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CHEMICALLY MEDIATED COMPETITION BETWEEN MICROBES AND ANIMALS: MICROBES AS CONSUMERS IN FOOD WEBS

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Abstract. Microbes are known to affect ecosystems and communities as decomposers, pathogens, and mutualists. However, they also may function as classic consumers and competitors with animals if they chemically deter larger consumers from using rich food-falls such as carrion, fruits, and seeds that can represent critical windfalls to both microbes and animals. Microbes often use chemicals (i.e., antibiotics) to compete against other microbes. Thus using chemicals against larger competitors might be expected and could redirect significant energy subsidies from upper trophic levels to the detrital pathway. When we baited traps in a coastal marine ecosystem with fresh vs. microbe-laden fish carrion, fresh carrion attracted 2.6 times as many animals per trap as microbe-laden carrion. This resulted from fresh carrion being found more frequently and from attracting more animals when found. Microbe-laden carrion was four times more likely to be uncolonized by large consumers than was fresh carrion. In the lab, the most common animal found in our traps (the stone crab *Menippe mercenaria*) ate fresh carrion 2.4 times more frequently than microbe-laden carrion. Bacteria-removal experiments and feeding bioassays using organic extracts of microbe-laden carrion showed that bacteria produced noxious chemicals that deterred animal consumers. Thus bacteria compete with large animal scavengers by rendering carcasses chemically repugnant. Because food-fall resources such as carrion are major food subsidies in many ecosystems, chemically mediated competition between microbes and animals could be an important, common, but underappreciated interaction within many communities.

Key words: bacteria; carrion; chemical ecology; competition; detritus; energy subsidy; food web; intraguild predation; microbes; scavengers; trophic.

Introduction

Microbes affect population, community, and ecosystem-level processes as pathogens (Dobson and Hudson 1986, Packer and Clay 2000), mutualists (Boucher et al. 1982, Smith and Read 1997), and decomposers (Moore et al. 2004). Beginning with the original conceptualization of energy flow and trophic dynamics in food webs (Lindeman 1942), microbial decomposers typically have been relegated to the role of nutrient recyclers and are rarely included in food web theory as consumers or as competitors with animals for resources (Polis and Winemiller 1996, Moore et al. 2004). However, if microbes rapidly colonize rich food resources and make them repugnant to animal scavengers (Janzen 1977), then microbes compete with animals for food and, therefore, could also be included in food webs as competitors for rich resources and not just as recyclers of nutrients and energy.

Food-falls such as animal carcasses, seeds, and plant detritus represent critical energy and nutrient subsidies for forested streams and rivers (Wallace et al. 1997, Helfield and Naiman 2002), coastal bays and estuaries (Duggins et al. 1989), oceanic desert islands (Polis and Hurd 1995), and some temperate forests (Yang 2004). For the Earth's largest ecosystem, the deep sea, such food-falls are the primary source of energy input (Etter and Mullineaux 2001). These food windfalls are rapidly colonized by both animals and microbes, potentially resulting in intense competition (Janzen 1977, Britton and Morton 1994, DeVault et al. 2003). Microbes often use antibiotic chemicals to prevent competing microbes from taking their resources (Vining 1990), but they could also use chemicals against potential animal competitors (Janzen 1977). Because animals that eat food-falls will consume not only microbial resources, but also the microbes growing on these resources,

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microbes should benefit from producing chemicals that repel animal consumers as well as microbial competitors. If such defenses occur, they could shift these resources from generalist consumers to more specialized scavengers adapted to compete with microbes, or they could redirect energy and nutrient flow within ecosystems, thereby shifting critical energy or nutrient subsidies from upper level consumers to the detrital pathway.

Despite the potential importance of chemically mediated microbe–animal competition (Janzen 1977, Hochberg and Lawton 1990) and correlative patterns suggesting its presence (Cipollini and Stiles 1993, Crist and Friese 1993), we know of no direct, experimental demonstration of this interaction. Therefore, using fish carrion as a model system for testing questions about microbe–animal competition, we investigated how marine microbes alter the attractiveness and palatability of carrion to common consumers in a coastal salt marsh ecosystem in the southeastern United States. We used field experiments and laboratory feeding assays to address how bacteria mediate: (1) the probability that large consumers will be attracted to, or find, a food resource; (2) the density of consumers that will be attracted to a found resource; and (3) the probability that different consumers will eat that resource once it is located.

Methods

Trapping and feeding experiments with fresh vs. microbe-laden carrion

To determine how microbial colonization affected the attractiveness of carrion to animals, we baited crab traps with either (1) freshly thawed menhaden, *Brevoortia tyrannus*, fish carcasses (fresh carrion), or (2) menhaden carcasses following 40–48 h of microbial colonization (microbe-laden carrion) and quantified capture rates. To create microbe-laden carrion, fresh menhaden carcasses were held for 40–48 h in an outdoor wading pool containing 10 cm of marsh sediment, while receiving flow-through seawater (6 L/min) from the Wilmington River estuary, and while covered with a shade cloth to moderate temperature. We used direct bacterial cell counts via DAPI staining (Porter and Feig 1980) to determine how bacterial populations increased during the aging process. Fresh carcasses were kept frozen until two hours before trap deployment.

Crab traps baited with fresh vs. microbe-laden fish carcasses were interspersed subtidally at distances of ~30 m in the tidal creeks of Wassaw Sound (31°58′ N/80°58′ W), Georgia, USA (see Plate 1). After 20–24 h, traps were collected, and all captured animals identified. Traps were rebaited and deployed in a different tidal creek each day for five days (n = 18–20 traps per treatment per day or $n = 98$ traps for each treatment over five days). We pooled data across sampling days as there were no significant differences among days for total animals caught. We used Fisher's exact test to determine differences in capture frequency between traps baited with fresh vs. microbe-laden carrion and t tests to evaluate differences in the abundance of animals captured. To address the generality of our results and the possible effects of shorter time periods of microbial colonization, we also performed less intensive sampling with menhaden carrion that had been aged for 20–24 h ($n = 38$ traps per treatment).

