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ORIGINAL PAPER

Fatty acid profiles of feeding and fasting bears: estimating calibration coefficients, the timeframe of diet estimates, and selective mobilization during hibernation

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Abstract

Accurate information on diet composition is central to understanding and conserving carnivore populations. Quantitative fatty acid signature analysis (QFASA) has emerged as a powerful tool for estimating the diets of predators, but ambiguities remain about the timeframe of QFASA estimates and the need to account for species-specific patterns of metabolism. We conducted a series of feeding experiments with four juvenile male brown bears (*Ursus arctos*) to (1) track the timing of changes in adipose tissue composition and QFASA diet estimates in response to a change in diet and (2) quantify the relationship between consumer and diet FA composition (i.e., determine "calibration coefficients"). Bears were fed three compositionally distinct diets for 90–120 days each. Two marine-based diets were intended to approximate the lipid content and composition of the wild diet of polar bears (*U. maritimus*). Bear adipose tissue composition changed quickly in the direction of the diet and showed evidence of stabilization after 60 days. During hibernation, FA profiles were initially stable but diet estimates after 10 weeks were sensitive to calibration coefficients. Calibration coefficients derived from the marine-based diets were broadly similar to each other and to published values from marine-fed mink (*Mustela vison*), which have been used as a model for free-ranging polar bears. For growing bears on a high-fat diet, the temporal window for QFASA estimates was 30–90 days. Although our results reinforce the importance of accurate calibration, the similarities across taxa and diets suggest it may be feasible to develop a generalized QFASA approach for mammalian carnivores.

Keywords Foraging ecology · Brown bear · Hibernation · Polar bear · QFASA

Introduction

The ability to locate and capture preferred prey is closely tied to the survival and reproductive rates of top predators (Peterson et al. 1998; Fuller and Sievert 2001; Chevallier et al. 2020). Climate warming, and other anthropogenic drivers of environmental change, can disrupt patterns of prey abundance and availability with negative consequences on predator population dynamics (Regehr et al. 2007; Northrup et al. 2012). Accurate information on diet composition and patterns of prey selection is thus central to understanding carnivore ecology and to the design and implementation of effective conservation strategies (Sierro and Arlettaz 1997; Parrish et al. 2002).

A variety of methods have been developed to estimate the diet composition of free-ranging predators, including direct observation (Stirling 1974), stomach and fecal content analysis (Barnett et al. 2010; Klare et al. 2011) and, more recently, biochemical tracer methods (Fry 2006; Budge et al.

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2006). Quantitative fatty acid signature analysis (QFASA) generates estimates of individual diets by comparing the fatty acid (FA) composition of predator and prey (Iverson et al. 2004). QFASA has emerged as an especially useful tool for estimating the diets of marine (Beck et al. 2007; Budge et al. 2012) and Arctic predators (Thiemann et al. 2008; Wang et al. 2010; Haynes et al. 2015), because of the diversity of dietary FA and high lipid content of potential prey, respectively.

Accuracy of QFASA is contingent on the use of appropriate calibration coefficients (CCs) that account for FA-specific patterns of metabolism in the predator (Meynier et al. 2010; Budge et al. 2012). CCs are calculated as simple ratios of the abundance of a given FA in the tissue of the predator relative to the abundance in the diet, after tissue-diet equilibration (Iverson et al. 2004). CCs can then be applied to either the predator or prey FA data prior to running the QFASA model (Bromaghin et al. 2015). Because metabolic patterns may be species-specific, and potentially influenced by the gross composition of the diet (Rosen and Tollit 2012), a lack of accurate and appropriate CCs can limit the utility of QFASA as an investigative tool. Bromaghin et al. (2017) developed a model that allows for simultaneous estimation of both predator diet composition and CCs using only predator and prey FA data, which may eliminate the need for empirically derived CCs. However, a single set of CCs may not be appropriate for all groups of predators in a population and a better understanding of the influence of diet composition and nutritional status (i.e., whether an animal is gaining, losing, or maintaining body mass) on CCs and diet estimates is needed to determine how estimated CCs should be applied across groups of animals.

The temporal window of QFASA diet estimates also remains uncertain. Iverson et al. (2004) assumed that the blubber of captive gray seals (*Halichoerus grypus*) reflected diet consumed over the preceding 3–5 months. Budge et al. (2004) showed that radiolabeled FA consumed in the diet were deposited in gray seal blubber within 12 h. Adipose tissue thus represents an integration of recent and long-term diet and most studies using QFASA assume that results reflect diet over a period of "weeks to months" (Beck et al. 2005; Budge et al. 2006; Galicia et al. 2015; McKinney et al. 2017). However, the ambiguity of this timeframe limits ecological insights.

The temporal window of QFASA estimates may also depend on the energy balance of the individual. Although few studies have been conducted, the rate of FA turnover in mammalian adipose tissue is likely correlated with the rate of lipid intake (Anderson et al. 1972), but will also be affected by other physiological functions such as growth and lactation (Foglia et al. 1994; Nordstrom et al. 2008). A fasting animal will mobilize stored fat, but if that mobilization is selective (i.e., some FA are preferentially mobilized or conserved; e.g., Florant et al. 1990; Hill and Florant 1999; Raclot 2003), QFASA estimates may not accurately reflect integrated diet composition.

