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Tracing geographic and temporal trafficking patterns for marijuana in Alaska using stable isotopes (C, N, O and H)

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ABSTRACT

A large proportion of Alaska Bureau of Alcohol and Drug Enforcement's time is spent controlling the production and distribution of marijuana. Marijuana in Alaska can originate from within (e.g., Fairbanks and the Matanuska-Susitna Valley) or from outside Alaska (e.g., Latin America, Canada and other locations in the United States of America). However it is difficult to track the supply proportions from various potential geographic areas in remote areas of the globe, such as Alaska. This is due to an insufficient ability to trace the source regions from which confiscated marijuana was originally grown. We analyzed multiple stable isotopes (C, N, O and H) in marijuana confiscated in Alaska, to identify the likely geographic source from which the marijuana originated. Fifty-six of the marijuana samples were from known grow locations in Alaska. These samples exhibited stable oxygen and hydrogen isotope ratios (δ^{18} O and δ D) of 10.4‰ to 37.0‰ and -203.1‰ to -136.7‰, respectively. Stable carbon and nitrogen isotope ratios (δ^{13} C and δ^{15} N) of the same samples ranged from -53.8% to -26.4% and -12.5% to 12.1%, respectively. We use these data to compare with stable isotope analyses of marijuana confiscated in Alaska, but from unknown grow locations, which were found to have δ^{18} O and δ D ranging from 10.0% to 34.5% and -214.6% to -107.5%, respectively. The large range of data suggests that the samples originated from multiple sources ranging from low to high latitudes. A large range in δ^{15} N values from the samples was also evident (-5.0% to 14.7%). Most intriguing of all was the unexpected large range in the stable carbon isotope compositions of the samples (-61.8% to -24.6%). Twelve of the samples were found to have an exceedingly low δ^{13} C values (-36.1% to -61.8%) compared to typical δ^{13} C values of other plants using C3 photosynthesis. Interior growing conditions (e.g., hydroponic and green house) and a variety of CO₂ sources (e.g., CO₂ from tanks and fermentation CO₂ generators) supplied to growing marijuana (in addition to atmospheric CO_2) that are sometimes used to improve marijuana yields likely account for these exceptionally low δ^{13} C values. Our project has implications for monitoring broad scale trafficking patterns over time in relatively remote regions such as Alaska, where the resources allocated for law enforcement must be utilized over a wide geographic area.

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1. Introduction

Marijuana confiscated in Alaska is known to originate either from within the state (e.g., Fairbanks and the Matanuska-Susitna Valley) [1] or from numerous areas outside the state (e.g., Latin America, Canada and the contiguous United States) [2]. However, the supply proportions from different geographic areas are not well known. This is due to an insufficient ability to trace source regions from which confiscated marijuana was originally grown.

Stable isotope techniques are commonly utilized in environmental studies, and can provide information regarding biogeochemical cycles, environments, and migration patterns of animals and people [3–7]. The stable isotopic signatures preserved in plants and animals can reveal valuable information regarding how and where the material was synthesized [8–10]. Stable carbon and nitrogen isotope analytical techniques have been employed as forensic tools for identifying the source of marijuana [11–14]. However, stable oxygen and hydrogen isotopes in marijuana have not to date been conducted.

Stable carbon isotope ratios of plants, expressed as δ^{13} C values, are primarily related to the photosynthetic pathway used by a plant [15]. A range of δ^{13} C values have been reported for marijuana from the United States, Mexico, Colombia, and Brazil (\sim -31‰ to -24‰) [11-14], which are typical for a plant using the C₃ photosynthetic pathway [16]. The primary factor controlling the δ^{13} C of C₃ plants is the carbon isotopic

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composition of the atmospheric CO₂ used by plants during photosynthesis [17,18]. The δ^{13} C of atmospheric CO₂ is essentially uniform at a global scale, and fractionation associated with C₃ photosynthesis accounts for the difference between atmospheric CO₂ and C₃ plant biomass. The slight change in the δ^{13} C of atmospheric CO_2 (-0.1 to -0.2% per decade) due to the burning of fossil fuels can be taken into account when analyzing archived samples from the early 1900s to the present day [19,20]. Variation in the δ^{13} C of atmospheric CO₂ can also occur at a local/ habitat scale [21] with lower δ^{13} C values (as low as -16.4‰) being attributed to a canopy effect [21]. Variation in the δ^{13} C of C₃ plants beyond the influence of the δ^{13} C of atmospheric CO₂ can be related to local growing conditions and plant physiological features such as stomatal conductance and water-use-efficiency [18]. In essence, C₃ plants growing in dry conditions can tend to have higher δ^{13} C values compared with plants growing in wetter conditions [16,22]. For example, a range of δ^{13} C values have been reported for marijuana plants collected from various source locations in Brazil [11-13]. Samples of marijuana from humid regions were found to have δ^{13} C values in the range of -32% to -25%, while those from dry regions were in the range of -28%to -25‰ [11-13].

The stable nitrogen isotope ratios of plants, expressed as δ^{15} N values, can be related to a number of factors, including water stress, nitrogen limitation, and the δ^{15} N of the nitrogen sources available [23]. Broadly speaking, organic fertilizers tend to have a higher δ^{15} N (+10 to +20‰), compared with industrial fertilizers (-4 to +4‰) [24–26]. Most plants have δ^{15} N values ranging between -5 to +2‰ [27]. Marijuana from various source locations in Brazil exhibit a range of δ^{15} N from ~10‰ to ~-4‰ [11,13]. Some low δ^{15} N values (e.g., below ~2‰) were attributed to marijuana cultivation in nutrient-poor soils, where nitrogen must be supplied either through industrial fertilizers, or with green manure, i.e., N-fixing plants such as legumes [11,13].

The stable oxygen and hydrogen isotope composition of plant tissues can be related to the water that was used to synthesize those tissues [28,29]. Plants, such as marijuana, take up water, some of which is involved in plant biochemical pathways (e.g., photosynthesis) and subsequently becomes part of a plant's chemical composition [28,30]. The stable isotopic composition of a plant can therefore retain an isotopic 'fingerprint' of the water that was supplied to it when it was growing [28]. These 'isotopic fingerprints' can subsequently be related to geographic locations based on the geographic pattern of oxygen and hydrogen isotopes in water, as global patterns of stable oxygen and hydrogen isotope in precipitation [31] are known to influence the stable isotopic composition of domestic water supplies [32-34]. For example, the δ^{18} O of drinking water in Fairbanks ($\sim -19\%$) is significantly lower than that of tap water in New York ($\sim -8.5\%$) [7]. To date we are not aware of any δ^{18} O and δ D data derived from measurements of marijuana.