To assess the palatability of fresh and microbe-laden carrion, we conducted feeding assays using the stone crab *Menippe mercenaria*, the lesser blue crab *Callinectes similis*, and the striped hermit crab *Clibanarius vittatus*, because these animals are abundant consumers in estuarine environments (Ruppert and Fox 1988) and were frequently caught in our traps. The gelatinous consistency of microbe-laden carrion made directly feeding it to consumers difficult. To circumvent this problem, we made experimental foods by blending fresh or microbe-laden carrion with water (1:1 volume/volume) and 2% alginic acid by fish wet mass (Hay et al. 1998), rendering a paste that removed textural differences between foods, but should have retained the chemical and nutritional differences. Fresh or microbe-laden paste was extruded from a 10-mL syringe into 0.25 mol/L calcium chloride solution that reacts with the alginic acid to firm the paste into a "noodle" with the consistency of cooked pasta. We then offered 3-cm-long fresh and microbe-laden carrion noodles to stone crabs and lesser blue crabs, scoring whether these were eaten or rejected (Hay et al. 1998). Because stone crabs and lesser blue crabs generally either ate all or rejected all of one or both food types, we did not measure the amount of each food eaten by either consumer, but simply recorded the frequency of acceptance vs. rejection of the food pellet. Data were analyzed using Fisher's exact tests.

Because striped hermit crabs fed more slowly than the other crabs, we offered the hermit crabs portions of fresh vs. microbe-laden carrion that they could feed on over several hours instead of scoring assays as immediate acceptance or rejection. We coated 3-cm-long beaded cable ties with either fresh or microbe-laden carrion paste and soaked them in calcium chloride for 30 s. Cable ties coated with either fresh or microbe-laden carrion were offered simultaneously to striped hermit crabs housed in 250-mL containers. At 15-min intervals over 3 h, we scored each food as 0%, 25%, 50%, 75%, or 100% eaten (measured as the length of cable tie from which the food coating had been removed), and harvested replicates when individual crabs had consumed $\geq$50% of either food. We analyzed the proportion of hermit crabs feeding on either type of food with a Fisher's exact test, and evaluated the amount of each food eaten as a proportion of the total amount eaten using a paired t test. When analyzing the amount of food eaten, we eliminated replicates where no feeding occurred.

To determine if bacteria directly affected the palatability of microbe-laden carrion, we used the broad spectrum antibiotic chloramphenicol to minimize bacterial growth during the 2-d aging period and then conducted feeding assays using: (1) fresh carrion; (2) aged, microbe-laden carrion; and (3) aged, antibiotic-treated carrion. Carrion was held for 40–48 h in 20-L containers filled with either seawater (aged, microbe-laden treatment) or a 70 mg/L solution of chloramphenicol in seawater (aged, antibiotic treatment). Water in the containers was renewed every 3–4 h to prevent hypoxia and to renew the chloramphenicol solution. Fresh carrion was kept frozen and thawed immediately prior to use for feeding assays. Direct cell counts of bacteria using DAPI staining (Porter and Feig 1980) showed that the antibiotic treatment effectively retarded bacterial growth, as cell counts on antibiotic-treated carrion were below detection levels. Feeding assays were conducted using the same methods used for fresh vs. microbe-laden carrion. To test for changes in food palatability mediated directly by chloramphenicol (as opposed to through its effects on microbes), we incorporated chloramphenicol at the test concentration (70 mg/L) into artificial food made from fresh carrion and fed it to stone crabs. Pieces of fresh carrion either treated with antibiotic or not treated with antibiotic were offered simultaneously to stone crabs by placing the pieces on opposite sides of 4-L containers housing single stone crabs. Each stone crab was scored as to whether it first ate the untreated or the antibiotic-treated carrion. We used Fisher's exact test to determine if stone crabs chose one food type over the other. In this assay, if stone crabs chose antibiotic-treated carrion significantly $>50\%$ of the time, then chloramphenicol stimulated feeding; if they chose controls significantly $>50\%$ of the time, then the antibiotic suppressed feeding.

Because treatment with the antibiotic preserved carrion palatability, we also conducted a trapping experiment using aged, microbe-laden carrion and aged, antibiotic-treated carrion to determine if reducing bacterial growth affected consumer ability to find carrion, as opposed to its palatability once found. Aged, microbe-laden carrion and aged, antibiotic-treated carrion were generated and a trapping experiment with these foods was conducted using the same methods as used for the trapping experiment with fresh vs. microbe-laden foods ($n = 40$ traps per treatment). We analyzed the frequency of traps that captured animals with Fisher's exact test and the number of animals caught per trap with a t test. We used directed P values with $\gamma/\alpha = 0.8$ for these tests as suggested by Rice and Gaines (1994), based on the prediction that suppressing bacterial growth would increase attractiveness as it had increased palatability.

Food quality (measured as protein or nitrogen levels) often affects food palatability and the effectiveness of chemical defenses (Cruz-Rivera and Hay 2003). Aging carrion in water could leach compounds used as feeding cues, so that aging alone, unrelated to microbial processes, might make microbe-laden foods less palatable. To test this possibility, we determined protein concentrations for fresh and microbe-laden carrion using the Bradford assay (Bradford 1976) and then made foods differing even more than this to determine if this would cause food rejection. Dried samples of fresh and microbe-laden carrion ($n = 3$ for each carrion type) were ground to a powder, digested overnight with NaOH, combined with Bradford reagent, analyzed on a spectrophotometer, and compared to a standard curve using bovine serum albumin to calculate protein concentrations. To determine if a decrease in nutritional content explained the unpalatability of microbe-laden carrion, we manipulated the concentration of fresh fish flesh in an artificial food, making one food with 50% less value than the other, and assessed effects on feeding choices. We conducted feeding assays with stone crabs using artificial foods made with 1:1 vs. 2:1 (volume/volume) water : fresh carrion.