Polar bears (*Ursus maritimus*) are wide-ranging top predators that rely on annual sea ice for access to their marine mammal prey (Stirling and McEwan 1975; Stirling and Archibald 1977; Thiemann et al. 2008). Polar bear population dynamics have been negatively affected by climate warming, primarily mediated by disruptions in prey availability (Derocher et al. 2004; Regehr et al. 2007; Lunn et al. 2016; Pagano et al. 2018). Thus, accurate information on polar bear diet composition is central to understanding the ecological effects of Arctic climate warming (McKinney et al. 2013; Rode et al. 2014; Pilfold et al. 2015). Given the vast distribution and low density of polar bear populations, as well as their high-fat, marine-based diet, QFASA has emerged as an especially powerful tool in understanding polar bear foraging ecology (Iverson et al. 2006; Thiemann et al. 2008; Galicia et al. 2016; Bourque et al. 2020). Controlled feeding studies of polar bears are often limited by small sample sizes and logistical constraints associated with housing polar bears in zoos and aquaria (Rode et al. 2016). Thus, studies of polar bears using QFASA have largely relied on data from model species, primarily mink (*Mustela vison*), fed a known marine-based diet (Thiemann 2006; Galicia et al. 2015; McKinney et al. 2017). However, the validity of the mink model for polar bears has rarely been tested (but see Bromaghin et al. 2017).

Brown bears (*U. arctos*) are biologically similar to polar bears in many respects, owing to their close evolutionary relationship (Welch et al. 2014; Cahill et al. 2015, 2018). Where ecological circumstances allow, the two species may use shared resources (Miller et al. 2006; Doupe et al. 2007; Barnas et al. 2020) and even interbreed (Pongracz et al. 2017). Brown bears may therefore serve as a more appropriate model than mink for understanding patterns of metabolism relevant to dietary analysis in polar bears.

We conducted a series of controlled feeding studies using four juvenile brown bears, with the following objectives: (1) quantify the relationship between diet and predator FA for bears on a diet nutritionally similar to that of wild polar bears; (2) develop CCs to improve QFASA diet estimation for free-ranging bears; (3) estimate the timeframe for QFASA derived diet estimates; (4) determine changes in FA profiles during fasting/hibernation (e.g., selective mobilization or conservation of specific FA).

Materials and methods

Captive feeding and fasting trials

We conducted controlled feeding experiments using four juvenile male brown bears at the Washington State

University Bear Research, Education, and Conservation Center. Juvenile bears in a dedicated research center allowed us to isolate the bears from alternative food items (i.e., plants) that are often present within the exhibits of captive bears and allowed us to obtain tissue samples at regular intervals, a sampling protocol that would not be compatible with the husbandry requirements of older bears or those in zoos.

Beginning in May 2011, all bears were fed the same series of three diets over 2 years (Table 1). The Trial 1 diet consisted of dry dog food (Science Diet, Hill's Pet Nutrition, Inc., Topeka, KS, USA) enriched with calcium, vitamin E and minerals. The diets used in Trials 2 and 3 were comprised of dry dog food supplemented with oil derived from salmon (JEdwards International Inc., bulk wild Alaskan salmon oil) and anchovy (*Engraulis ringens*; JEdwards International, Inc., omega-3 fish oil), respectively. Trial 4 was a fasting period during which bears were in hibernation. Trials 1 and 2 occurred in year 1 when the males were first-year cubs (age 5 months at Trial 1 start) and Trials 3 and 4 occurred in year 2 when the males were yearlings (age 1.5 years at Trial 3 start). Oil-supplemented diets were prepared in batches by homogenizing dog food pellets and marine fish oil at a wet weight ratio of ca. 2:1. Marine fish oil accounted for 82 and 81% of the dietary lipid for Trials 2 and 3, respectively (Table 1). These diets were constructed to approximate the lipid content and FA composition of the wild diet of polar bears while meeting the animals' micronutrient requirements. Wild polar bears preferentially consume the blubber of seals (Stirling 1974; Stirling and Archibald 1977), and captive studies have suggested that polar bears will selectively consume up to 80% blubber (unpublished data cited in Best 1985). Nevertheless, polar bears on the sea ice also scavenge prey remains and will consume some seals almost entirely (Stirling and Archibald 1977). It is thus difficult to estimate the lipid content of the typical wild diet. We used a total lipid content of ca. 40% in Trials 2 and 3 because it was the maximum achievable while still producing a homogenous mixture. The three different experimental diets provided an opportunity to calculate CCs for diets with different FA profiles and different lipid contents (Budge et al. 2020).

Bears were fed ad libitum during all trials. The bears were fed the Trial 1 diet for 90 days and then immediately switched to the Trial 2 diet for another 90 days. The experiment was stopped, and bears were fed a mixed diet prior to winter hibernation. Trial 3 was initiated when bears emerged from hibernation in spring 2012 and were fed dog food supplemented with anchovy oil. Trial 3 lasted 120 days to increase the chances bear FAs would equilibrate with the diet, after which bears were again fed a mixed diet for 4 weeks prior to winter hibernation (Trial 4; see Table 1). All food were withdrawn at the beginning of Trial 4, but bears had access to water ad libitum.

