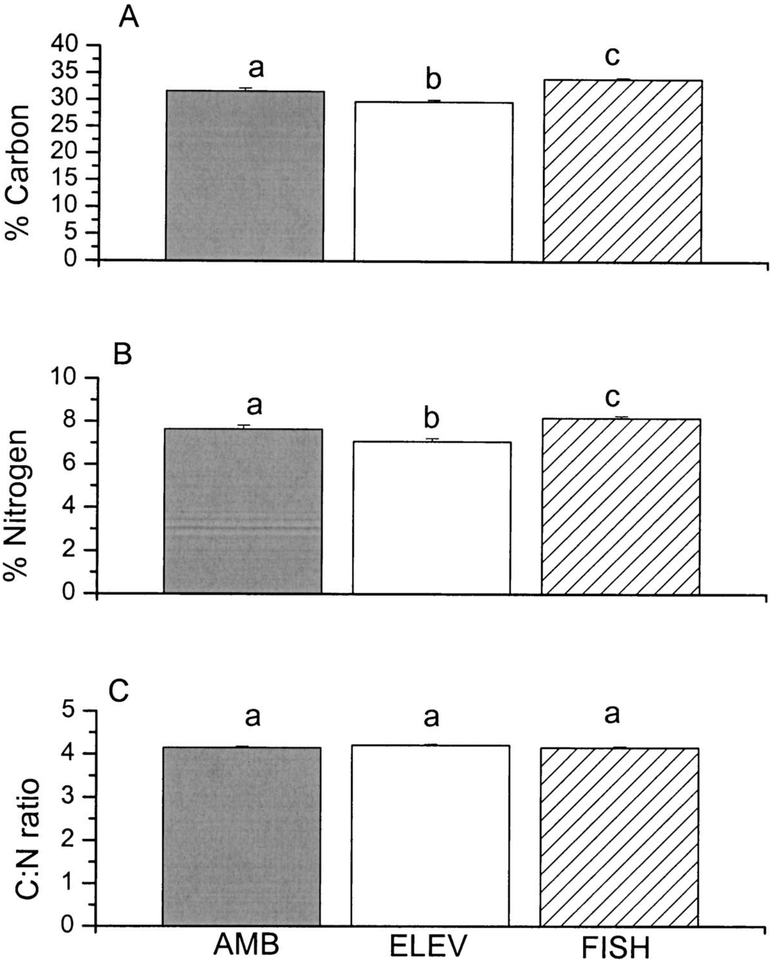

FIG. 4. Mean +1 SE % C (A), % N (B), and C:N ratio (C) of crayfish tissues for each food treatment after 10 mo of growth. Only crayfish that survived the entire duration of the growth experiment were used for tissue analysis. Growth condition abbreviations are as in Fig. 1. $n = 38$ for AMB, $n = 30$ for ELEV, and $n = 36$ for FISH. Bars with the same letter are not significantly different.

source (Fig. 7B) in the AC or AE treatments. Crayfish did not demonstrate a preference in the EC treatment. Crayfish offered leached detritus showed no differences in initial arm choice (Table 1) or in time in each arm or at each source for any treatments (Fig. 7A, B).

FISH growth condition.—Crayfish reared in the FISH growth condition preferred AMB detritus to CON when offered fresh detritus (Fig. 8A, B). This preference was shown by time spent in each arm (Fig. 8A) and at each source (Fig. 8B) in the AC treatments. Crayfish showed no other preferences in any detritus preparation or preference treatment.

Discussion

Crayfish growth variables were linked to detritus preferences. Crayfish growth, survival, molt frequency, % protein, and % lipids were higher when crayfish were fed AMB detritus than when fed ELEV detritus. These differences were reflected in preferences shown by crayfish for AMB fresh detritus and DOM. Our study had 2 advantages over previous work that has attempted to link crayfish feeding preferences with the nutritional quality of detrital food sources. First, data from our present study were used to draw direct conclusions about the nu-