The chemistry of microbe-laden carrion and effects on consumer feeding

We blended 400 mL of microbe-laden carrion with deionized water (1:1 volume/volume), extracted the resulting slurry with 400 mL ethyl ether for 3 h, and then concentrated the ethyl ether extract under a stream of nitrogen, instead of under vacuum, in an effort to lessen the evaporative loss of volatile chemicals (Kicklighter et al. 2004). After the ethyl ether extraction, we exhaustively extracted this microbe-laden carrion slurry using a 1:1 water : methanol solvent mixture and then a 2:1 dichloromethane : methanol mixture to obtain the remainder of the crude extract that was not extractable with ether. These two extractions were dried under vacuum and combined. Extracts were incorporated into test foods using minimal amounts of carrier solvent (usually ethyl ether; Hay et al. 1998) and fed to stone crabs. Control foods were prepared with carrier solvent but without extract. Because bacteria on carrion commonly produce volatile metabolites (Janzen 1977, Frazier and Westhoff 1988) that are lost during extraction and separation, extracts were initially added to test food at three times the volumetric concentration of their yield following extraction, as recommended by a previous study working with volatile chemical defenses (Kicklighter et al. 2004).

We used stone crab feeding deterrence to guide fractionation of the ethyl ether extract employing normal phase silica gel column chromatography and HPLC. Constituents of the final deterrent fraction from HPLC could not be separated further due to very similar polarities of the multiple compounds in this fraction. Major components of this active fraction were identified using ^{1}H and ^{13}C NMR spectroscopy of the native compounds and gas chromatography/mass spectroscopy (GC/MS) of methyl ester derivatives. GC/MS analysis of reductive ozonolysis products enabled determination

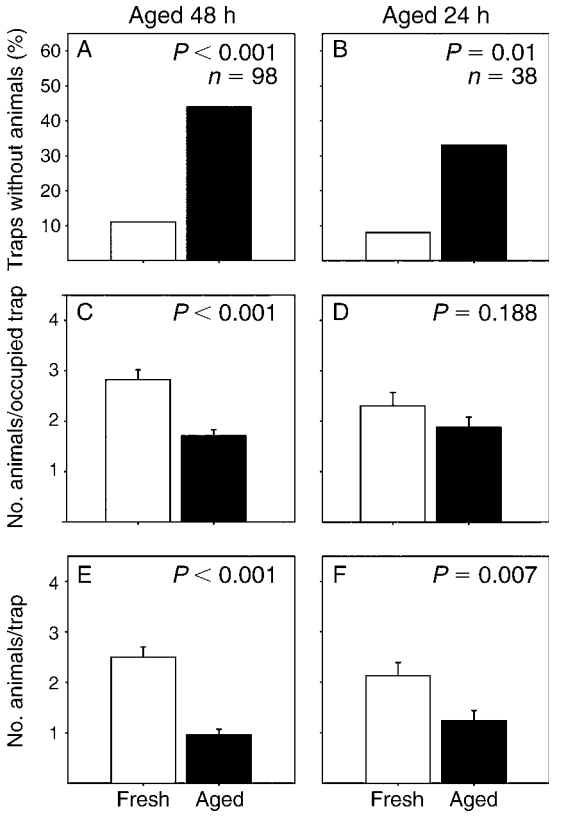

FIG. 1. (A, B) Frequency of empty traps, (C, D) total number of animals per occupied trap, and (E, F) total number of animals per trap overall when baited with either fresh carrion or microbe-laden carrion that had been aged for either 48 h or 24 h. Bars for panels C–F represent means + SE.

of double bond positions. Because the main components of the deterrent fraction were non-esterified fatty acids, we quantified total non-esterified fatty acids from a total lipid extraction of microbe-laden carrion (Bligh and Dyer 1959) and of our ethyl ether extract using the ACS-ACOD-MEHA method for non-esterified fatty acids as outlined in the NEFA C test kit (Wako Chemicals, Richmond, Virginia, USA). This method allowed us to determine the efficiency of our ethyl ether extraction scheme for non-esterified fatty acids from microbe-laden carrion.

Analysis of bacterial communities on fresh and microbe-laden carrion

To compare bacterial communities on fresh and microbe-laden carrion, we used 16S rRNA gene clone libraries (Pace et al. 1986) to identify bacteria from each carrion type. Total nucleic acids were extracted and purified from 0.5 g (wet mass) samples of microbe-laden and fresh menhaden ($n = 3$). Purified DNA samples were PCR amplified using primers specific for domain-level bacteria, (i.e., 27F and 1522R; Johnson 1994) and using appropriate reaction mixes and incubation conditions (Mills et al. 2003). Amplified products were analyzed on 1.0% agarose gels run in TBE buffer, stained with ethidium bromide, and UV illuminated. Amplicons were pooled from three to five reactions and purified with the Qiaquick gel extraction kit (Qiagen, Valencia, California, USA).