Sample collection

Diet samples were collected at the beginning of each feeding trial. One sample of dog food was collected from each batch of homogenized pellets (one in 2011, two in 2012) and a sample of marine fish oil was collected from each barrel used (one for salmon, two for anchovy). Bears were immobilized with Telazol (tiletamine HCl and zolazepam HCl; Fort Dodge, IA) prior to collecting adipose tissue samples using a 6 mm biopsy punch inserted through a small incision in the skin, approximately 15 cm lateral to the base of the tail. We collected an adipose tissue biopsy from each bear 30–45 days after the initiation of a feeding trial and at the beginning of the fasting/hibernation period in 2012. Sampling was repeated ca. every 2 weeks during feeding trials and every 21–65 days during hibernation. Final samples were collected at the end of each trial. Samples were stored at − 80 ºC until analysis. All sampling and handling procedures were reviewed and approved by the Washington State University Institutional Animal Care and Use Committee.

Laboratory analysis

Lipid was quantitatively extracted from adipose tissue biopsies and dog food samples using 2:1 chloroform:methanol according to Folch et al. (1957) as modified by Iverson et al. (2001). FA methyl esters (FAME) were prepared from lipid extracts and dietary oil samples using H_2SO_4 as a catalyst (Budge et al. 2006) and analyzed in duplicate on a Perkin Elmer Autosystem II Capillary gas chromatograph fitted

Table 1 Duration and composition of diets fed to four juvenile male brown bears during four experimental feeding trials. Lipid proportions are on an as-fed basis (% wet matter)

Feeding trial	Diet	Lipid from dog food (%)	Lipid from fish oil (%)	Total lipid (% dry matter)	Trial duration (days)
Trial 1	Dog food	100	0	10.8	90
Trial 2	Dog food + salmon oil	18.3	81.7	39.8	90
Trial 3	Dog food + anchovy oil	19.0	81.0	40.2	120
Trial 4	None (hibernation)	–	–	–	140

with a flame ionization detector and a flexible fused silica column (30 m × 0.25 mm ID) coated with 50% cyanopropyl polysiloxane (0.25 µm film thickness; Agilent Technologies, DB-23; Palo Alto, CA, USA). We inspected each chromatogram manually and corrected any erroneously identified peaks. FAs were measured as the mass percent of all FAs in the extracted lipid sample and are described according to carbon chain length:number of double bonds and location (n-*x*) of the first double bond relative to the terminal methyl group.

Calibration coefficients and diet estimates

The FA composition of Trial 2 and 3 diets was calculated by combining the FA values of the dog food and marine fish oil samples according to their relative lipid contributions (Table 1). Direct measurement of the FA composition of combined dog food and fish oil was precluded by separation of lipid and non-lipid components in homogenized diet samples. We identified up to 70 FA in the samples, but some FA were present in only trace amounts. We, therefore, limited the analyses to FA identified in at least one diet at > 0.1% of the total (as per Budge et al. 2012). This full FA set included 45 FAs that accounted for a mean of 98.9% (range 98.2–99.5%) of total bear FA. The full FA set was rescaled to sum to 100% across all diets and bears.

Calibration coefficients were calculated by dividing the percentage of a given FA in each bear by the percentage of the same FA in the diet, averaged across all four bears (Iverson et al. 2004). We used the final (i.e., day 90 or 120) FA value for each bear to calculate CCs. Thus, we generated a separate set of CCs for each of the three experimental diets. We also compared our results to two sets of CCs generated from studies of captive mink; one set from mink fed a controlled diet supplemented with herring, seal oil or poultry ($n = 37$, hereafter called "Mink (all)"), and another set using only those mink fed herring or seal oil ($n = 21$, hereafter called "marine-fed mink"). Both mink sets have been used previously to estimate the diets of polar bears (see Thiemann 2006 and Thiemann et al. 2008 for details).

We used QFASA (Iverson et al. 2004) to generate diet estimates for each bear every time they were sampled. Briefly, QFASA models the FA composition of a predator as a linear mixture of potential prey signatures and estimates diet composition by minimizing the distance between the observed and modeled predator, after applying CCs. We used the Aitchison distance measure and generated estimates in the prey space (see Bromaghin et al. 2015). We could not use a single set of FA to estimate the diets of all bears in the study because some FA were not present in one or more of the experimental diets or the mink CC sets (Table 2). Therefore, diets of bears in Trial 1 were estimated using the Full FA set, minus those FA < 0.1% in either the Trial 1 diet or Trial 1 bears, yielding a set of 18 FA. For bears in Trials 2, 3, and 4, we used a modeling set of 22 FA that was patterned after Florko et al. (2020) set of 29 FA, minus 7 FA that were not present in our data set (i.e., < 0.1%; 16:2n-6, 16:4n-3, 16:4n-1, 18:3n-1, 20:3n-3, 22:1n-7, 22:4n-3). Although the choice of FA could potentially affect the performance of QFASA, our goal was to develop CCs and test their performance under standardized conditions. Other studies have found little difference in diet estimates generated from different FA sets (Meynier et al. 2010; Wang et al. 2010). All QFASA estimates were generated in R (version 4.0.0, R Core Team 2020) using the qfasar package (Bromaghin 2017).