We aimed to examine: (1) whether marijuana confiscated from different known grow locations within Alaska have different isotopic signatures, (2) whether the oxygen and hydrogen isotope composition of marijuana from different known grow locations within Alaska reflect the isotopic composition of the domestic water supply at a location, (3) how marijuana confiscated in Alaska from unknown origins compares with marijuana isotopic data from known locations in Alaska (this study) and other locations described in the published literature, and (4) whether the source of production of marijuana confiscated in Alaska has changed over multiple years in the recent past (2003–2008). To achieve these aims we analyzed a suite of marijuana samples from known grow locations in Alaska to complement stable isotope data derived from analyses of marijuana samples confiscated in Alaska from unknown origins.

2. Methods

2.1. Sample acquisition

Samples of marijuana plants confiscated in Alaska were supplied to the University of Alaska Fairbanks (UAF), Alaska Stable Isotope Facility (ASIF) via a chain of custody involving the University of Alaska Fairbanks Police Department. The chain of custody and analytical protocols were authorized by the Drug Enforcement Administration (DEA) and a record of the registration (DEA registration number RW0324551) is held on record with the UAF Office of Research Integrity. The ASIF analytical procedures resulted in the total destruction of the supplied sample material. Fifty-six of the marijuana samples were confiscated from grows of known origin in Alaska and in many of these cases (n = 23) a water sample was also collected from the location. These samples of known grow locations came from sites that we group as Interior Alaska (including the towns of Fairbanks, North Pole, and Two Rivers), and Southcentral Alaska (including the towns of Girdwood, Kasilof, Anchor Point, Kenai, Sterling, Nikiski, Soldotna, Ninilchik, and Homer) (Fig. 1). The majority (n = 122) of the marijuana samples were confiscated in Alaska but were from unknown origin (i.e., confiscated during trafficking).

2.2. Stable isotope analysis

The stable carbon, nitrogen, oxygen and hydrogen isotope composition of the total organic content of the marijuana samples collected were conducted at the ASIF. All samples were dried in the laboratory for at least 5 days prior to analyses. Because samples were not always homogeneous, every effort was made to analyze the most representative composition for each sample. Subsamples were taken from each marijuana sample for oxygen and hydrogen isotope analyses, and stable carbon and nitrogen isotope analyses were conducted on the remaining material. Analyses of all samples were conducted in (at least) triplicate to allow statistical comparisons and to assess sample precision. The process involved weighing (using a microbalance – Elemental Microanalysis Limited, Devon, UK) small quantities $(\sim$ 300–400 μ m) of marijuana into a tin capsule. Stable isotope ratios are reported in standard δ notation, as parts per thousand (‰) relative to Vienna Pee Dee Belemnite (VPDB) for carbon, atmospheric air (AT-air.) for nitrogen, and Vienna Standard Mean Ocean Water (VSMOW) for oxygen and hydrogen. The stable carbon and nitrogen isotopic compositions were determined by combusting the samples in a Costech ESC 4010 elemental analyzer interfaced via a ThermoConflo III to a Thermo Delta+XP IRMS. For δ^{13} C and δ^{15} N analytical precision (defined here as one standard deviation derived from the results of running multiple standards throughout the run) was determined by conducting multiple analyses (n = 29) of a homogenous peptone standard during the run, and was 0.14 for δ^{13} C and 0.28 for δ^{15} N.

For δ^{18} O and δ D analysis of marijuana, the protocol was consistent with previously published protocols for the analysis of organic samples [35], where each sample was analyzed using a Thermo Thermal Conversion Elemental Analyzer (TC/EA) attached via a Conflo III to a Thermo Delta V Plus IRMS. We acknowledged the issues related to hydrogen exchange as described by others [36]. In this regard we adopted a method consistent with that outlined by others [36], where samples were "air equilibrated" with ambient laboratory air moisture in the ASIF at room temperature for >96 h prior to δ D analysis. After >96 h of comparative equilibration, all samples and standards were loaded into the auto sampler carousel of the TC/EA-IRMS system described above. Analytical precisions (1 σ) based on multiple (n = 10) analyses of benzoic acid (Fisher Scientific, Lot No



Fig. 1. Locations in Alaska referred to in this study.

947459) analyzed during the run of samples were 0.16 for δ^{18} O and 1.50 for δ D analysis.

Water samples were collected from some of the marijuana grow locations by law enforcement officers and supplied to ASIF. In some cases, this water came directly from a tap; in other cases, the water was sampled from a watering container adjacent to the live plants. These "grow waters" were analyzed for $\delta^{18}O$ and δD in triplicate using the TC/EA-IRMS described above and following the protocols described in detail elsewhere [7]. Analytical precision (1 σ) was 0.2‰ and 2‰ for $\delta^{18}O$ and δD respectively. All $\delta^{18}O$ and δD values for water are reported in per mil (‰) relative to VSMOW.

3. Results

3.1. Stable isotope analyses of marijuana samples from known origins

A total of fifty-six marijuana samples from grow operations uncovered by police officers around Alaska (i.e., known origin) were supplied to ASIF. The carbon and nitrogen isotopic compositions of these samples showed a large range of values (Fig. 2). δ^{13} C ranged from -54% to -26%, while δ^{15} N ranged between -13%and +12. Samples grown in interior Alaska generally exhibited more negative $\delta^{15}N$ and $\delta^{13}C$ signatures, while samples from southcentral Alaska were higher in both δ^{15} N and δ^{13} C (Fig. 2). The δ^{18} O and δ D of the marijuana from known origins also displayed distinct geographic signatures (Fig. 3). The mean δ^{18} O of samples from interior Alaska was 14.9‰ (1σ = 3.2‰), and the mean δ D was -174.4% (1 σ = 15.1‰). Marijuana samples from southcentral Alaska exhibited distinctly higher mean values for δ^{18} O and δ D: 25.2‰ (1 σ = 5.8‰) and -158.2‰ (1 σ = 14.2‰) respectively (Fig. 3). Oxygen and hydrogen analyses were performed on separate subsamples from those used for carbon and nitrogen analyses. In some cases, sufficient sample material was not available for the secondary (carbon and nitrogen) analysis. This, along with occasional autosampler malfunctions, is the cause for slight differences in the number of data points between δ^{18} O versus δD plots and $\delta^{13}C$ versus $\delta^{15}N$ plots (i.e., Figs. 2 and 3). These minor gaps in our dataset are apparent in Table 1.