Purified pooled amplicons representing 16S rRNA gene sequences were cloned into the TOPO TA cloning vector pCR2.1 according to manufacturer's instructions (Invitrogen, Carlsbad, California, USA). Cloned inserts were subsequently PCR amplified with primers specific for the pCR2.1 cloning vector (i.e., M13F/R; Mills et al. 2003) and digested (2 h at 37°C) with MspI and HhaI (Promega, Madison, Wisconsin, USA). Clones from fresh ($n = 44$) and microbe-laden carrion ($n = 47$) libraries were grouped into 56 unique phylotypes as determined by restriction fragment length polymorphism analysis. Each phylotype was sequenced. We used LIBSHUFF analysis (Singleton et al. 2001) to determine differences between the bacterial communities on fresh and microbe-laden carrion. The 26 16S rRNA gene nucleotide sequences have been deposited in the GenBank database under accession numbers AY682046–AY682071 (*available online*).[8]

RESULTS

When we compared animal captures for traps baited with fresh carrion vs. microbe-laden carrion aged for 48 h, only 11% of traps baited with fresh carrion failed to capture scavenging animals while 44% of traps baited with microbe-laden carrion contained no scavenging animals (Fig. 1A, $P < 0.001$, $n = 98$; Fisher's exact test). Thus, microbe-laden carrion was 4 times more likely than fresh carrion to escape detection or use by scavengers. For traps that were colonized by scavengers, those baited with fresh carrion captured 1.6 times more animals than those baited with microbe-laden carrion (Fig. 1C, $P < 0.001$, t test). When averaged across all traps, those baited with fresh carrion captured 2.6 times more animals than ones baited with microbe-laden carrion (Fig. 1E, $P < 0.001$).

When we repeated this experiment with carrion aged for 24 h (but using only 38 sets of traps), we obtained similar results. Traps baited with microbe-laden carrion were 4 times more likely to escape detection by animals (Fig. 1B, $P = 0.01$), while fresh carrion captured 1.8 times more animals than microbe-laden carrion (Fig. 1F, $P = 0.007$). Considering only traps that did capture animals, densities were 23% higher in traps with fresh carrion, but this difference was not significant at this reduced sample size (Fig. 1D, $P = 0.188$). Overall, whether we allowed 24 h or 48 h for microbial colonization, microbe-laden carrion was less likely to be found by large consumers, and attracted fewer consumers (Fig. 1).

[8] ⟨http://www.psc.edu/general/software/packages/genbank/genbank.html⟩

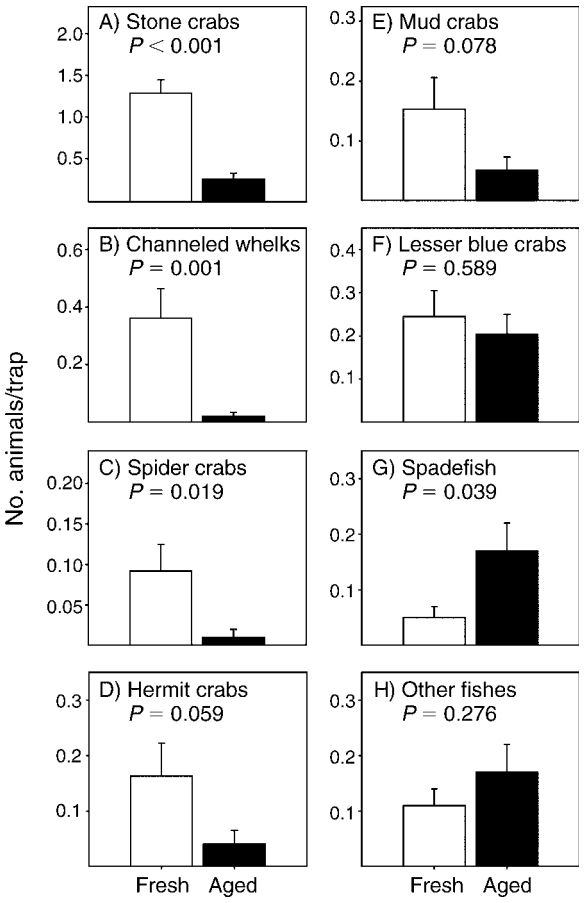

FIG. 2. Animals caught per crab trap (mean + SE) for individual animal species or groups in traps baited with either fresh carrion or microbe-laden carrion (aged 48 h); $n = 98$ for each comparison. Note the different y-axis scales.

Of the 339 animals captured in the well-replicated experiment using carrion aged for 48 h, 74% were crabs, 15% were fish, and 12% were gastropods. Thus, the majority of scavengers were species that typically use olfaction to locate their food (Weissburg and Zimmer-faust 1993, Smee and Weissburg 2006). The stone crab *Menippe mercenaria* was by far the most abundant species, comprising 45% of all captures. Because of its large size, it would have comprised a much larger percentage of total consumer biomass. Other common species included the lesser blue crab *Callinectes similis* (13% of captures), and the channeled whelk *Busycon canaliculatum* (11% of captures). Hermit crabs *Clibanarius vittatus* and *Pagurus pollicaris*, the mud crab *Panopeus herbstii*, and spider crabs *Libinia* spp. together comprised 15% of captures. Fresh carcasses attracted 5 times more stone crabs ($P < 0.001$, $n = 98$), 17.5 times more channeled whelks ($P = 0.001$), 9 times more spider crabs ($P = 0.019$), 4 times more hermit crabs ($P = 0.059$), and 3 times more mud crabs ($P = 0.078$; Fig. 2A–E, respectively), than microbe-laden carrion. Capture of lesser blue crabs did not differ between carrion types ($P = 0.589$; Fig. 2F). Juvenile Atlantic spadefish (*Chaetodipterus faber*) were preferentially attracted to the microbe-laden carcasses ($P = 0.039$; Fig. 2G), while other fishes were relatively uncommon and did not significantly differ in their attraction to microbe-laden vs. fresh carrion ($P = 0.276$; Fig. 2H). The spadefish was the only consumer significantly attracted to microbe-laden carrion, and it comprised only 6% of the total captures.