Statistical analysis

To assess the accuracy of QFASA diet estimates, we calculated the sums of the absolute differences between the actual and estimated proportions for each food type, following the equation (Budge et al. 2012):

$$\begin{aligned}\text{Sum of differences} = \Big(&\left|\text{actual}_{\text{dog food}} - \text{estimated}_{\text{dog food}}\right| \\ &+ \left|\text{actual}_{\text{salmon oil}} - \text{estimated}_{\text{salmon oil}}\right| \\ &+ \left|\text{actual}_{\text{anchovy oil}} - \text{estimated}_{\text{anchovy oil}}\right|\Big).\end{aligned}$$

We constructed a linear mixed model to assess the effect of CC set on the sum of differences for final diet estimates, with CC as a fixed factor and Bear ID as a random factor. We compared QFASA model outputs using CCs from all three feeding trials, plus the two mink CC sets described above. The diet estimates for a given feeding trial that were generated using CCs derived from that same trial were used as an idealized benchmark against which other CC sets could be compared. We also used a linear mixed model to investigate whether and how sums of differences changed over time, with sampling date as a fixed factor and Bear ID as a random factor. We used quantile–quantile plots to assess the normality of residuals and, where necessary, sums of differences were log transformed to meet model assumptions. Parameter p values were generated using Wald tests and we used Tukey post-hoc contrasts to compare group means. All statistical analyses were performed in R (version 4.0.0, R Core Team 2020). We used the nlme package to construct linear mixed models and the multcomp package to perform post hoc tests with statistical significance set at $\alpha = 0.05$.

Results

Diet composition

The three experimental diets differed in their FA composition. The Trial 1 diet was comprised of dog food (Table 1)

Table 2 FA composition of diets and bears and resulting calibration coefficients (CC) calculated from three controlled feeding studies of four juvenile brown bears