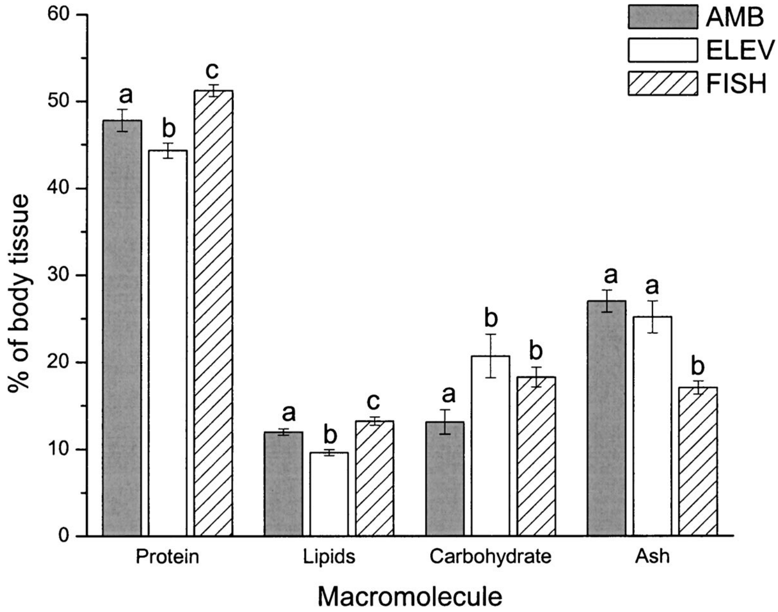

FIG. 5. Mean (±1 SE) % macromolecular composition of crayfish tissues. Only crayfish that survived the entire duration of the growth experiment were used for tissue analysis. Growth condition abbreviations are as in Fig. 1. $n = 38$ for AMB, $n = 30$ for ELEV, and $n = 36$ for FISH. Bars with the same letter are not significantly different.

tritional quality of types of detritus for crayfish, rather than speculating about nutritional quality from chemical measurements of the leaf litter (as in Adams et al. 2003). AMB detritus was higher in nutritional value and lower in secondary defensive compounds (see Table 1 in Adams et al. 2003 for measurements of detritus chemical composition), and AMB detritus yielded better crayfish growth responses than ELEV detritus. Second, unlike our previous work (Adams et al. 2003), our present study demonstrated that previous experience with detrital food types did not appear to alter the preference of crayfish for AMB detritus. We acknowledge that only pref-

TABLE 1. Percent initial arm choice in all preference treatments for each testing condition. Abbreviations for growth condition (AMB, ELEV, FISH), preference treatments (AE, AC, EC), and DOM are as in Fig. 1. Abbreviations for arm are: A = AMB stimulus present, E = ELEV stimulus present, C = no-stimulus control present. Bold font indicates $p < 0.05$. – indicates preference treatment not appropriate to arm.

		Detritus preparation								
		Fresh			Leached			DOM		
Growth condition	Arm	AC	EC	AE	AC	EC	AE	AC	EC	AE
AMB	A	**100**	–	**80**	70	–	70	**90**	–	**100**
	E	–	50	**20**	–	60	30	–	**90**	**0**
	C	**0**	50	–	30	40	–	**10**	**10**	–
ELEV	A	**100**	–	**80**	50	–	40	**100**	–	**80**
	E	–	50	**20**	–	40	60	–	70	**20**
	C	**0**	50	–	50	60	–	**0**	30	–
FISH	A	70	–	40	30	–	40	50	–	40
	E	–	50	60	–	60	60	–	50	60
	C	30	50	–	70	40	–	50	50	–

