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Trans fatty acids provide evidence of anthropogenic feeding by black bears

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Abstract: Bears (*Ursus* spp.) that become conditioned to anthropogenic food sources pose a risk to human safety and generally need to be relocated, rehabilitated, or destroyed. Identifying food-conditioned bears may be difficult if the animal is not captured or killed while immediately engaged in the nuisance behavior. Fatty acid signature analysis has been used to examine the dietary habits of bears and other carnivores and is based on the predictable incorporation of ingested fatty acids into the consumer's fat stores. Unusual fatty acids that are available in only a few food types may be particularly useful dietary markers. In this study, we tested the hypothesis that *trans* fatty acids present in many processed foods could serve as markers of anthropogenic foraging by black bears (*Ursus americanus*). Among 13 bears that were killed in western and central Colorado, *trans* fatty acids were more abundant in conflict bears than in non-conflict bears. Further, the abundance of *trans* fatty acids in bear fat appeared to be correlated with the intensity of bear–human conflict. We conclude that the trophic transfer of *trans* fatty acids can provide valuable insights into the ecological, demographic, and anthropogenic factors that contribute to bear–human conflict.

Key words: bear–human conflict, biomarker, Colorado, diet, fatty acid, foraging, human–wildlife conflicts, management, nuisance, road-kill, *Ursus americanus*

Conflicts with black bears (*Ursus americanus*) are becoming more frequent throughout the range of the species in North America (Hristienko and McDonald 2007, Brown and Conover 2008, Conover 2008, Cotton 2008, Lemelin 2008). Bear–human conflicts often center on anthropogenic food sources, including garbage, bird seed in feeders, crops, or livestock (Johnson and Griffel 1982, Garshelis 1989, Gore and Knuth 2006, Baruch-Mordo 2007, Leigh and Chamberlain 2008; Figures 1 and 2). Bears that become conditioned to these foods pose the greatest risk to human safety (Herrero 1970, Gunther 1994). Understanding the factors that contribute to bears becoming food-conditioned is therefore essential to mitigating bear–human conflicts (Conover 2002).

Management actions aimed at nuisance bears include lethal and nonlethal controls that may be expensive to implement, result in negative public opinion, or have questionable efficacy (Linnell et al. 1997, Beckmann et al. 2004, Treves and Naughton-Treves 2005). Although the assumption often is made that the individual bear receiving the management action is the same one that caused the initial conflict, positively identifying a food-conditioned bear may be difficult. In many cases, a bear is trapped or shot in the vicinity of a conflict sometime after the problem has occurred (Madison 2008, Beckmann and Lackey 2008). Consequently, there may be some uncertainty as

Figure 1: Evidence of bear activity at a dumpster.

Figure 2. Nuisance bears that are athropogenic feeders can be identified by *trans* fatty acid biomarkers found in their fat. This black bear has learned where to find an easy meal. (*Photos courtesy Matt Gronke-ck.*)

to whether the bear that was ultimately trapped or shot is the same one that was involved in the initial conflict. This uncertainty may result in some non-problem bears being unnecessarily destroyed, as well as real problem bears not being adequately managed (Wolfe 2008).

Fatty acid signature analysis is a powerful tool for studying the ecology of bears and other predators (Iverson et al. 2001*a*, 2006; Thiemann et al. unpublished data). Fatty acids, which are the primary components of most lipids, are comprised of a hydrocarbon chain containing a variable number of double bonds. The length of the hydrocarbon chain, along with the number and position of double bonds, determine the physical and biochemical properties of the fatty acid. Mammalian carnivores have a limited ability to synthesize fatty acids and, as a result, many dietary fatty acids are predictably incorporated into a consumer's adipose tissue (Beynen et al. 1980, Cook 1991, Galli and Risé 2006). The relationship between the fatty acid composition of the consumer and its diet can then be used to make inferences about the foraging habits of free-ranging animals (e.g., Ackman and Eaton 1966; Reidinger et al. 1985; Iverson et al. 2001*a*, 2004). In some ecosystems, unusual fatty acids available in only a few types of food may serve as dietary markers and provide particularly detailed information on animal diets (Budge et al. 2007, Thiemann et al. 2007).