3.2. Stable isotope analyses of site water

In cases where a water sample accompanied a known-origin marijuana sample, we analyzed the water for δ^{18} O and δ D. These



Fig. 2. Carbon and nitrogen isotopic composition of marijuana samples with known grow locations. Data are grouped by geographic region. Solid lines indicate the threshold for indoor versus outdoor-grown samples [15], and organic versus inorganic fertilizers [37]. Dotted lines represent the median values for nitrate and ammonia fertilizers [42].



Fig. 3. Oxygen and hydrogen isotopic composition of marijuana samples with known grow locations. Data are grouped by geographic region.

data are shown, grouped by geographic region, in Fig. 4. The mean δ^{18} O and δ D for southcentral Alaska samples were -14.1% ($1\sigma = 3.3\%$) and -112.2% ($1\sigma = 18.3\%$) respectively. The mean δ^{18} O and δ D for interior Alaska samples were -20.8 ($1\sigma = 2.1\%$) and -160.41 ($1\sigma = 9.7\%$), respectively. A line drawn through these points yields an equation of δ D = $7.42 \times \delta^{18}$ O – 7.67. Analyses that plot off this line likely reflect evaporative effects, which might be a result of water storage in an open container or basin at the grow location, for some period of time prior to sample collection.

3.3. Stable isotope analyses of marijuana samples seized from unknown origins

The δ^{15} N values of marijuana samples of unknown origin ranged from -5% to 15‰, with a mean value of $\sim 2\%$ (Fig. 5). Variation within a sample was typically small, and less than analytical precision. The δ^{13} C of the marijuana showed a large total range from -62% to $\sim -25\%$. δ^{13} C, δ^{15} N, δ^{18} O and δ D values for all samples, along with location (if known) are tabulated in Table 1. The low δ^{13} C values (i.e., <-35%) were not solely found from analyses of samples with consistently high or low δ^{18} O. Very low δ^{13} C values were more commonly found in marijuana plants with a δ^{18} O of between 15‰ and 21‰. The two samples with the very lowest δ^{13} C values (-52‰ and -62‰) had δ^{18} O values of 21‰ and 19‰. The δ^{18} O and δ D of marijuana samples of unknown origins ranged from $\sim 10\%$ to $\sim 35\%$, while the δD of the samples ranged from $\sim -215\%$ to -108% (Fig. 6). Some of the samples showed considerable within-sample variability (up to 11%) in terms of δ^{18} O relative to other marijuana samples in the collection and analytical precision. Considerable δD variability was also evident for some of the samples (up to $\sim 40\%$). This was possibly due to heterogeneities within the sample.

3.4. Marijuana trafficking patterns over time

Fig. 7 shows isotopic trends in marijuana of unknown origin, confiscated by police from 2003 to 2008 in the Alaska. The isotopic composition of marijuana appears to be relatively constant over the past 5 years, with some notable exceptions. The carbon isotope record exhibits consistently low values over the 2003–2006 period, trending toward higher δ^{13} C in recent years, with the 2007 and 2008 populations showing significantly different mean δ^{13} C relative to previous years (Fig. 7a). δ^{15} N, meanwhile, displays a very slight trend toward lower values, particularly during the years

Table 1

Oxygen, hydrogen, carbon and nitrogen isotopic compositions of all marijuana samples analyzed for this study. Source location, if known, is indicated. n.d. = no data available.