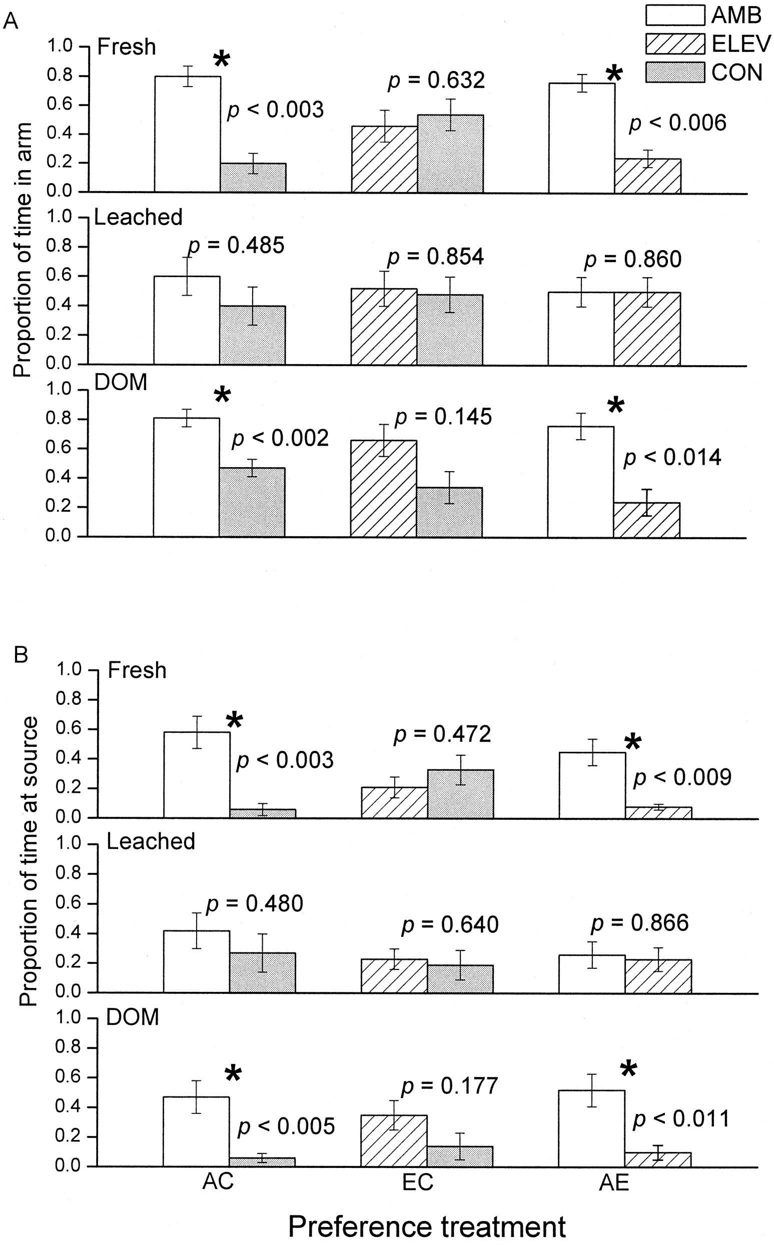

FIG. 6. Mean (±1 SE) proportion of time in each arm of the Y-maze (A), and at each food source (B) for all detritus preparations and preference treatments within the ambient-CO_2 (AMB) growth condition. See text for details of experimental design and treatment combinations. X-axis labels indicate the stimulus in each arm of the Y-maze during the preference tests. Preference treatment abbreviations are as in Fig. 1. $* = p < 0.05$.

erence patterns were evaluated in our study (i.e., relative amount of time in each arm of the Y-maze), not the quantitative relationship between the amount of time spent in an arm of the maze and growth condition of the crayfish. Nevertheless, crayfish with exposure to detritus preferred AMB detritus over ELEV detritus. Thus, the preference of crayfish for AMB detritus in