In feeding assays, stone crabs ate fresh carrion 2.5 times more frequently than microbe-laden carrion ($P = 0.001$, $n = 15$, Fig. 3A) while lesser blue crabs fed indiscriminately on the two carrion types ($P = 0.229$, $n = 18$; Fig. 3C). Striped hermit crabs ate fresh carrion 2.3 times more frequently than microbe-laden carrion (14 of 17 vs. 6 of 17 feeding, respectively; $P = 0.013$; Fisher's exact test) and consumed 5.4 times more of the fresh than microbe-laden carrion ($P < 0.001$, $n = 14$; Fig. 3B). Bacterial cell counts using DAPI staining showed that bacteria were 250 times more abundant on microbe-

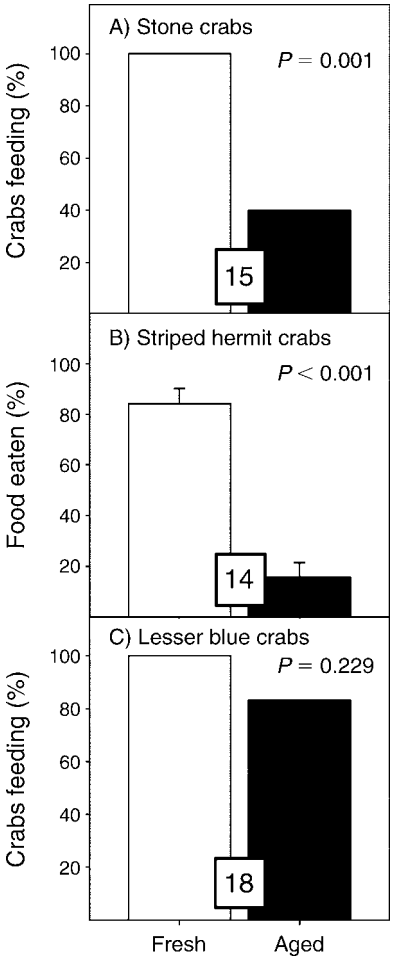

FIG. 3. Feeding assays with fresh carrion vs. microbe-laden carrion aged for 48 hours and fed to (A) stone crabs, (B) striped hermit crabs (mean + SE), and (C) lesser blue crabs. Inset boxes give the sample size for each assay.

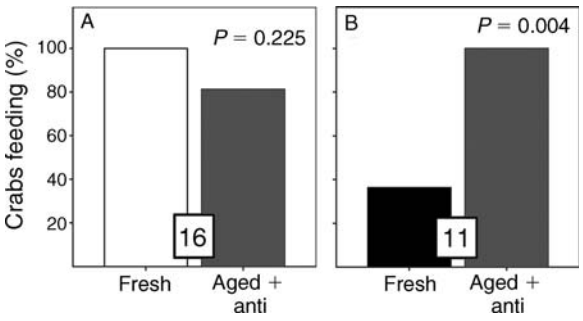

FIG. 4. Feeding assays with stone crabs (A) on fresh carrion vs. aged, antibiotic-treated ("aged + anti") carrion and (B) on aged, microbe-laden carrion vs. aged, antibiotic-treated carrion. Inset boxes give the sample size.

laden vs. fresh carcasses (2.5×10^9 vs. 9.8×10^6 cells/mL, respectively; $P < 0.001$, $n = 3$, t test).

When we used the antibiotic chloramphenicol to minimize bacterial growth during the aging period, stone crabs ate aged, antibiotic-treated carrion as readily as fresh carrion ($P = 0.225$, $n = 16$, Fig. 4A), and in preference to aged, bacteria-laden carrion ($P = 0.004$, $n = 11$, Fig. 4B). When added to fresh carrion, chloramphenicol alone neither stimulated nor depressed feeding compared to fresh carrion without chloramphenicol ($P = 0.722$, $n = 16$; Fisher's exact test). Although the protein content of carrion decreased 16% during the 48-h aging period (dropping from 65.3% ± 1.9% of fresh carrion dry mass to 55.0% ± 0.7% for microbe-laden carrion; $P = 0.004$, $n = 5$, t test), this lower nutritional value did not explain unpalatability of the microbe-laden food; 100% of stone crabs ($n = 15$) consumed experimental foods even when protein concentrations (and caloric value) were reduced by 50%.

When we surveyed animals attracted to either aged and microbe-laden or aged and antibiotic-treated carrion, the traps baited with aged, antibiotic-treated carrion captured animals more frequently than traps baited with aged, microbe-laden carrion (27 of 40 vs. 18 of 40 traps catching animals, respectively; directed $P = 0.043$, $n = 40$, Fisher's exact test). Thus, microbial colonization, as opposed to aging or leaching of stimulants alone, can affect the probability of food-falls being found by consumers. Treating carrion with antibiotics raised the mean number of animals captured per trap by 56% compared with untreated carrion, but this increase was not statistically significant (0.70 ± 0.14 vs. 1.10 ± 0.21 [mean ± SE] animals per trap; directed $P = 0.076$, $n = 40$, t test). Aged, antibiotic-treated carrion caught fewer consumers than did fresh carrion (1.10 ± .21 vs. 3.28 ± 0.36 animals per trap, respectively; $P < 0.001$, $n = 40$, t test), indicating that aging alone probably leached stimulatory compounds that animals use to locate desirable foods.

When we incorporated chemical extracts of microbe-laden carrion into fresh carrion, the ethyl ether extract of microbe-laden carrion rendered fresh carrion less palatable to stone crabs ($P = 0.045$, $n = 18$), while the remaining organic-soluble extract did not ($P > 0.99$, $n = 12$; Fig. 5A). Using bioassay-guided fractionation with stone crabs as the consumers, we tracked deterrence to one fraction that was composed primarily of non-esterified fatty acids (Fig. 5B–D), with Z-5 hexadecenoic acid, Z-9 octadecenoic acid, octadecanoic acid, hexadecanoic acid, and tetradecanoic acid comprising >90% of the mass of this fraction. Quantification of total non-esterified fatty acids in microbe-laden carrion and in our ether extract showed that our tests of non-esterified fatty acids in the ethyl ether extract at putatively 3 times natural concentration actually represented only 1.5 times natural concentration. Although high concentrations of fatty acids make foods rancid and unpalatable to many animals (Janzen 1977, Frazier and Westhoff 1988), when these specific fatty acids were combined at test concentrations equal to the concentrations of the fraction that deterred crab feeding (Fig. 5D), these fatty acids alone did not deter stone crab feeding as 100% of stone crabs ($n = 16$) consumed both the treatment and control food.