ASIF ID	Source location	δ^{18} O (‰)	1σ stdev (‰)	δ^2 H (‰)	1σ stdev (‰)	δ^{13} C (‰)	1σ stdev (‰)	δ^{15} N (‰)	1σ stdev (‰)
06-023	Anchor point	22.67	1 95	-168 39	1.80	_28 33	0.31	0.11	0.27
05-0401 (M1)	Fairbanks	n.d.	n.d.	n.d.	n.d.	-29.24	0.40	0.66	0.76
06-016	Fairbanks	14.48	0.85	-168.44	1.77	-34.84	1.38	0.35	0.09
06-022	Fairbanks	16.12	1.98	-203.06	5.20	-31.47	0.24	-1.36	0.58
07-001	Fairbanks	21.73	2.06	-166.28	8.89	-27.46	0.69	-0.23	0.30
08-031	Fairbanks	13.30	2.01	-176.68	3.08	-40.20	0.10	-2.36	0.14
08-034	Fairbanks	14.32	11.0. 3 23	-105.40	5.14 1.23	-28.88	0.14	0.05	0.04
09-020	Fairbanks	17.03	2.36	-172.18	15.04	-47.39	0.62	0.44	0.67
09-022	Fairbanks	18.62	1.59	-198.65	9.13	-41.37	0.13	-0.23	0.26
09-023	Fairbanks	19.93	1.01	-171.38	1.80	-35.13	0.16	4.02	0.23
09-034	Fairbanks	13.89	1.69	-162.07	2.00	-33.16	0.03	8.15	0.05
09-039	Fairbanks	17.92	2.08	-159.45	2.06	-27.87	0.11	9.88	0.10
09-040	Fairbanks	14.11	0.71	-166.88	5.89	-34.41	0.73	6.30 8.04	0.01
09-044	Fairbanks	17.84	4.21	-165.66	7 84	-27.04 -40.21	0.55	2 31	0.62
09-047	Fairbanks	21.23	0.45	-151.34	3.00	-30.63	0.45	-12.51	0.92
10-14-05 (M1)	Fairbanks	19.41	0.37	-169.20	2.79	-35.80	0.42	1.96	0.55
05-88442	Fairbanks	17.92	4.25	-174.97	7.23	-40.59	0.28	-6.01	0.64
Pete's Steese	Fairbanks	19.35	0.90	-155.08	1.59	-25.82	0.37	0.96	0.36
06-020	Girdwood	17.49	0.87	-161.12	10.00	-41.62	0.39	7.12	0.31
08-029	Kasilof	34.51 19.95	1.13	-154.41 -188.18	3.22	-31.25	0.04	1.35	0.91
06-027	Kenai	29 55	3.21	-159.18	8 12	-39.95	0.45	-0.05	0.55
07-029	Kenai	30.93	1.95	-147.88	1.50	-26.38	0.20	0.46	0.09
08-021	Kenai	n.d.	n.d.	n.d.	n.d.	-35.45	0.17	7.81	0.47
08-030	Kenai	36.99	1.93	-136.69	3.54	-27.94	0.22	10.22	0.71
09-017	NE of fairbanks	18.04	2.13	-197.25	1.36	-32.72	0.51	2.11	0.20
06-026	Nikiski	24.38	0.86	-173.52	7.64	-31.44	0.23	0.55	0.79
07-045 08-022	Nikiski	24.99 n.d	2.01 n.d	-138.32 nd	6.09 n d	-28.10 -31.74	0.15	3.98	0.08
08-025	Nikiski	n.d.	n.d.	n.d.	n.d.	-35.06	0.53	-0.02	0.50
08-026	Nikiski	n.d.	n.d.	n.d.	n.d.	-53.83	1.01	-4.21	0.77
08-028	Nikiski	28.73	0.99	-146.14	2.07	-35.21	0.13	1.89	0.35
07-047	Ninilchik	19.13	3.34	-152.19	2.28	-33.08	0.08	12.08	0.30
08-024	No info	n.d.	n.d.	n.d.	n.d.	-33.63	2.34	0.44	1.11
06-017	North pole	14.72	0.67	-188.57	1.50	-48.18	0.11	-0.87	0.19
08-036	North pole	12.64	0.12	-100.93 -199.29	4 87	-43.32	0.00	2.27	0.00
09-018	North pole	17.58	0.79	-191.05	0.41	-34.85	0.24	3.96	0.40
09-046	North pole	15.44	1.47	-194.79	4.26	-36.94	0.36	6.57	0.62
07-051	Soldotna	22.16	1.05	-153.71	3.64	-29.37	0.67	1.94	0.41
08-023	Soldotna	n.d.	n.d.	n.d.	n.d.	-30.04	0.28	3.81	0.18
08-027	Soldotna	26.73	0.97	-163.88	2.91	-32.04	2.18	1.14	1.33
08-028	Sterling	22.08	0.74	-170.20	0.02	-32.33	0.17	5.17 4.11	0.61
09-019	Tanacross	12.69	1.60	-205.99	4.79	-32.25	0.15	6.64	0.43
09-032	Tok	11.42	1.06	-171.75	8.04	-40.77	0.13	1.52	0.31
09-033	Tok	11.73	1.06	-164.01	2.08	-41.53	0.18	1.98	0.15
09-035	Tok	11.56	0.25	-162.28	4.28	-39.73	0.16	1.43	0.29
09-036	Tok	10.39	0.29	-170.49	6.97	-39.37	0.22	5.53	6.68
09-037	Tok	11.25	0.72	-157.07	11.81	-42.47	0.16	2.07	0.10
09-041	Tok	13.92	0.56	-163.61	2.31	-38.56	0.07	1.25	0.15
09-042	Tok	11.17	0.55	-170.01	5.41	-41.22	0.05	1.65	0.20
09-043	Tok	11.07	1.42	-168.62	6.39	-39.64	0.40	1.93	0.27
Two Rivers 8-18-05	Two rivers	20.04	1.18	-155.15	11.75	-25.04	0.66	4.18	0.57
03-0410 2701	Unknown	15.41	0.64	-172.82	6.35	-49.58	0.25	5.23	0.09
03-05/4 2/01	Unknown	22.72	2.22	-181.74	9.58	-35.34	0.29	1.47	0.23
03-0738 2302	Unknown	20.70	2.08	-107.45 -139.48	6.94	-31.40 -44.08	0.20	2.64	0.18
03-0738 2313	Unknown	26.87	3.37	-138.44	14.65	-33.56	0.81	3.08	0.47
03-0831 2302	Unknown	11.93	0.20	-168.89	4.51	-32.66	0.71	2.41	0.14
04-0255 2304	Unknown	12.95	0.65	-166.53	2.51	-36.60	1.91	2.00	0.29
04-0328 2602	Unknown	18.65	1.03	-170.18	2.67	-34.53	0.34	4.44	0.25
04-0397 2602	Unknown	22.98	0.03	-172.81	20.86	-29.72	0.18	1.88	1.10
04-0001 2301	Unknown	26.02	3.45	-180.69	4 31	-40./4 _33.73	0.72	3.44 0.08	1.13
05-0240	Unknown	16.75	2.11	-152.37	5.53	-33.41	0.28	7.14	0.44
05-0252 2301	Unknown	14.17	1.70	-154.05	1.07	-33.47	0.13	4.47	0.22
05-0260	Unknown	10.36	1.49	-158.11	5.86	-39.21	0.37	2.25	0.32
05-0297	Unknown	20.27	2.32	-140.58	4.47	-36.74	0.30	-0.06	0.71
05-0481	Unknown	15.64	1.62	-183.92	7.38	-29.36	1.04	0.32	0.51
05-0481 10-18-05	Unknown	10.00	0.86	-183.57	0.95 p.d	-31.16	0.52 n.d	-1.07	0.40 p.d
05-0534 05-0534 n/a	Unknown	19.08 22.20	0.46	-185.59 -202.62	n.a. 1.02	-30.40 -29.17	n.a. 0 39	4.00 2.05	n.a. 0.69

Table 1 (Continued)