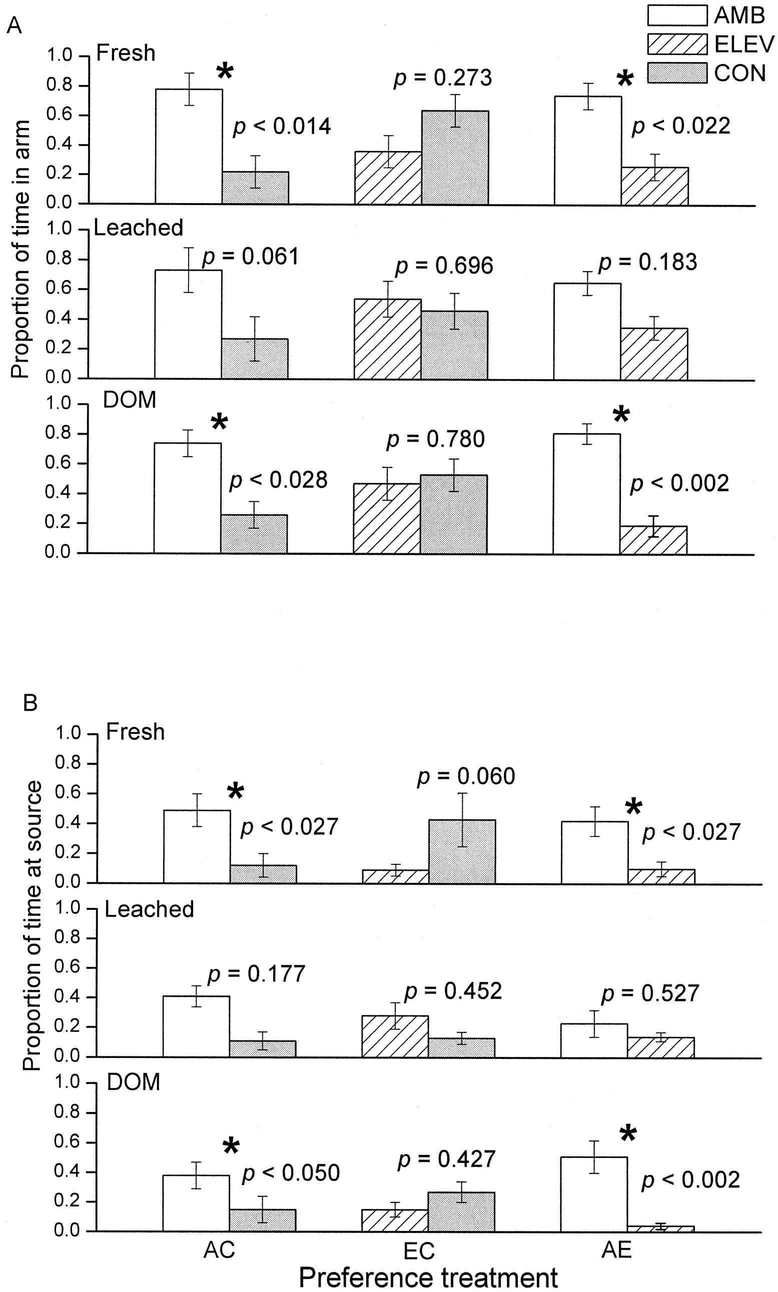

FIG. 7. Mean (±1 SE) proportion of time in each arm of the Y-maze (A), and at each food source (B) for all detritus preparations and preference treatments within the elevated-CO_2 (ELEV) growth condition. See text for details of experimental design and treatment combinations. X-axis labels indicate the stimulus in each arm of the Y-maze during the preference tests. Preference treatment abbreviations are as in Fig. 1. * = $p < 0.05$.