When we used 16S rRNA gene clone libraries to quantify the bacterial communities on fresh and microbe-laden carrion, clone sequences related to aerobic bacteria from the classes *Gammaproteobacteria* and *Alphaproteobacteria* represented >70% of the clone library from fresh carrion ($n = 44$ clones; Fig. 6A). In

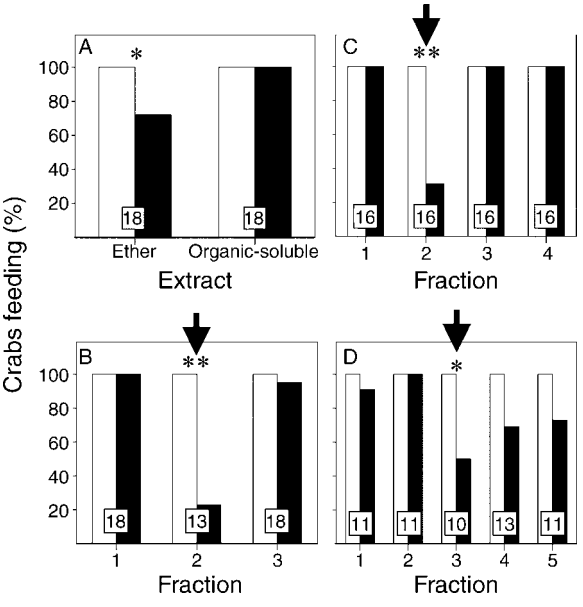

FIG. 5. Effects of organic extracts from microbe-laden carrion (aged 48 h) on stone crabs feeding for (A) the ethyl ether or the remaining organic-soluble extracts, (B) the initial-column fractions from the ethyl ether separation, (C) the second-column fractions, and (D) the HPLC fractions. Assays are for control (open bars) vs. extract or fraction-treated foods (solid bars). Arrows represent the fraction chosen for subsequent fractionation. Inset boxes give the sample size. Significance levels: *$P < 0.05$; **$P < 0.01$.

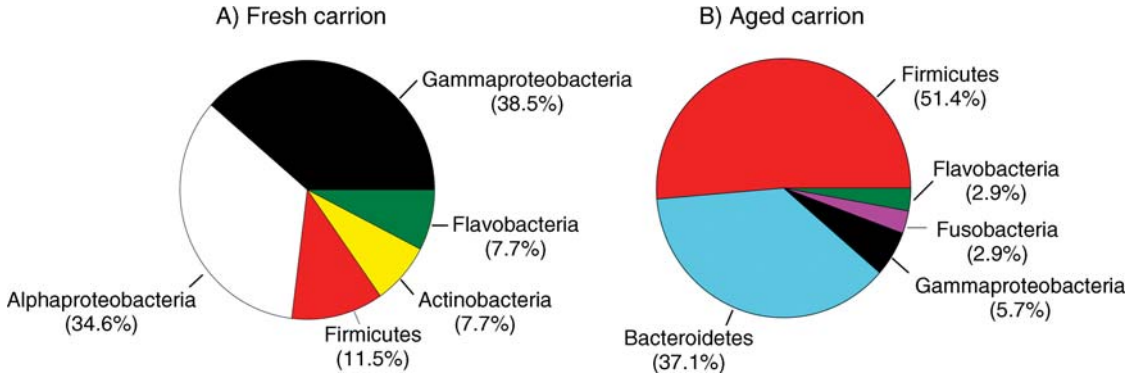

FIG. 6. Molecular analyses of bacterial populations on (A) fresh carrion and (B) microbe-laden carrion (aged 48 h). Data represent the percentage of clones isolated from that carrion type represented by a specific bacterial taxonomic group. Microbial communities differed significantly between fresh and microbe-laden carrion ($P < 0.025$), as determined by LIBSHUFF analysis (Singleton et al. 2001).

contrast, 89% of clones derived from microbe-laden carrion ($n = 47$ total clones) were related to anaerobic bacteria from the phyla *Firmicutes* (51% of clones) and *Bacteroidetes* (38% of clones; Fig. 6B). LIBSHUFF analysis (Singleton et al. 2001) showed that bacterial communities on fresh vs. microbe-laden carrion differed significantly ($P < 0.025$).

DISCUSSION

Both field surveys and laboratory feeding assays demonstrated that increased bacterial densities were associated with diminished attractiveness and diminished palatability of microbe-laden carrion to most animal consumers. In the field, microbe-laden carrion aged for either 24 hours or 48 hours escaped detection by large consumers (as evidenced by trap success) about four times more frequently than fresh carrion, and it attracted only 36–54% as many total numbers of consumers (Fig. 1). Microbe-laden carrion attracted significantly fewer stone crabs, channeled whelks, and spider crabs, and tended to attract fewer mud crabs and hermit crabs (Fig. 2). Lesser blue crabs did not differentiate between food types, while the Atlantic spadefish was the only species significantly attracted to microbe-laden carrion (Fig. 2). In the laboratory, feeding by the most common consumer captured in our field assays (stone crabs) was strongly suppressed by microbe-laden vs. fresh carrion, as was feeding by a less common hermit crab; in contrast, feeding by the infrequently collected lesser blue crab was equivalent on these foods (Fig. 3). Our data from a marine ecosystem are consistent with field studies in terrestrial ecosystems showing a rapid decrease in scavenging by animals under conditions where microbes are likely to be at high densities in carcasses (Houston 1986, DeVault et al. 2004, Selva et al. 2005).