Despite recent concerns about their effects on human health, *trans* fatty acids are present in many commercially prepared food products (Enig et al. 1983, Exler et al. 1995, Albers et al. 2008). These unusual fatty acids are synthesized during the hydrogenation process that converts oils into semisolid fats, giving them greater biochemical stability and a longer shelf-life (Emken 1984). The consumption of *trans* fatty acids has been linked to increased serum cholesterol levels and greater risk of cardiovascular disease in humans (Mensink and Katan 1990, Willett et al. 1993). *Trans* fatty acids are also present in ruminants' fats and may potentially be obtained by wild bears through the consumption of wild or domestic animals (Cordain et al. 2002, Rule et al. 2002). However, species that synthesize *trans* fatty acids in the rumen and store it in their fat generally have smaller quantities and different isomers of these fatty acids than are found in processed human foods (Knight et al. 2004, Ledoux et al. 2007).

Trans fatty acids may serve as useful biomarkers because they cannot be endogenously synthesized by carnivores and because dietary *trans* fatty acids are readily incorporated into adipose tissue (Ohlrogge et al. 1981, Chen et al. 1995). In this study, we examined the potential use of *trans* fatty acids as biomarkers for anthropogenic feeding by black bears. We hypothesized that anthropogenic *trans* fatty acids would be most abundant in those bears

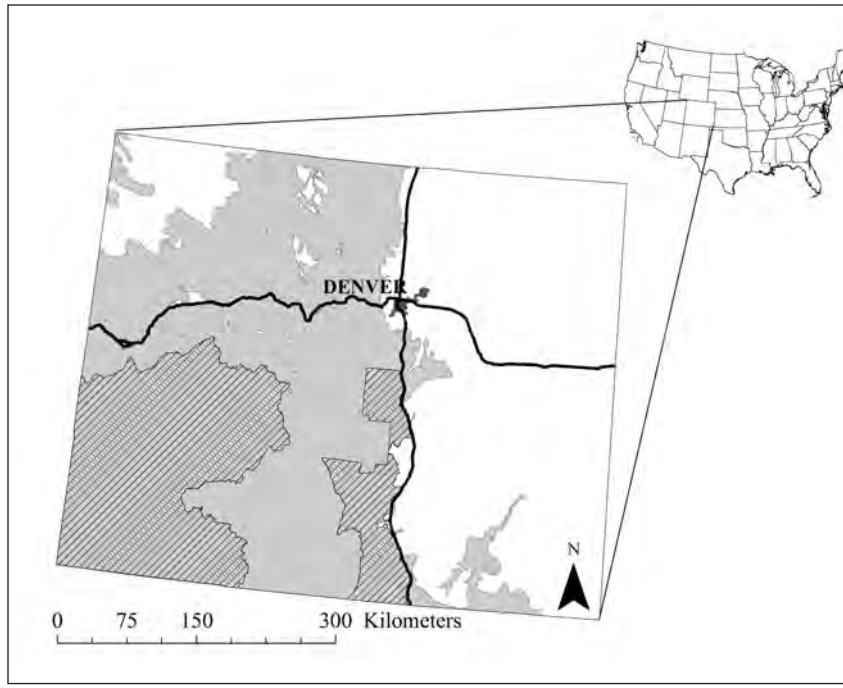

Figure 3. Map of the study area (hatched) in southwestern Colorado where black bears were sampled during spring and summer, 2004. Light-shaded areas represent the habitat range of black bears. (*Map courtesy Colorado Division of Wildlife, 2006*)

that conflicted most directly with humans, as compared to bears killed indiscriminately by collisions with vehicles or by legal harvest.