ASIF ID	Source location	δ^{18} O (‰)	1σ stdev (‰)	$\delta^2 { m H}~(\infty)$	1σ stdev (‰)	δ^{13} C (‰)	1σ stdev (‰)	δ^{15} N (‰)	1σ stdev (‰)
05-0540	Unknown	15.76	3.49	-193.56	5.35	-34.70	0.18	9.42	0.89
05-0584 2303	Unknown	19.21	0.44	-173.77	5.98	-26.50	0.27	14.71	0.12
05-0592 2001	Unknown	19.27	0.81	-167.00	2.95	-61.83	0.37	0.01	0.60
05-0595 2505	Unknown	21.86	0.57	-152.54	12.81	-32.47	0.93	0.77	0.41
06-0017 2802	Unknown	13.83	0.62	-147.03	5.20	-44.93	0.16	1.51	0.13
06-0017 2802	Unknown	23.01	0.27	-149.08 -171.71	21.36	-39.55	0.05	_1 99	0.00
06-0027 2804	Unknown	20.55	0.24	-139.85	10.84	n.d.	n.d.	n.d.	n.d.
06-003	Unknown	15.47	0.99	-167.58	0.31	n.d.	n.d.	n.d.	n.d.
06-004	Unknown	19.05	0.49	-170.36	2.29	-33.99	n.d.	-0.13	n.d.
06-005	Unknown	15.08	0.75	-190.86	10.20	-30.40	0.35	3.34	0.26
06-006	Unknown	20.40	0.76	-150.93	7.94	-29.96	0.61	2.15	0.43
06-007	Unknown	20.34	1.06	-146.76	1.92	-31.90	0.99	2.34	0.05
06-008	Unknown	22.39	1.84	-149.42	24.60	-33./3	0.13	1.37	0.27
06-010	Unknown	14.45	2.54	-200.49	2.81	-30.80	0.00	2.50	0.00
06-011	Unknown	18.39	0.40	-145.93	2.31	-34.25	0.17	-0.48	0.33
06-012	Unknown	21.07	3.55	-148.22	2.91	-27.97	0.09	0.91	0.05
06-013	Unknown	17.33	1.71	-191.58	3.45	-39.97	0.60	1.70	0.09
06-014	Unknown	21.35	1.09	-154.32	3.04	-30.74	0.38	2.36	0.21
06-019	Unknown	12.24	1.76	-186.85	4.84	-45.09	0.22	10.06	0.48
06-024	Unknown	22.54	6.10	-181.35	4.67	-34.18	0.00	-2.76	0.00
06-025	Unknown	18.20 24.37	1.24	-166.69 -155.81	2.55	-37.92	0.29	-0.94	0.48
06-030	Unknown	17.26	1.01	-175.35	9.91	-40.69	0.00	5.13	0.00
07-003	Unknown	24.20	0.85	-149.46	0.97	-29.78	0.35	1.80	0.31
07-004	Unknown	20.43	1.55	-157.58	2.93	-33.72	0.49	1.31	0.87
07-006	Unknown	20.85	2.40	-164.99	6.28	-28.15	0.00	3.75	0.00
07-007	Unknown	16.65	1.66	-162.97	8.77	-32.60	0.57	2.56	0.32
07-008	Unknown	17.16	1.05	-181.25	7.85	-42.35	0.61	2.72	0.33
07-009	Unknown	23.72	1.60	-146.26	0.34 2.20	-34.33	0.20	-0.45	0.26
07-010	Unknown	18 97	1.74	-140.88 -180.18	1.84	-30.32	0.35	0.15	0.09
07-012	Unknown	21.01	2.00	-158.46	8.50	-34.24	0.32	2.67	0.40
07-013	Unknown	16.41	0.38	-182.40	5.21	-29.97	0.59	0.48	0.11
07-014	Unknown	21.60	0.85	-162.70	4.91	-33.74	0.22	1.29	0.11
07-015	Unknown	17.33	0.30	-168.26	1.83	-38.60	0.14	1.09	0.35
07-016	Unknown	24.53	0.56	-159.18	3.70	-29.42	n.d.	2.24	n.d.
07-017	Unknown	26.43	0.04	-155.34	3.37	-27.22	0.69 p.d	5.98 p.d	0.11 nd
07-018	Unknown	19.00	1.62	-155.60	15 10		n.d.	1 34	n.d.
07-020	Unknown	21.89	0.81	-160.23	2.88	-33.20	0.53	3.61	0.13
07-021	Unknown	21.24	0.55	-132.49	0.62	-52.39	0.06	1.33	0.07
07-022	Unknown	18.89	0.56	-174.25	8.48	-30.35	0.16	1.72	0.32
07-023	Unknown	17.11	1.14	-167.59	0.64	-30.34	0.25	3.20	0.46
07-024	Unknown	26.09	0.87	-139.75	1.26	-28.39	0.51	1.72	0.18
07-025	Unknown	20.38	1.70	-145.96	1.12	-26.79	0.62	2.23	0.12
07-020	Unknown	21.31	1 70	-149.01 -149.30	0.87	-32.47	0.05	0.80	0.09
07-028	Unknown	20.41	1.34	-145.33	10.27	-27.31	n.d.	0.04	n.d.
07-030	Unknown	21.69	0.70	-136.63	5.70	-30.37	0.24	0.25	0.11
07-031	Unknown	16.72	0.42	-172.05	3.48	-40.17	0.17	2.59	0.25
07-032	Unknown	21.46	0.79	-134.53	4.88	-37.17	0.45	0.59	0.13
07-033	Unknown	25.39	1.89	-162.08	1.47	-31.54	0.36	1.20	0.15
07-034	Unknown	20.80	2.00 4.53	-151.79 -161.51	1.52	-28.80 -29.28	0.30	1.94	0.01
07-036	Unknown	24.28	1.31	-135.28	3.37	-40.13	n.d.	3.24	n.d.
07-037	Unknown	22.20	3.34	-160.47	5.00	-30.37	0.65	1.62	0.56
07-038	Unknown	17.99	0.42	-143.10	3.74	-41.21	0.51	1.97	0.27
07-039	Unknown	23.31	1.76	-156.51	17.73	-34.44	1.32	2.93	0.17
07-040	Unknown	23.04	6.15	-145.13	7.86	-27.69	0.76	1.94	0.34
07-041	Unknown	18.33	1.90	-168.18	6./2 2.22	-36.68	n.a. 0.42	-2.88	n.d. 0.27
07-042	Unknown	17.33	0.47	-165.09	6 36	-34.26	0.43	-4 95	0.06
07-044	Unknown	15.66	0.40	-164.28	5.01	-40.29	0.69	2.53	0.34
07-046	Unknown	16.34	0.42	-153.14	5.47	-29.97	0.64	1.78	0.34
07-048	Unknown	22.44	0.28	-138.00	1.62	-28.98	0.71	1.82	0.14
07-052	Unknown	11.38	0.48	-174.45	0.88	n.d.	n.d.	n.d.	n.d.
08-001	Unknown	11.11	1.11	-162.45	8.02	-39.74	0.25	3.17	0.29
08-002	Unknown	20.96	0.17	-184.43	8.79	-36.74	n.d.	3.33	n.d. 0.62
08-004	Unknown	22.48	1.11	-148.83	2.98	-20.00	0.51	3.80	0.15
08-005	Unknown	20.31	1.95	-152.40	16.72	-29.20	0.61	1.58	0.45
08-006	Unknown	14.20	1.09	-179.42	0.53	-25.75	0.30	4.64	0.28
08-007	Unknown	n.d.	n.d.	n.d.	n.d.	-30.87	0.59	2.61	0.17
08-008	Unknown	n.d.	n.d.	n.d.	n.d.	-26.45	0.76	1.61	0.38
08-009	Unknown	15.03	1.15	-185.71	7.71	-43.54	0.76	1.03	0.67