Aged, microbe-laden carrion could attract fewer scavengers and be less palatable due to microbes chemically defending their resource or due to leaching of feeding stimulants during the aging process. When we used an antibiotic to keep bacterial populations low during the aging process, aged, antibiotic-treated carrion captured more consumers in the field than did untreated carrion aged for an equal duration, suggesting that bacteria directly reduce the attractiveness of aged carrion to animal consumers. However, aged carrion treated with antibiotic attracted fewer animals than fresh carrion, suggesting that physical degradation alone also decreased carrion attractiveness, probably due to leaching of water-soluble chemicals that are olfactory cues for scavenging animals (Weissburg et al. 2002). In feeding assays, aged carrion treated with antibiotics remained palatable to stone crabs (Fig. 4B). Further, stone crabs were not deterred by the decreased nutritional quality of microbe-laden carrion; protein content of the microbe-laden carrion decreased by only 16% during the aging process, but stone crabs readily ate foods in which we decreased food quality by 50%. Thus bacteria, as opposed to physical degradation processes, made carrion unpalatable to animal consumers, whereas both bacteria and physical degradation played roles in decreasing the rate at which consumers located and colonized carrion.

The level of bacterial colonization that decreased carrion attractiveness (Figs. 1 and 2) and deterred feeding by stone crabs and striped hermit crabs (Fig. 3A, B) did not affect foraging in the field (Fig. 2F) or feeding in the lab (Fig. 3C) by lesser blue crabs, consumers that often eat foods that are repugnant to other animals (Fig. 3C; Kicklighter et al. 2004). Blue crabs (*Callinectes* spp.) are voracious omnivores (Rosas et al. 1994) and may have adaptations, much like carrion-scavenging raptors (Houston 1979), that allow them to find and feed on foods that most animals find repulsive. In addition, Atlantic spadefish were significantly attracted to microbe-laden carrion (Fig. 2G), a behavior that contrasted sharply to the other consumers in our study. However, spadefish typically eat sponges (Randall 1967), which can house bacteria in densities up to 10^8–10^{10} bacteria per gram of sponge wet mass, concentra-

PLATE 1. D. Burkepile (left) and B. Woodson (driving) transporting crab traps that will be baited with either fresh or rotten menhaden and deployed in tidal creeks near Skidaway Island, Georgia, USA. Photo credit: J. D. Parker.

tions two to four orders of magnitude greater than in seawater (Hentschel et al. 2006). These microbe–sponge consortia commonly produce noxious secondary metabolites that deter feeding by many consumers (Pawlik et al. 1995). Thus, sponge feeding may predispose Atlantic spadefish to a tolerance for microbes and their noxious metabolites. While consumers clearly differ in their willingness to consume microbe-infested food, bacterial colonization appears to render carcasses unpalatable to all but the most gastrointestinally robust consumers.

Analysis of bacterial communities showed dramatic differences between fresh and microbe-laden carrion. Fresh carrion was dominated by aerobic bacteria from the classes *Gammaproteobacteria* and *Alphaproteobacteria* (Fig. 6A) while microbe-laden carrion was dominated by anaerobic bacteria from the phyla *Firmicutes* and *Bacteroidetes* (Fig. 6B). The phylum *Firmicutes* contains the genera *Bacillus* and *Clostridium*, both of which colonize animal carcasses and produce objectionable, volatile, and toxic chemicals that cause food spoilage and violent illnesses in humans (i.e., botulism; Frazier and Westhoff 1988). These changes in bacterial community composition coincided with a >250 times increase in bacterial density. Thus, after two days of aging, the bacterial consortium on carrion changed dramatically in both density and composition (from a bacterial community dominated by benign aerobes to one dominated by objectionable anaerobes that rendered carcasses repugnant and unpalatable; Fig. 4). This change apparently occurs within 24 hours or less, as judged by the decrease in trapping success using carrion aged for 24 hours (Fig. 1B, F).

Bacteria may make carcasses unpalatable by producing toxic chemicals that cause vomiting, diarrhea, and a reorganization of the gut flora or by producing nontoxic compounds that indicate the presence of invasive pathogenic microbes that can infect the blood and organs of the consumer (Snydman 1989). These foodborne infections (such as *Salmonella, Listeria,* and some *Escherichia coli* infections) can cause severe sepsis, meningitis, and even death (Snydman 1989, Wachi et al. 2005). Thus, scavengers may not eat aging carrion not only to avoid the post-ingestive consequences of consuming toxic bacterial chemicals but also to avoid severe bacterial infections (Janzen 1977). That scavengers may be able to sense and avoid pathogenic microbes is suggested by the fact that animals avoid scavenging ungulates killed by rinderpest (Morris 1932, as cited in Janzen 1977) and scavenge disease-killed carcasses less often than those killed by predators (Selva et al. 2005).

In our study, a chemical mechanism for carrion unpalatability was suggested because microbe-laden carrion and its organic extract smelled strongly of skunk, rotten eggs, and body odor. When we fed organic extracts of microbe-laden carrion to stone crabs, the ethyl ether extract was significantly deterrent (Fig. 5A). Because microbe-laden carrion was colonized by a consortium of bacteria (Fig. 6B), deterrence was likely due to the effects of many compounds in the resulting chemical "cocktail" produced by microbes. Despite this,

we were able to use bioassays-guided separations to follow deterrent chemicals through three separation steps using column chromatography and HPLC (Fig. 5B–D). A fraction of the extract remained strongly deterrent despite considerable loss of volatile compounds responsible for the objectionable odor during each stage of the extraction, separation, and bioassay process (i.e., the odor decreased considerably during this process). Composition of this deterrent fraction was >90% non-esterified fatty acids; however, when these specific fatty acids were tested directly, they were not unpalatable to stone crabs. Thus, the chemical deterrence that we show could be due to low concentration compounds that were undetectable in the deterrent fraction, or to these lesser compounds acting in concert with the compounds that we identified.