Study area and methods

Black bears were sampled in southwestern Colorado (Figure 3) during the late spring and summer (May–September) of 2004. Adipose tissue samples were collected opportunistically from black bears that either (1) were killed after being struck by a vehicle (road-kill), (2) shot during legal harvest, (3) were destroyed by USDA/Wildlife Services or Colorado Division of Wildlife officers after the animals came into conflict with humans or livestock, or (4) died due to unknown causes unrelated to conflict control. Details about the type of bear–human conflict were collected for each bear and are summarized in Table 1. Standard operating procedures for euthanizing conflict bears in Colorado are described in an administrative directive (Colorado Division of Wildlife 2002), and the procedure for processing adipose tissue samples was approved by the Animal Care and Use Committee at the USDA-WS-National Wildlife Research Center (QA-1207).

Bear age was determined by sectioning a vestigial premolar tooth (Stoneberg and Jonkel 1966, Willey 1974), and weight was visually estimated by wildlife officers. Each bear was dissected immediately after being killed or discovered. A minimum 1–2 cm^3 of subcutaneous fat was collected from the mid-section of each bear and stored at -20°C until analysis (Thiemann et al. 2006). In the lab, lipid was extracted and isolated from the tissue using a modified Folch extraction (Folch et al. 1957, Iverson et al. 2001*b*), and fatty acid methyl esters were prepared using HCl in methanol (Christie 1973, Thiemann et al. 2004). Fatty acid methyl esters were identified using gas chromatography-mass spectroscopy (GC-MS; Agilent 6890 GC/5973 MS equipped with an Agilent HP-88 column, Agilent Technologies, Palo Alto, Cal., USA) and quantified using an internal standard, tridecanoic acid (13:0). Fatty acid concentrations are expressed as the mass percentage of total fatty acids in the sample and individual fatty acids are referred to using the standard nomenclature *A*:*B*n-*C*, where *A* is the number of carbon atoms in the hydrocarbon chain, *B* is the number of double bonds, and *C* is the position of the first double bond, relative to the terminal methyl group (IUPAC-IUB 1978).

Table 1. Description of black bears (*Ursus americanus*) killed in Colorado in late spring and summer, 2004. Samples of adipose tissue from each bear were used in this study.

Bear no.	Sex	Age (years)	Estimated mass (kg)	Date killed (2004)	Comments	Nuisance category
1	Male	8	160	July 31	Shot after repeatedly breaking into tents between July 17 and July 31, 2004	Dwelling
2	Male	7	100	August 5	Trapped and shot after breaking into multiple mobile homes between July 4 and Aug 5, 2004	Dwelling
3	Male	12	180	July 1	Shot after depredating sheep	Livestock
4	Male	1	50	August 26	Shot after depredating livestock	Livestock
5	Female		90	August 28	Shot after depredating sheep	Livestock
6	Male	2	100	August 29	Shot after depredating sheep	Livestock
7	Female	4		May 22	Struck and killed by a vehicle	Road-kill
8	Male		140	July 19	Shot after being struck and injured by a vehicle	Road-kill
9	Female	1		August 8	Struck and killed by a vehicle	Road-kill
10	Female	3	90	September 29	Struck and killed by a vehicle	Road-kill
11	Female	12	90	August 22	Struck and killed by a vehicle	Road-kill
12	Male	0		August 9	Possible poisoning; cub was observed feeding and then fell over and was unable to get up.	Injury
13	Male	5	100	September 2	Shot and killed by a hunter	Harvest

Rigorous statistical analyses were precluded by small sample sizes and the opportunistic nature of our sampling regime. However, to test the hypothesis that bears coming into conflict with humans had higher levels of anthropogenic *trans* fatty acids than non-conflict bears, we divided bears into "conflict" (those destroyed after breaking into dwellings or predating livestock, $n = 6$) and "non-conflict" (all other bears, $n = 7$) categories. These groups were compared using a one-tailed *t*-test (SPSS, $\alpha = 0.05$).