Fatty acid	Trial 1: Dog food					Trial 2: Dog food + salmon oil					Trial 3: Dog food + anchovy oil						
	Diet (%)	Final bear FA (%)		CC		Diet (%)	Final bear FA (%)		CC		Diet (%)	Final bear FA (%)		CC		Mink (all) CC	Mink (marine) CC
		Mean	SD	Mean	SD		Mean	SD	Mean	SD		Mean	SD	Mean	SD		
Saturated																	
14:0[a]	0.71	0.86	0.05	1.22	0.06	4.33	2.25	0.12	0.52	0.03	6.06	3.20	0.22	0.53	0.04	1.37	0.81
i-15:0	0.00	0.37	0.02	–	–	0.17	0.48	0.04	2.74	0.24	0.18	0.51	0.03	2.86	0.15	0.70	0.63
15:0	0.06	0.28	0.01	4.79	0.23	0.40	0.43	0.01	1.09	0.02	0.43	0.48	0.02	1.11	0.05	0.80	0.78
16:0[a]	19.79	17.51	0.56	0.88	0.03	14.31	16.58	0.63	1.16	0.04	17.26	17.34	0.89	1.00	0.05	0.96	1.06
i-17:0	0.03	0.71	0.03	24.61	1.17	0.21	0.66	0.09	3.15	0.43	0.17	0.47	0.05	2.72	0.31	–	–
ai-17:0	0.02	0.08	0.01	3.93	0.47	0.09	0.11	0.01	1.25	0.16	0.18	0.14	0.01	0.79	0.04	0.75	0.70
17:0[a]	0.22	0.50	0.01	2.23	0.06	0.30	0.42	0.04	1.38	0.15	0.48	0.38	0.01	0.78	0.02	0.77	0.85
18:0[a]	7.90	5.55	0.81	0.70	0.10	3.40	2.66	0.42	0.78	0.12	4.38	2.63	0.24	0.60	0.06	0.72	0.79
20:0[a]	0.19	0.14	0.01	0.72	0.07	0.15	0.11	0.00	0.74	0.02	0.26	0.13	0.02	0.51	0.07	0.75	0.71
Monounsaturated																	
16:1n-11	0.04	0.04	0.00	0.88	0.03	0.43	0.26	0.02	0.60	0.06	0.38	0.23	0.02	0.59	0.05	0.91	0.95
16:1n-9[a]	0.29	0.35	0.02	1.20	0.05	0.27	0.30	0.02	1.13	0.07	0.26	0.30	0.02	1.16	0.08	0.99	1.13
16:1n-7[a]	2.71	5.21	0.59	1.92	0.22	5.28	8.92	0.79	1.69	0.15	8.17	10.86	0.21	1.33	0.03	1.44	1.24
16:1n-5	0.03	0.09	0.01	2.58	0.34	0.31	0.24	0.02	0.77	0.05	0.18	0.19	0.01	1.05	0.04	0.79	0.73
17:1b	0.00	0.01	0.00	–	–	0.33	0.15	0.01	0.45	0.03	0.20	0.16	0.01	0.78	0.05	0.90	0.88
17:1[a]	0.15	0.49	0.05	3.23	0.33	0.31	0.60	0.05	1.92	0.17	0.18	0.60	0.05	3.37	0.28	1.17	1.16
18:1n-13	0.03	0.06	0.01	2.09	0.20	0.12	0.21	0.01	1.78	0.11	0.09	0.04	0.07	1.64	–	0.72	0.45
18:1n-11[a]	0.12	0.12	0.02	0.99	0.13	0.77	0.74	0.07	0.95	0.09	0.11	0.31	0.06	2.78	0.55	3.65	5.47
18:1n-9[a]	33.34	43.39	1.01	1.30	0.03	17.47	30.85	0.60	1.77	0.03	13.90	27.64	1.35	1.99	0.10	1.41	1.64
18:1n-7[a]	2.01	2.51	0.16	1.25	0.08	3.07	2.94	0.12	0.96	0.04	3.10	2.99	0.07	0.97	0.02	1.33	1.40
18:1n-5	0.09	0.15	0.02	1.62	0.22	0.54	0.43	0.04	0.80	0.07	0.12	0.21	0.02	1.70	0.20	0.96	0.87
20:1n-11	0.06	0.07	0.01	1.25	0.10	5.46	3.70	0.14	0.68	0.03	0.12	1.70	0.37	14.63	3.17	4.39	4.52
20:1n-9[a]	0.50	0.49	0.02	0.98	0.04	2.72	1.77	0.06	0.65	0.02	0.85	1.20	0.15	1.40	0.18	1.69	1.27
20:1n-7	0.03	0.04	0.01	1.30	0.20	0.34	0.21	0.02	0.62	0.05	0.25	0.19	0.01	0.73	0.04	1.78	1.20
22:1n-11	0.00	0.01	0.01	–	–	7.62	2.87	0.24	0.38	0.03	0.42	1.01	0.25	2.42	0.60	0.67	0.33
22:1n-9	0.09	0.03	0.00	0.32	0.03	0.83	0.34	0.02	0.41	0.03	0.18	0.14	0.03	0.78	0.15	0.60	0.54
24:1	0.02	0.02	0.01	1.20	0.62	0.67	0.18	0.03	0.27	0.05	0.32	0.00	0.00	–	–	0.17	0.16
Polyunsaturated																	
16:2n-4	0.00	0.00	0.00	–	–	0.35	0.19	0.01	0.53	0.04	0.90	0.45	0.02	0.51	0.03	0.89	0.50
16:3n-4	0.01	0.00	0.00	–	–	0.26	0.03	0.01	0.11	0.04	1.11	0.15	0.02	0.14	0.01	0.52	0.25
18:2n-6[a]	27.15	17.50	1.31	0.64	0.05	6.29	8.32	0.68	1.32	0.11	6.09	8.42	0.62	1.38	0.10	1.12	1.29
18:2n-4	0.02	0.01	0.01	0.96	0.66	0.14	0.08	0.01	0.61	0.05	0.31	0.17	0.01	0.57	0.03	2.03	0.73

Table 2 (continued)

Fatty acid	Trial 1: Dog food					Trial 2: Dog food + salmon oil					Trial 3: Dog food + anchovy oil					Mink (all) CC	Mink (marine) CC
	Diet (%)	Final bear FA (%)		CC		Diet (%)	Final bear FA (%)		CC		Diet (%)	Final bear FA (%)		CC			
		Mean	SD	Mean	SD		Mean	SD	Mean	SD		Mean	SD	Mean	SD		
18:3n-6	0.10	0.21	0.02	2.09	0.21	0.09	0.11	0.02	1.29	0.24	0.31	0.21	0.02	0.67	0.05	0.71	0.75
18:3n-4	0.03	0.06	0.01	2.16	0.18	0.12	0.22	0.02	1.91	0.17	0.15	0.47	0.04	3.14	0.30	1.61	1.56
18:3n-3[a]	3.09	1.60	0.12	0.52	0.04	1.38	1.16	0.04	0.84	0.03	1.16	1.13	0.07	0.98	0.06	0.62	0.70
18:4n-3	0.02	0.04	0.01	2.87	0.57	2.13	0.64	0.03	0.30	0.01	2.11	0.77	0.07	0.37	0.03	0.48	0.30
18:4n-1	0.06	0.05	0.01	0.83	0.15	0.17	0.27	0.04	1.55	0.22	0.22	0.65	0.09	3.03	0.40	0.60	0.48
20:2n-6[a]	0.35	0.31	0.02	0.89	0.06	0.37	0.30	0.00	0.80	0.01	0.30	0.34	0.02	1.14	0.06	0.93	0.92
20:3n-6[a]	0.12	0.20	0.01	1.64	0.05	0.12	0.14	0.01	1.19	0.08	0.21	0.16	0.01	0.75	0.03	0.72	0.73
20:4n-6[a]	0.34	0.47	0.04	1.36	0.12	0.49	0.38	0.02	0.78	0.05	1.06	0.59	0.06	0.55	0.06	0.37	0.44
20:4n-3	0.00	0.04	0.01	–	–	1.08	0.98	0.10	0.91	0.09	0.67	0.68	0.05	1.01	0.07	0.76	0.51
20:5n-3	0.06	0.05	0.00	0.82	0.06	7.97	2.73	0.14	0.34	0.02	15.09	5.15	0.65	0.34	0.04	0.19	0.14
21:5n-3	0.00	0.01	0.01	–	–	0.36	0.19	0.01	0.53	0.02	0.61	0.30	0.02	0.49	0.03	0.70	0.46
22:4n-6[a]	0.12	0.20	0.01	1.68	0.12	0.09	0.11	0.00	1.27	0.01	0.16	0.13	0.01	0.81	0.06	0.89	0.97
22:5n-6	0.02	0.03	0.00	1.26	0.16	0.09	0.08	0.00	0.88	0.05	0.28	0.13	0.01	0.48	0.04	0.70	0.81
22:5n-3	0.05	0.09	0.01	1.88	0.17	1.59	1.41	0.13	0.89	0.08	1.67	1.53	0.06	0.92	0.04	0.87	0.88
22:6n-3	0.04	0.05	0.02	1.28	0.41	7.03	4.25	0.20	0.60	0.03	9.37	5.52	0.36	0.59	0.04	0.64	0.59
∑ saturated	28.93	26.00				23.35	23.70				29.41	25.29					
∑ monounsaturated	39.51	53.09				46.54	54.72				28.83	47.75					
∑ polyunsaturated	31.57	20.91				30.11	21.58				41.76	26.96					
Total (%)	100	100				100	100				100	100					