Table 1 (Continued)

ASIF ID	Source location	δ^{18} O (‰)	1σ stdev (‰)	$\delta^2 \mathrm{H}~(\infty)$	1σ stdev (‰)	δ^{13} C (‰)	1σ stdev (‰)	δ^{15} N (‰)	1σ stdev (‰)
08-010	Unknown	20.04	0.84	-157.56	2.34	-32.05	0.37	1.76	0.35
08-011	Unknown	n.d.	n.d.	n.d.	n.d.	-32.74	0.08	0.45	0.43
08-012	Unknown	n.d.	n.d.	n.d.	n.d.	-33.50	0.22	-1.51	0.55
08-013	Unknown	n.d.	n.d.	n.d.	n.d.	-30.63	0.19	3.17	0.23
08-014	Unknown	n.d.	n.d.	n.d.	n.d.	-32.66	0.20	-1.61	0.30
08-015	Unknown	n.d.	n.d.	n.d.	n.d.	-27.12	0.33	1.44	0.25
08-016	Unknown	n.d.	n.d.	n.d.	n.d.	-27.92	0.30	1.79	0.57
08-017	Unknown	n.d.	n.d.	n.d.	n.d.	-39.31	0.04	1.96	0.19
08-018	Unknown	n.d.	n.d.	n.d.	n.d.	-35.10	0.73	5.38	0.64
08-019	Unknown	n.d.	n.d.	n.d.	n.d.	-40.75	1.28	1.76	0.54
08-020	Unknown	26.64	1.05	-164.73	4.82	-32.39	0.26	0.88	0.16
08-032	Unknown	19.48	2.76	-184.14	0.26	n.d.	n.d.	n.d.	n.d.
08-038	Unknown	25.21	0.23	-107.47	10.48	-25.82	0.15	1.09	0.21
08-039	Unknown	18.86	1.84	-154.71	4.37	-37.08	0.22	2.56	1.67
08-040	Unknown	20.57	1.09	-158.07	23.63	-28.64	0.28	0.14	0.60
08-041	Unknown	19.23	2.07	-158.82	6.63	-26.64	0.37	0.86	0.13
08-042	Unknown	17.97	2.60	-194.60	14.91	-30.07	0.11	3.62	0.22
08-043	Unknown	n.d.	n.d.	n.d.	n.d.	-31.98	0.26	1.44	0.42
08-044	Unknown	n.d.	n.d.	n.d.	n.d.	-33.60	0.19	-0.36	0.25
08-045	Unknown	n.d.	n.d.	n.d.	n.d.	-30.97	0.37	3.69	0.43
08-046	Unknown	n.d.	n.d.	n.d.	n.d.	-24.62	0.42	6.43	0.26
08-047	Unknown	n.d.	n.d.	n.d.	n.d.	-33.97	0.12	-0.09	0.04
09-001	Unknown	18.19	2.88	-154.83	20.98	-31.25	0.29	2.54	0.40
09-002	Unknown	16.80	1.44	-153.69	2.43	-33.69	0.13	2.30	0.05
09-003	Unknown	16.72	1.53	-138.21	7.74	-44.62	0.96	2.15	0.16
09-004	Unknown	13.24	1.36	-202.52	4.63	-31.86	0.72	0.75	0.30
09-005	Unknown	16.92	2.36	-148.69	18.39	-29.19	0.19	2.02	0.91
09-006	Unknown	22.24	3.76	-189.37	9.01	-48.83	0.43	-2.48	0.24
09-007	Unknown	28.09	2.18	-151.37	12.24	-27.36	0.34	-0.25	0.51
09-008	Unknown	12.46	2.94	-214.60	8.35	-33.65	0.26	2.05	0.40
09-009	Unknown	17.51	2.84	-211.71	15.32	-30.38	0.26	1.98	0.01
09-010	Unknown	13.12	1.42	-195.82	6.15	-30.16	0.32	6.36	0.33
09-011	Unknown	22.61	2.64	-189.67	11.65	-28.47	0.40	3.15	0.25
09-012	Unknown	n.d.	n.d.	n.d.	n.d.	-37.87	0.07	-0.32	0.23
09-013	Unknown	n.d.	n.d.	n.d.	n.d.	-29.19	0.23	7.41	0.84
09-014	Unknown	21.80	0.26	-161.54	8.32	-38.81	0.72	1.26	0.05
09-024	Unknown	24.26	3.57	-128.58	4.57	-32.05	n.d.	8.68	n.d.
09-025	Unknown	24.16	4.60	-158.09	7.67	-32.53	0.32	2.05	0.07
09-026	Unknown	19.79	0.45	-127.62	4.30	-45.72	0.44	0.64	0.04
09-027	Unknown	22.44	1.59	-142.99	4.39	-26.37	0.75	2.26	0.50
09-028	Unknown	24.60	2.35	-128.71	6.40	-32.69	0.35	1.03	0.41
09-029	Unknown	22.64	1.39	-182.21	9.33	-37.49	0.23	2.77	0.27
09-030	Unknown	22.47	1.45	-143.66	2.11	-34.27	0.04	-0.35	0.54
09-031	Unknown	23.37	3.25	-153.92	16.22	-36.39	0.37	0.71	0.35
9760DD	Unknown	19.14	0.00	-119.72	0.00	-42.14	0.18	3.24	0.18
05-96825 12-21-05	Unknown	21.79	0.37	-138.39	6.14	-32.57	0.56	1.53	0.43
nc-1	Unknown	19.77	0.39	-173.41	5.53	-32.95	0.26	0.99	0.62
nc-2	Unknown	34.53	0.37	-165.81	3.10	-31.12	0.31	2.22	0.13