Results

The fatty acid profiles of all 13 bears are presented in Table 2. A total of 9 different fatty acids were identified, with four of these (16:0, 18:0, *cis*18:1n-9, *cis*18:2n-6) accounting for >95% of total fatty acids across all samples. Two different *trans* fatty acids were identified in black bear fat: *trans*18:1n-9 and *trans*18:2n-6. Of these, *trans*18:1n-9 was most abundant, accounting for 1.13 ± 0.25% (mean ± SEM; Table 2) of total fatty acids across all samples. The abundance of *trans*18:1n-9 was more than twice as high in conflict bears (1.60 ± 0.30%) than in non-conflict bears (0.73 ± 0.34%; $t_{11} = 1.972$, $P = 0.037$).

Table 2. Fatty acid composition (mass % of total fatty acids) of 13 black bears killed in Colorado during spring and summer, 2004, sorted by nuisance category.

Nuisance Category	Bear no.	Sex	14:0	16:0	18:0	*trans* 18:1n-9	*cis* 18:1n-9	*trans* 18:2n-6	*cis* 18:2n-6	18:3n-3	20:3n-3
Dwelling	1	M	1.56	28.26	9.92	2.53	42.73	0.00	14.69	0.31	0.00
	2	M	0.94	17.83	6.09	1.42	34.39	0.00	37.14	2.19	0.00
Livestock	3	M	1.36	32.18	18.73	1.29	42.76	0.00	2.94	0.74	0.00
	4	M	0.61	12.35	6.21	1.19	45.98	0.11	32.48	0.93	0.13
	5	F	1.38	21.76	8.33	2.41	35.44	0.00	29.91	0.77	0.00
	6	M	1.42	45.01	10.40	0.76	38.09	0.00	3.74	0.58	0.00
Road-kill	7	F	0.69	13.56	5.69	1.60	38.05	0.20	39.79	0.42	0.00
	8	M	1.93	30.88	9.71	0.00	49.29	0.00	8.20	0.00	0.00
	9	F	1.39	30.68	9.30	0.00	23.62	1.44	31.03	2.53	0.00
	10	F	0.83	17.47	4.75	0.94	39.67	0.00	35.27	0.95	0.12
	11	F	1.65	36.47	8.34	2.14	40.04	0.00	10.86	0.50	0.00
Injury	12	M	1.03	19.95	8.85	0.42	35.11	0.00	32.97	1.66	0.00
Harvest	13	M	0.87	22.94	6.12	0.00	50.07	0.00	17.49	2.50	0.00
		Mean	**1.21**	**25.33**	**8.65**	**1.13**	**39.63**	**0.14**	**22.81**	**1.08**	**0.02**
		SE	**0.11**	**2.65**	**0.98**	**0.25**	**1.94**	**0.11**	**3.73**	**0.24**	**0.01**

Discussion

Diet composition has a strong influence on the fatty acid profiles of black bears and other monogastric carnivores (Iverson et al. 2001*a*, 2004). Although some fatty acids can be synthesized endogenously from non-lipid precursors, many dietary fatty acids are predictably incorporated into a consumer's fat stores. Because black bears are unable to synthesize *trans* fatty acids, the *trans*18:1n-9 and *trans*18:2n-6 we observed in black bears must have originated in the diet. The fact that conflict and non-conflict bears had different levels of *trans* fatty acids suggests a relationship between bear diets and bear–human conflict.

Free-ranging bears may obtain *trans* fatty acids from different dietary sources. However, the predominant *trans* fatty acid isomer we identified in black bears (*trans*18:1n-9) is characteristic of the hydrogenated vegetable oil found in many processed foods (Ohlrogge et al. 1981, Chen et al. 1995, Ledoux et al. 2007). The muscle and fat of wild and domesticated ruminants, including mule deer (*Odocoileus hemionus*), antelope (*Antilicapra americana*), elk (*Cervus elaphus*), beef cattle (*Bos taurus*) and sheep (*Ovis aries*) contain small amounts of *trans*18:2 (<2% of total fatty acids) and *trans*18:1 (<3%; Cordain et al. 2002, Rule et al. 2002, Purchas et al. 2005, Valvo et al. 2005). However, the predominant *trans* fat isomer in ruminant fat is *trans*18:1n-7 (Parodi 1976, Ledoux et al. 2007), which we did not detect in black bears. Furthermore, the abundance of *trans* fatty acids in ruminants is much smaller than in processed human foods. Margarine, potato chips, and commercially-prepared chocolate chip cookies may contain 22-32% *trans*18:1 (expressed as mass % of total fatty acids; Exler et al. 1995); a concentration roughly 2 orders of magnitude higher than in ruminants.