Bold type denotes the 22 fatty acids used to generate QFASA estimates for bears in Trial 2–4

[a]18 fatty acids used to generate QFASA estimates for bears in Trial 1

and had 10.8% lipid (dry matter basis). The FA composition was dominated by three FA: 16:0, 18:1n-9 and 18:2n-6 accounted for 80.3% of total extractable lipid. The diet for Trial 2 was comprised of dog food supplemented with salmon oil and although it had a similar ratio of saturated: monounsaturated:polyunsaturated FA as Trial 1 (Table 2), its composition was more balanced across FA (Fig. 1). The diet used in Trial 3 was comprised of dog food supplemented with anchovy oil and had the highest proportion of polyunsaturated FA, which accounted for > 40% of total lipid (Table 2).

Changes in bear FA profiles during feeding and fasting

The relative abundance of most FA was stable over the course of Trial 1, which reflects the fact that bears were fed dog food prior to the start of the trial. However, some FA did show gradual change over Trial 1 (e.g., 16:1n-7, Fig S1), indicating de novo synthesis or mobilization. Once switched to the Trial 2 diet, bear FA profiles changed rapidly in the direction of the new diet. The most rapid change occurred between day 1 and day 45, with more gradual change evident in subsequent samples. During Trial 3, bear FA profiles again changed rapidly in response to the new diet, with large shifts in FA occurring between day 1 and day 29, with more gradual changes thereafter and evidence of stabilization after day 62 (Fig. 2).

There was variability in FA patterns during hibernation (Trial 4). FA profiles were generally stable during the first 3 weeks of hibernation, but beyond that some FA increased (e.g., 18:1n-9), some decreased (e.g., 20:5n-3), while others remained stable (e.g., 18:2n-6; see Table 3; Fig. 2). Of the 45 total FA, 20 decreased during hibernation (mean change in % total FA: − 0.39 ± 0.76, range − 2.92 to − 0.01) and 25 increased (mean change in % total FA: 0.31 ± 0.73, range 0.003–3.66). The FA showing the largest proportional changes (i.e., > 52%) were only present in small amounts (i.e., < 1% of total FA). Of the FA accounting for > 1% of total FA, 20:5n-3 showed the greatest proportional change, declining by 51.5% over 140 days of hibernation (Table 3).

Calibration coefficients

Trial 1 yielded CC values for 38 FA, a smaller number than Trial 2 (45 FA) or Trial 3 (44 FA) because of the more limited diversity of FA in the Trial 1 diet. Trial 1 CCs were generally comparable with Trial 2 and 3, with some exceptions (Fig. 3). CC values from Trials 1 and 2 were virtually identical for 16:1n-9, 18:0, 18:1n-11, and 20:0. In contrast, the calibration for i-17:0 generated from Trial 1 was 37 times higher than the value generated from Trial 2. Trial 1 CCs were consistently higher than either of the other feeding trials for the long chain polyunsaturated FA 22:4n-6, 22:5n-6, 22:5n-3, and 22:6n-3.

CCs generated from Trials 2 and 3 were generally similar, again with some exceptions. The largest difference was in the CC for 20:1n-11, which was more than 20 times larger from Trial 3 than from Trial 2. The CCs generated from captive brown bears were also comparable to those generated from captive mink, also fed a marine based diet (Fig. 3), with a few exceptions. For example, the CC value for 18:1n-13 generated from Trial 3 was 3.6 times larger than the marine-fed mink value, whereas the marine-fed mink CC for 18:1n-11 was 3.8 times larger than the value from Trial 2.