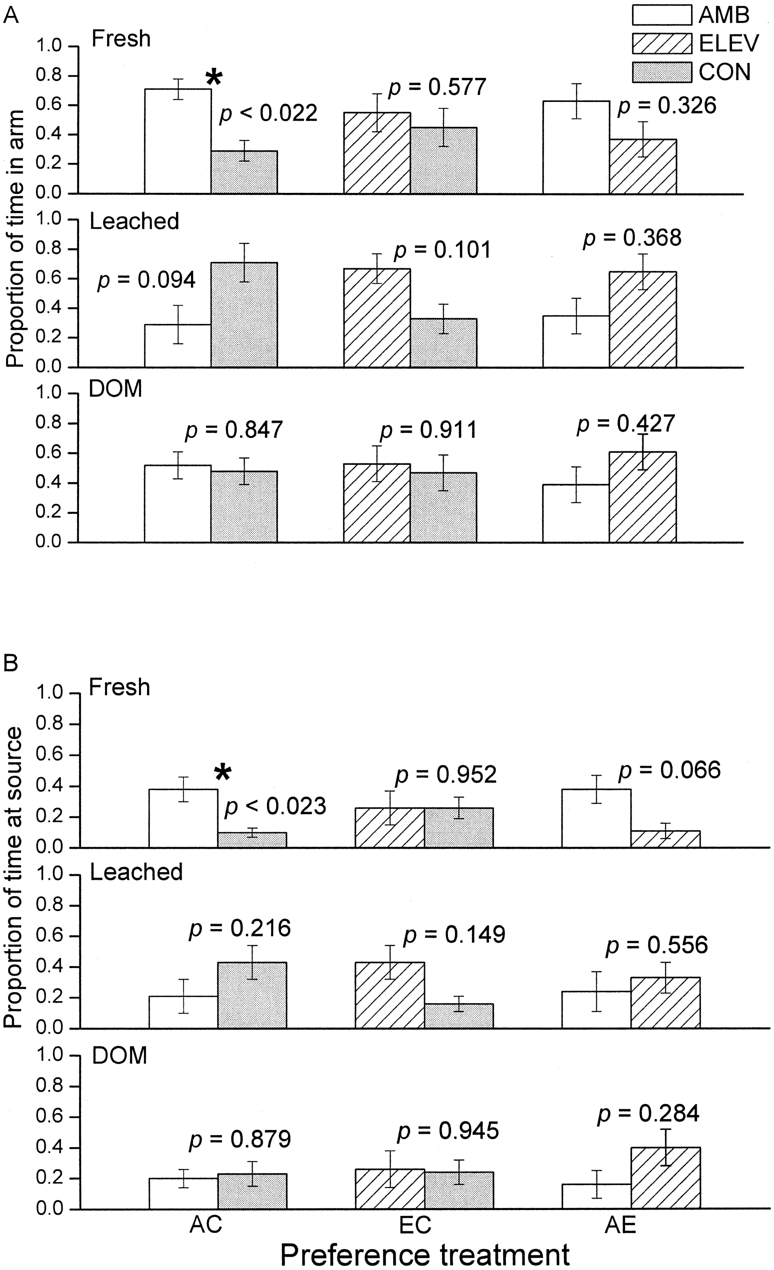

FIG. 8. Mean (±1 SE) proportion of time in each arm of the Y-maze (A), and at each food source (B) for all detritus preparations and preference treatments within the fish (FISH) growth condition. See text for details of experimental design and treatment combinations. X-axis labels indicate the stimulus in each arm of the Y-maze during the preference tests. Preference treatment abbreviations are as in Fig. 1. * = $p < 0.05$.

the behavioral experiments can be attributed to the nutritional value of detritus rather than to acclimation to a previously experienced food source.

AMB and ELEV detritus differ chemically. Aspen litter produced under ELEV conditions has more % C and less % N per unit leaf area than litter produced under AMB conditions, and these chemical differences result in a higher C:N ratio and decreased nutritional value for ELEV detritus relative to AMB detritus (Tuchman et al. 2002, Adams et al. 2003). In addition, the relative concentrations of secondary compounds and lignin are higher in ELEV detritus than in AMB detritus. These compounds may reduce palatability and digestibility of litter, thereby affecting preferences as has been demonstrated for terrestrial herbivores (Lincoln et al. 1993), aquatic detritivores (Tuchman et al. 2002), and crayfish feeding on macrophytes exhibiting natural variation in these compounds (Lodge 1991, Bolser et al. 1998).

The chemical differences between AMB and ELEV detritus were reflected in differences in crayfish physiology between the AMB and ELEV growth conditions. In the ELEV growth condition, RGR and molt frequency decreased and mortality increased. These responses have been observed for other aquatic arthropods fed aspen litter produced under AMB or ELEV conditions (Tuchman et al. 2002, 2003, NCT unpublished data). In our study, changes also were observed in the chemical composition of crayfish body tissues. Percent lipid and % protein were lower and % carbohydrate was higher in crayfish in the ELEV growth condition than in the AMB growth condition. Thus, the observed selection preference for AMB detritus may prevent reduced growth and malnutrition.