Considering the higher level of *trans*18:1n-9 in conflict bears than in non-conflict bears and its greater availability in human food than in ungulate prey, our results indicate that *trans*18:1n-9 can serve as a marker of anthropogenic foraging by black bears. Because the abundance of *trans*18:2 in human processed foods is low (2–7% of total fatty acids in margarine, potato chips, and cookies; Exler et al. 1995) compared to *trans*18:1, the presence of *trans*18:1n-9 but not *trans*18:2n-6 in most bears in this study is also consistent with anthropogenic foraging. Bear 9 showed the reverse trend and had the highest level of *trans*18:2n-6 but no detectable amounts of *trans*18:1n-9. This could be a result of feeding on ruminants (which may be higher in *trans*18:2 than *trans*18:1; Cordain et al. 2002) or feeding on some specific human foods. For instance, doughnuts may contain >2% *trans*18:2, but zero *trans*18:1 (Exler et al. 1995).

We acknowledge that some wildlife managers may have trapped conflict bears using anthropogenic foods containing *trans* fatty acids. Although immediate consumption of trap bait (i.e., within 24 hours) may have influenced the fatty acid composition of some blood components (Cooper et al. 2005), adipose tissue composition reflects dietary patterns over a span of weeks to months (Kirsch et al. 2000, Iverson et al. 2004). Therefore, the *trans* fatty acids we identified in the adipose tissue of black bears are indicative of longer term feeding on human foods and garbage.

Our dataset was too limited to examine demographic differences in black bear fatty acids (Table 1). However, bears of different age and sex classes may have different probabilities of anthropogenic foraging (Rogers et al. 1976), and, with greater sample sizes, these trends should be apparent in black bear fatty acids. Because *trans* fatty acids undergo metabolic turnover and do not accumulate in tissues over time (Ohlrogge et al. 1981), age-specific trends in *trans* fatty acids should reflect the amount of recent (rather than lifetime) anthropogenic foraging.

Although statistical comparisons were limited to conflict *vs.* non-conflict bears, there appeared to be a correlation between conflict category and the abundance of anthropogenic *trans* fatty acids in black bears. The 2 bears that had entered human dwellings posed the greatest risk to human safety and clearly had access to human food and garbage. These bears had repeatedly entered tents and mobile homes and had likely come to associate human dwellings with food rewards. The frequent consumption of human food and garbage accounts for the relatively high levels of *trans* fatty acids in these 2 bears (Figure 4a).

The bears that were killed after depredating livestock represent a more intermediate