Fig. 4. Oxygen and hydrogen isotopic composition of domestic water samples, collected from locations of marijuana grows.

2006 and 2007 (Fig. 7b). The oxygen isotope record trends neither positive nor negative, and no single year is significantly different from another (Fig. 7c). The δ^{18} O instead reflects an approximately equal divide between interior and southcentral Alaskan source regions that has remained relatively constant over the past 5 years.

4. Discussion

4.1. Stable isotope analyses of marijuana samples from known origin

The large range of δ^{13} C and δ^{15} N of marijuana samples grown in Alaska suggests a wide variety of growing conditions. To place constraints on these growth conditions, our results are plotted relative to environmental parameters for marijuana isotopes from the literature [14,37]. δ^{13} C values above -29% indicate outdoor growth and values below -32% indicate indoor growth [14]. This threshold is based on theoretical models of carbon isotope fractionation in plants [15], and supported by empirical observations [38]. As would be expected for Alaska, the majority of our results plot within the indoor-grown field, with only a few samples plotting in the outdoor-grown range of δ^{13} C values (Fig. 2), which



Fig. 5. Carbon and nitrogen isotopic composition of marijuana samples of unknown origin, confiscated in Fairbanks. Solid lines indicate the threshold for indoor versus outdoor-grown samples [15], and organic versus inorganic fertilizers [37]. Dotted lines represent the median values for nitrate and ammonia fertilizers [42].

originated from southcentral Alaska. Nitrogen isotope ratios of our known-origin samples indicate the use of predominantly inorganic fertilizers ($\delta^{15}N < 7_{\infty}$; [37]). The threshold for organic versus inorganic fertilizers is based on previous work documenting the nitrogen isotope composition of marijuana plants grown with a variety of fertilizers [37]. Only two samples from our dataset, originating from southcentral Alaska, appear to have been grown using organic fertilizers such as manure. It is possible that in coastal Alaskan towns where fishing is a major industry, domestic plants such as marijuana might sometimes be fertilized using fishing waste and other marine debris, contributing to a higher plant $\delta^{15}N$ value.

 δ^{18} O and δ D for marijuana of known origin separate into distinct spatial fields when plotted by geographic source region (Fig. 3), however there is considerable variation within each region (up to 7‰ for interior Alaska samples, and up to 20‰ for southcentral Alaska samples). This variation could be a result of



Fig. 6. Oxygen and hydrogen isotopic composition of marijuana samples of unknown origin, confiscated in Fairbanks. Data points represent the average of three analyses, and error bars represent one standard deviation of the mean.

numerous factors, including: (1) Variations in chemical composition – depending on the genetics and cultivation procedures, marijuana can contain anywhere between 3% and 20% tetrahydrocannibinol (THC; U.S. DEA). THC contains approximately 10% oxygen by weight, so differences in fractionation for THCwater and cellulose-water for ¹⁸O might contribute to overall variations in bulk δ^{18} O, depending on the concentration of THC in a given sample. However, our established DEA protocol limits the processing of samples prior to analysis and we are only able to report bulk δ^{18} O measurements on marijuana, rather than cellulose δ^{18} O. (2) Different growth conditions (i.e., humidity and temperature) may also affect the leaf cellulose δ^{18} O, and hence bulk δ^{18} O of marijuana samples. The δ^{18} O and δ D of cellulose can differ by up to 5‰ and 50‰, respectively, when grown in different levels of humidity (\sim 40% versus \sim 80%), using isotopically identical source waters [39]. Because growth conditions are largely unknown for our marijuana samples, we are unable to place constraints on variables such as temperature and humidity. (3) Differences in domestic water sources may also contribute to the variation in marijuana δ^{18} O. Surface water is used for about 70% of Alaska's domestic water supply [40], leading to potential seasonality effects. The prevalence of permafrost beneath the surface of Alaska (~85% [41]) makes drilling of wells difficult, and it is not uncommon for all inhabitants of a town or community to draw their water from a single source. Consequently, residents living in remote areas often haul their water from miles away. As such, the relationship between latitude and plant δ^{18} O is not necessarily quantifiable, although it is clear that different regions within Alaska have domestic water supplies with very distinctly different isotopic compositions (Fig. 4).

4.2. Stable carbon and nitrogen isotope analyses of marijuana samples seized from unknown origin

The broad range of δ^{13} C and δ^{15} N of marijuana samples of unknown origin (Fig. 5) suggests that marijuana ultimately bought and/or sold within Alaska is grown under a variety of environmental conditions. These values are consistent with those documented from the entire USA [14], which show a range of δ^{15} N from -5% to 20% and δ^{13} C from -52% to -22%. The majority of samples appear to have been grown indoors, using industrial fertilizers (Figs. 2 and 5). The very negative δ^{13} C values displayed by some of the marijuana samples are most likely the result of the plants being grown with an additional CO₂ source. In attempting to elevate the CO₂ concentration in enclosed spaces such as green houses, basements and cupboards, to increase yields, some marijuana growers supply their plants with a source of CO₂ in addition to atmospheric CO₂. This can take the form of CO₂ generated through fermentation or from CO₂ cylinders, which have more negative δ^{13} C values compared with atmospheric CO₂. Cylinders of CO₂ are frequently found in illegal marijuana grows in Alaska (Goetz, UAF police department personal communication). Low δ^{13} C values have also been documented in other parts of the United States (-51.8%; [14]), as evidence of indoor-grown plants being supplemented with CO₂. To date, our lowest measured δ^{13} C value for marijuana is -61.8%

Within-sample variation is very small, suggesting that the range of δ^{13} C and δ^{15} N is not simply a reflection of different parts of the plant. A nearly 1:1 relationship between leaf material and inflorescence (flowering part of the plant) material, with only a small offset (~1‰) for inflorescence relative to leaves has previously been documented [14]. Minimal variation within a sample is also consistent with findings of Shibuya [11,13], documenting stable isotope analyses on marijuana in Brazil, sampled from different tissues.