Furthermore, we probably underestimated the concentrations of important volatile chemicals in the deterrent ether extract (Fig. 5A), as previous work with volatile chemical defenses showed that similar experimental methods resulted in only 10% of some volatile chemicals being included in feeding assays if tested at concentrations mimicking natural yield (Kicklighter et al. 2004). Thus, feeding assays with the ether extract of microbe-laden carrion (Fig. 5A) and its fractions (Fig. 5B–D) may have been conservative due to the loss of amines, mercaptans, sulfides, and other small volatile compounds that could be important in mediating microbe–animal competition (Janzen 1977). Nevertheless, it is clear that bacteria, not aging per se, make carcasses chemically repugnant to the dominant consumer in this marine community (Figs. 4 and 5).

Microbes often use secondary metabolites (i.e., antibiotics) to compete with other microbes (Vining 1990), but secondary metabolites frequently play multiple ecological roles in deterring predators, preventing pathogen establishment, and vanquishing competitors (e.g., Schmitt et al. 1995, Kubanek et al. 2002). Thus, a consortium of competing bacteria on a rotting carcass may produce a chemical "cocktail" that also deters animal scavengers and therefore benefits the group of bacterial decomposers (Janzen 1977). However, group selection need not be invoked to explain these patterns as multiple species could be acting via selfish selection but benefit the consortia as a whole via positive interactions, as has been demonstrated in many ecological communities (Bertness and Callaway 1994, Bruno et al. 2003). Competitors, or predators and prey, often produce overall positive effects for each other without resorting to group selection (Hay et al. 2004), and microbiologists recently have made similar arguments for reciprocal positive interactions within microbial communities (Caldwell et al. 1997). Given the potential for mutualism, cooperation, and cheating within microbial consortia (Velicer 2003) where compounds produced by some species or clones may benefit other species or clones, more complex microbial investigations of interactions within these consortia are warranted. However, regardless of the selective mechanism responsible for these chemicals, we show that these chemicals produced by microbes make foods repugnant to scavenging animals and reduce the potential competitors for this critical resource.

Our study suggests that microbial putrefaction of food-falls is a chemical defense allowing microbes to compete not only with other microbes but also with grossly dissimilar organisms much larger than themselves. Such interkingdom competition is probably common, but underappreciated, (Hochberg and Lawton 1990, Cipollini and Stiles 1993, Crist and Friese 1993, Rohlfs et al. 2005), and this appears to be the first experimental demonstration of chemically mediated microbe–animal competition. Although chemically mediated competition between microbes and animals is not well documented, this interaction may be widespread wherever rich energy resources are available (Janzen 1977). Further, microbe–animal interactions may run the gamut from competitive to facilitative depending on the richness of the resource. For rich resources like carrion, fruits, and seeds, all of which can be immediately eaten by animals, microbes may need to defend these resources from consumers, and these energy-rich resources may be worth the cost of producing such defenses. However, for energy-poor resources like leaves, wood, and other plant detritus, such chemical defenses are neither known nor suggested by observational studies (Barlocher 1980). In fact, animals, such as freshwater invertebrates, often find these resources unattractive until they are first infected by microbes that condition these recalcitrant foods (Gessner et al. 1999). In these instances, microbes do not appear to compete with animals, but rather facilitate animal consumption of detritus.

In our study system, animals appear to frequently outcompete microbes in the race for carrion as evidenced by fresh carcasses being colonized rapidly by animals, thus giving microbes little time to putrefy them. This is to be expected in an ecosystem so rife with crabs and gastropods that it supports commercial fisheries for these groups. Such dense aggregations of consumers will be uncommon in deeper waters or shallow coastal systems with lower productivity. Carrion scavenging is less intense in other marine ecosystems (Britton and Morton 1994), with as little as 20% of carrion scavenged over several weeks at some locations (Witte 1999). Low rates of scavenging may also occur when carrion is initially provided in very large quantities that prevent local consumers from rapidly depleting it. Massive fish kills during harmful algal blooms or anoxic conditions and whale falls would be examples. For terrestrial systems, DeVault et al. (2003) show that scavengers remove 13–100% of carcasses depending on latitude, climate, habitat, and type of scavenger. Further, carcasses are scavenged slowly enough in terrestrial systems that a predictable suite of carrion-attendant arthropods specializes on this resource (Braack 1987,

Watson and Carlton 2005). Therefore, in areas where scavenging is slower, and particularly in areas where it is warm and humid, microbe–animal competition should intensify. In addition, microbe–animal competition over carrion may be more important in ecosystems where carcasses are large relative to the size of typical scavengers (i.e., large ungulate as opposed to rodent carcasses), or where massive, synchronous kills occur due to toxins, physical stresses, or disease outbreaks. In such cases, scavengers may not be able to completely use carcasses before microbes stake their claim to portions of them and prevent scavengers from taking the rest or limit scavenging to only those animals that are adapted to eating putrid carcasses.

Detrital subsidies such as dead plants, seeds, and carrion are important energy inputs that increase productivity both within and among ecosystems (Polis et al. 1997, Moore et al. 2004). For example, on islands in the Gulf of California, herbivores and detritivores are >100 times more abundant in areas that receive energy subsidies of carrion and drift algae from the ocean than in areas that do not, while predators (spiders) are six times more abundant in these subsidized areas (Polis and Hurd 1995). Energy subsidies on these islands can even affect the abundance of top predators such as coyotes that are up to 13.7 times more abundant in areas with marine subsidies and get up to 20% of their diet by scavenging marine carrion (Rose and Polis 1998). Microbe–animal competition for these resources could be pivotal, with detrital inputs either directly fueling higher trophic levels if animals thwart microbial defenses and consume most of the dead organic matter, or with detrital inputs facilitating primary productivity if microbes deter scavengers and shuttle the resources through the detrital pathway. When viewed in this context, microbial decomposers become larger, more dynamic players in food webs.

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