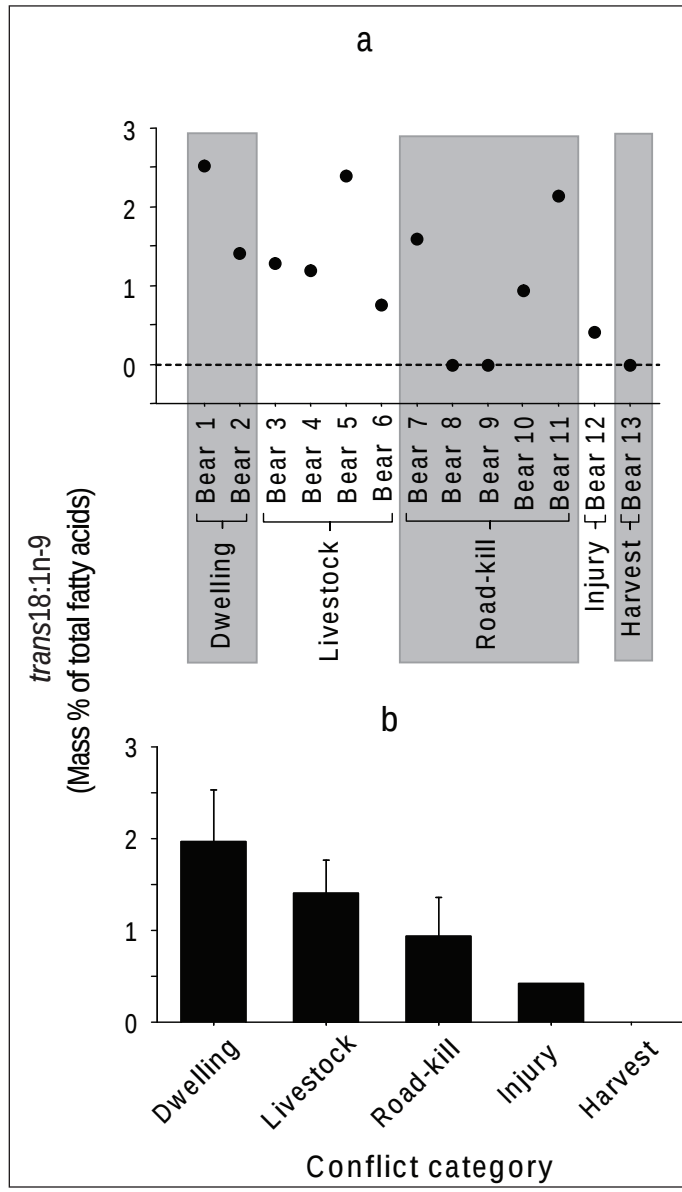

Figure 4. Abundance of *trans*18:1n-9 in the subcutaneous adipose tissue of black bears expressed (a) across all 13 individual bears and (b) as the average (+ 1 SEM) of each conflict category.

conflict category. These bears may have been primarily targeting animal prey, but the fact that they were foraging on farms and ranches put them in conflict with humans and may have provided access to anthropogenic foods. The intermediate-to-high levels of *trans*18:1n-9 in these bears (Figure 4) suggests they were foraging on garbage in addition to depredating livestock. Conversely, the bears killed after being struck by vehicles had, on average, 34% less *trans*18:1n-9 than the "livestock" bears (Figure 4b). Food-conditioned bears may be at higher risk of being hit by traffic, but roads still pose a threat to non-conflict bears with no history of food-association; 2 road-kill bears had undetectable levels of *trans*18:1n-9 (Figure 4a).

The conflict status of the injured bear (bear 12) was uncertain but there was little evidence to indicate this bear had become habituated to human food. However, its detectable (albeit low) level of *trans* fatty acid, along with the fact that it may have been poisoned (Table 1) suggest some history of anthropogenic foraging. As

expected, no *trans* fatty acids were detected in the bear killed during a legal harvest. This bear was shot in its natural habitat and therefore had given no indication of previous human interaction.

Management implications

Our results suggest that *trans* fatty acids can provide a diagnostic marker of anthropogenic food use by black bears. This may provide a powerful tool for wildlife officers and resource managers dealing with a suspected problem bear.s In many cases, after a bear–human conflict is reported, a bear is captured or killed sometime later in the general area where the conflict occurred. By collecting a small subcutaneous tissue sample from a captured bear and measuring the level of *trans* fatty acids (a process that can be completed in a few hours), wildlife agencies may be able to definitively identify bears that have a history of association with human food. In cases where a suspected problem bear is immediately killed on-site, fatty acid analysis may provide quantitative *post hoc* evidence of previous conflict behavior.

Although partially-hydrogenated *trans* fatty acids have been replaced in some processed foods, *trans* fatty acids are still abundant in many products (Albers et al. 2008). As long as these synthetic dietary compounds are present in human foods and garbage, they may remain a useful marker of anthropogenic food use by bears and other wildlife. In addition to improving the accuracy with which food-associated animals are identified for rehabilitation or removal from the population, further study of *trans* fatty acids in free-ranging animals may provide important insights into the demographic, ecological, and anthropogenic factors that contribute to human–wildlife conflicts.

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