Crayfish can discriminate between detritus produced under AMB and ELEV conditions, and they can select the more nutritious type of detritus using chemical signals (Adams et al. 2003). This ability remains even after crayfish have been reared on only one type of detritus. Crayfish reared on either type of detritus selected the most nutritious type of detritus in the preference tests; crayfish were attracted to AMB detritus in the fresh detritus and DOM leachate preparations. Regardless of detritus preparation, crayfish did not respond to ELEV detritus as a food source even when ELEV detritus was paired with a no-stimulus control. However, when detritus was leached, no preferences were exhibited for either AMB or ELEV detritus, presumably because the chemicals that cause attraction for crayfish are missing from leached litter. In our study, fine-scale discrimination of subtle differences in leaf litter chemistries conferred a physiological benefit to crayfish. Crayfish discriminated between 2 chemically similar stimuli (leaf litter from the same species of tree with subtle CO_2-induced modifications in chemistry), and showed preferences consistent with the food that provided a higher growth rate and lower chance of mortality.

Crayfish reared in the FISH growth condition selected the AMB arm over the CON arm and spent more time at the AMB source than the CON source. Crayfish reared in the FISH growth condition spent more time at the AMB source than the ELEV source in the AE preference treatment, but this result was not statistically significant ($p = 0.066$). Crayfish reared in the FISH growth condition may have been able to select the more nutritious food source, but may have been less motivated to eat detritus because they had never been exposed to it. It is possible that crayfish reared in the FISH growth condition did not readily perceive AMB or ELEV sources because they could not discriminate between AMB and ELEV sources when paired with CON sources. However, this conclusion is speculative because the result lacked statistical significance.

Chemosensory foraging behavior of crayfish and other crustaceans

The chemicals present in food sources can either initiate or deter crayfish feeding behavior, thereby influencing preference. Crayfish are differentially sensitive to a variety of compounds that induce feeding behavior, including amino acids and carbohydrates, with cellobiose and sucrose the most stimulatory for the crayfish *Orconectes rusticus* Girard (Tierney and Atema 1988). Behavioral evidence suggests that some crustaceans can detect the C:N ratio of plant detritus (Giddins et al. 1986, Skov and Hartnoll 2002). Evidence also suggests that crayfish feeding can be deterred by certain macrophyte defense compounds, including lignoid compounds, alkaloids, and tannins (Lodge 1991, Bolser et al. 1998, Kubanek et al. 2001). The concentration of tannins and other secondary defensive compounds is higher in leaf litter from *P. tremuloides* grown in ELEV conditions than in AMB conditions (Rier et al. 2002, Tuchman et al. 2002, Adams et al. 2003) and these compounds may have influenced preference.

Ecological implications

Crayfish are proposed keystone species in the aquatic ecosystems they inhabit, and they influence many trophic levels (Brönmark et al. 1992, Lodge et al. 1994, Usio 2000). Thus, the differences in growth, tissue composition, and mortality of crayfish observed in this experiment could have major effects on other organisms directly through predation and competition or indirectly through shredding activities. Crayfish shredding activity is integral to the transformation of C and energy stored in detritus to forms that can be used by other organisms (Momot 1995, Usio 2000). If crayfish consume nondetritus food sources to compensate for detritus of poor nutritional quality, then predation or grazing pressure on those other food sources might increase. For example, crayfish can have sizable effects on macrophyte and periphyton communities (Olsen et al. 1991, Lodge et al. 1994). Macroinvertebrate biomass can be reduced by direct crayfish consumption (Hanson et al. 1990, Lodge et al. 1994, Momot 1995) and by causing changes in available microhabitats through macrophyte and periphyton destruction (Usio 2000). Crayfish are connected to many groups in the food web because they play the roles of prey, predator, herbivore, and shredder (Griffith et al. 1994, Momot 1995, Usio 2000). Therefore, decreases in population density, biomass, and nutritional quality of crayfish, or alterations in their foraging activities may have far-ranging effects for other organisms in an elevated-CO_2 future.

Adams et al. (2003) speculated that a decrease in nutritional quality of detritus in an elevated-CO_2 future may induce a dietary shift in crayfish to include less detritus in the diet. If previous experience with detritus alters foraging decisions in crayfish, then a diet shift would be averted (e.g., crayfish would eat whatever was normally in the environment) and the effect on ecosystem C cycling would be diminished. However, our present study supports the notion that a dietary shift might occur. Crayfish make feeding decisions based on information about the present nutritional quality of food sources, not based on past experiences with food sources.

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