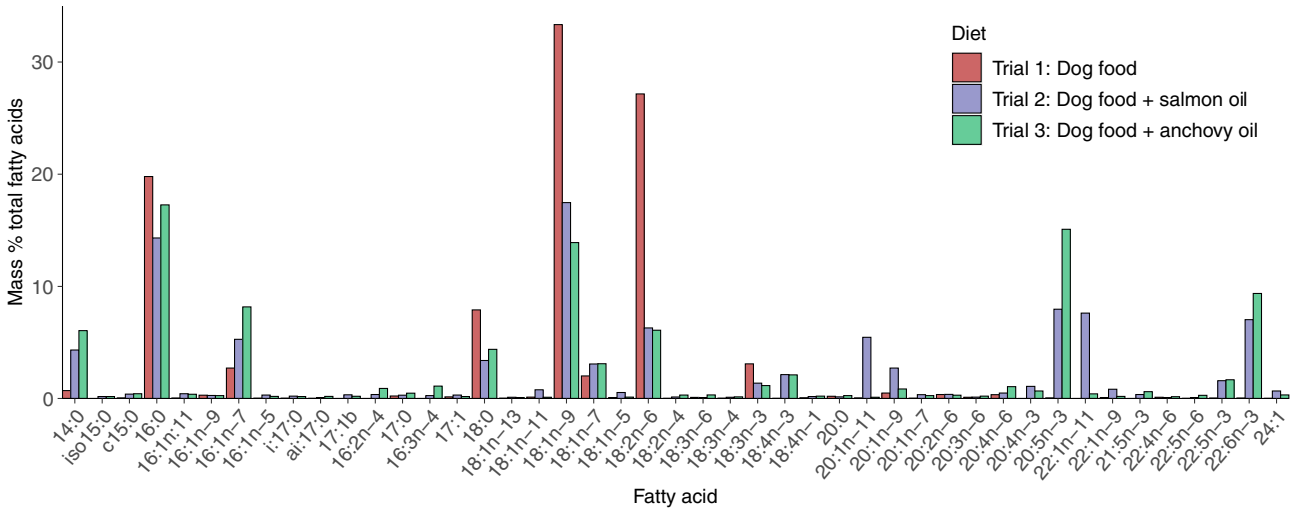

Fig. 1 Fatty acid composition of three experimental diets fed to brown bears

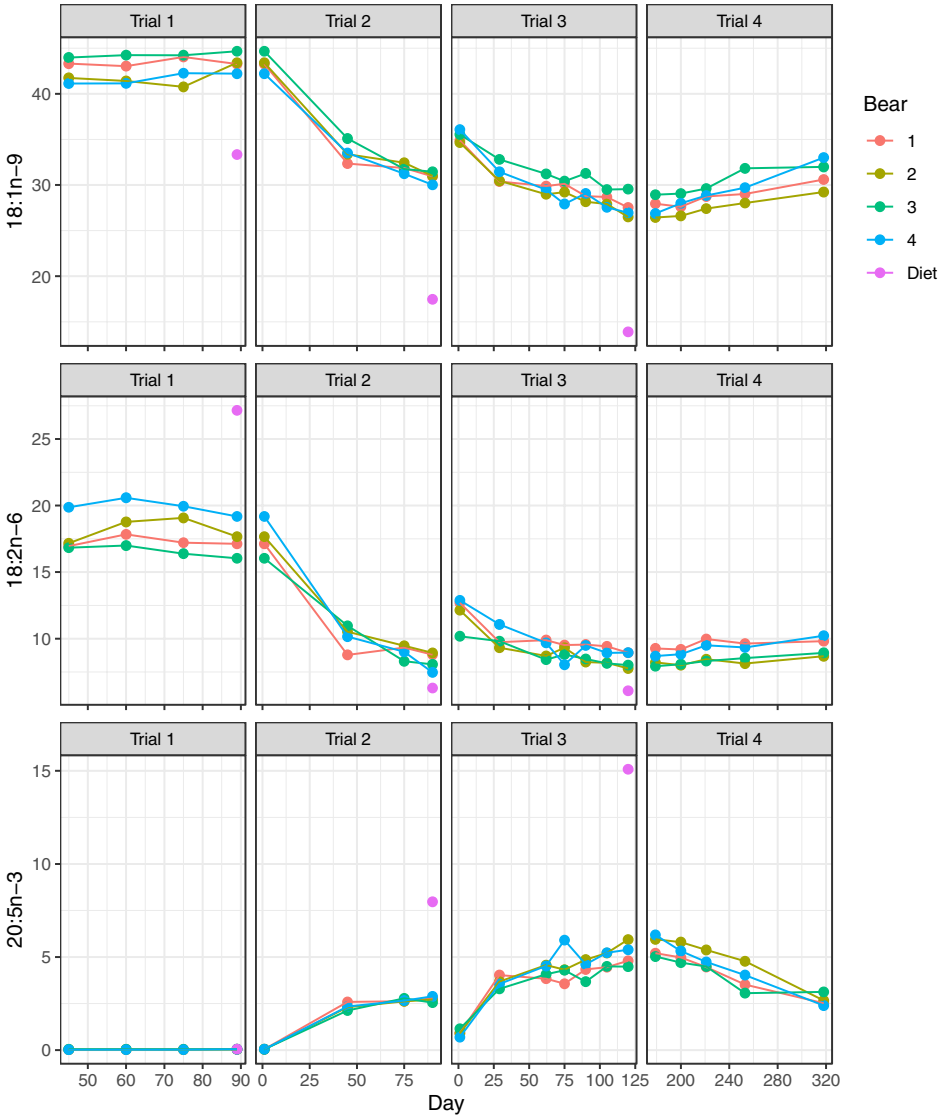

Fig. 2 Concentration (mass % of total FA) of selected FA in adipose tissue of four juvenile brown bears during three feeding trials, followed by hibernation (Trial 4). Pink circles indicate the FA composition of the experimental diets

QFASA estimates across CC

We used sums of differences between actual and estimated diets to assess the accuracy of QFASA diet estimates generated at the end of each feeding trial. The CC set used in QFASA modelling had a significant effect on the accuracy of diet estimates (linear mixed model, Trial 1: $F_{5,15} = 314.2$, $p < 0.001$; Trial 2: $F_{4,12} = 229.0$, $p < 0.001$; Trial 3: $F_{4,12} = 49.4$, $p < 0.001$). The diet composition for bears at the end of Trial 1 was most accurately estimated using Trial 3 CC (mean sum of diff: 0.012 ± 0.004); however, Trial 1 final diets were well-estimated regardless of the CC used (Fig. 4). The accuracy of estimates using mink (all) did not differ from using no CC, but all other comparisons were significantly different from each other (Tukey contrasts, all $p < 0.001$; Fig S2). The diet composition for bears at the end of Trial 2 was most accurately estimated using Trial 2 CC (mean sum of diff: 0.080 ± 0.052) but did not differ significantly from the estimates using marine-fed mink CC (0.152 ± 0.110; $p = 0.481$; Fig. 4). Sums of differences from all other CC sets were significantly different from each other (Tukey contrasts $p < 0.001$; Fig S2). The diet composition for bears at the end of Trial 3 was most accurately estimated using Trial 3 CC (mean sum of diff: 0.040 ± 0.014). Marine-fed mink CCs provided the second most accurate estimates (0.371 ± 0.131), although they were