Fig. 7. Carbon, nitrogen and oxygen isotopic composition of marijuana samples of unknown origin, confiscated in Fairbanks, shown versus year collected. Sample sets for each year are given a letter designation based on a *t*-test comparison between each year. Where a sample set has the same letter as another sample set, this denotes no statistical difference between the data sets ($p \ge 0.05$). Where sample sets have different letters, this denotes a significant statistical difference ($p \le 0.05$)

4.3. Stable isotope oxygen and hydrogen isotope analyses of marijuana samples seized from unknown origins

The majority of the samples analyzed from unknown origin exhibited δ^{18} O and δ D signatures that were within the range of those from known-Alaskan origin (Fig. 6). Among those, approximately half fell within the range corresponding to interior Alaska and half were in the range corresponding to southcentral Alaska. This could be indicative of several different scenarios. Firstly, most confiscated marijuana of unknown origin was grown within the state. Given Alaska's remote location this is likely. With shorter distances for marijuana to travel, transportation costs would be lower, as well as the probability of interception by law enforcement. Numerous large warehouses for marijuana grow operations have been reported in Alaska, some containing as many as 300 plants (Goetz, UAF police department personal communication). It is also possible that marijuana of unknown origin represents a mixture of Alaskan-grown and out-of-state derived plants. Accounting for the global distribution of δ^{18} O of precipitation [31,32], domestic water from other locations can have a similar isotopic composition as water from Alaska; therefore samples could even have originated from locations outside America. Even in more southerly latitudes such as Mexico, the continental and altitude effects could produce precipitation that is identical to precipitation from some places in Alaska. Moreover, the effect of humidity on δ^{18} O of cellulose [39] could produce variable plant δ^{18} O values, depending on geographic location. Consequently, it would be possible for a site in California with the proper combination of altitude, evaporation, and transpiration to produce a plant with δ^{18} O values in the same range as those found in Alaska. Analyses of marijuana samples confiscated at border control stations and from a range of other geographic locations will help to address this issue. Finally, chemical composition, as described above, may be a factor influencing the range of δ^{18} O and δ D values of marijuana of unknown origin. It is possible that marijuana grown outside Alaska has different THC content than that grown within the state, which could produce misleading δ^{18} O values for bulk measurements.

4.4. Trends in marijuana production

Our dataset is comprised of marijuana samples confiscated by local police for the past 6 years, allowing for a comparison of isotopic composition over time. Variations in the δ^{18} O, δ^{13} C, and δ^{15} N values of samples of unknown origin (Fig. 7) indicate potential trends in growth conditions and production procedures over the period from 2002 to 2008. The apparent rise in δ^{13} C could be a reflection of several factors, including sample bias where some years have more data points than others. The trend may also be reflecting a decreased reliance on supplemental CO₂ as part of growth procedures for marijuana cultivation. Because the addition of non-atmospheric CO₂ produces a plant δ^{13} C value that is depleted in ¹³C (discussed above), an increasing trend of δ^{13} C suggests this practice might have declined during our study period. Finally, the carbon isotope trend may also indicate a change in source region. Our Alaskan-grown samples show clear evidence for the use of supplemental CO₂, based on low δ^{13} C marijuana values (Fig. 2). If the source of trafficked marijuana in Alaska were shifting from dominantly indoor-grown plants to a greater percentage of outdoor-grown samples, this might also produce a δ^{13} C trend that increases through time. Meanwhile, the $\delta^{15}N$ of marijuana of unknown origin appears to decrease slightly over the period from 2002 to 2008, which may indicate an increased use of inorganic fertilizers in marijuana plant cultivation. Alternatively, this trend could reflect a shift toward a different source region for marijuana production, or might be simply an effect of sample bias, as mentioned above.

This type of temporal monitoring program of the isotope composition of marijuana, tracking changes in the isotope composition of marijuana confiscated and from unknown origins, could be set up at a number of other locations at a country or continental scale to track large scale trafficking patterns. A hypothetical shift from a trafficking pattern being supplied from Canada to one being supplied from Latin America to Colorado, for example, would be apparent in terms of the oxygen isotope composition of the marijuana. Our findings serve the additional purpose of characterizing the isotope composition of marijuana grown at a northerly extreme.

5. Conclusion

 δ^{13} C values for marijuana confiscated in Alaska suggest predominantly indoor growing conditions for samples of both known and unknown origin. Exceptionally low δ^{13} C values for some samples indicate the presence of a supplemental CO_2 source. most likely in the form of CO₂ supplied from gas cylinders. δ^{15} N values indicate that the majority of marijuana samples were grown using inorganic fertilizers, with evidence for organic fertilizers primarily restricted to more southern/coastal regions of Alaska. Marijuana grown in southcentral Alaska exhibits a distinctly higher mean δ^{18} O than that from interior Alaska, suggesting δ^{18} O of marijuana has the potential to be indicative of geography. Close correspondence between δ^{18} O and δ D of unknown and known origin marijuana isotope composition suggests there is a local connection between production and consumption of marijuana in Alaska. However, given the potential similarities in stable isotope ratios between Alaskan and non-Alaskan source water, as well as variations in plant composition (discussed above), we cannot rule out the possibility that many of these unknown samples originated from out of state. In order to fully evaluate the potential for stable isotopes of marijuana to be used as a geo-locator, a next step might include the construction of a database incorporating δ^{18} O, δ D, δ^{13} C, and $\delta^{15}N$ measurements on samples from other potential source regions such as British Columbia, California, and Mexico, Also, a comprehensive analysis of marijuana samples confiscated at border crossings into and out of Alaska would lend additional information regarding Alaskan versus non-Alaskan source regions.

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