less accurate than Trial 3 CC ($p < 0.001$) and were not significantly better than mink (all) CC (0.420 ± 0.131; $p = 1.000$; Fig. 4). There was also no difference in accuracy between mink (all) and Trial 2 (0.545 ± 0.181; $p = 0.108$). Sums of differences from all other CC sets were significantly different from each other (Tukey contrasts $p < 0.01$; Fig S2).

Table 3 Initial, final and change in concentration of fatty acids in the adipose tissue of four juvenile brown bears during 140 days of hibernation. Change values reflect mean changes within each bear. Fatty acids in bold type are also plotted in Fig. 3

Fatty acid	Start concentration (%)		Final concentration (%)		Total individual change		Percent individual change (%)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	2.61	0.25	2.35	0.26	− 0.27	0.13	− 10.12	5.12
i-15:0	0.50	0.02	0.52	0.05	0.02	0.06	3.65	12.79
15:0	0.47	0.02	0.42	0.03	− 0.05	0.03	− 11.50	5.42
16:0	16.05	1.27	14.88	0.89	− 1.16	0.63	− 7.11	3.41
16:1n-11	0.26	0.01	0.23	0.02	− 0.03	0.02	− 12.67	7.20
16:1n-9	0.29	0.02	0.32	0.01	0.03	0.01	10.75	2.90
16:1n-7	10.30	1.24	8.38	1.28	− 1.92	1.13	− 18.45	9.67
16:1n-5	0.19	0.02	0.16	0.01	− 0.03	0.02	− 17.54	10.01
i-17:0	0.56	0.07	0.57	0.05	0.01	0.05	2.14	8.96
ai-17:0	0.16	0.01	0.16	0.01	0.01	0.01	4.13	3.19
17:1b	0.21	0.00	0.22	0.02	0.01	0.02	6.88	9.33
16:2n-4	0.52	0.02	0.45	0.04	− 0.07	0.04	− 13.44	8.34
17:0	0.42	0.05	0.42	0.03	− 0.01	0.03	− 1.04	6.58
16:3n-4	0.15	0.01	0.12	0.02	− 0.03	0.01	− 18.97	6.83
17:1	0.54	0.08	0.47	0.05	− 0.07	0.03	− 12.80	5.32
18:0	2.62	0.58	3.06	0.43	0.45	0.38	18.98	16.52
18:1n-13	0.12	0.02	0.09	0.02	− 0.03	0.03	− 24.50	22.78
18:1n-11	0.33	0.02	0.44	0.04	0.11	0.03	34.18	9.28
18:1n-9	27.55	1.12	31.21	1.65	3.66	1.66	13.35	6.33
18:1n-7	3.21	0.18	3.31	0.11	0.09	0.09	2.97	3.21
18:1n-5	0.22	0.03	0.22	0.01	0.00	0.03	2.42	13.30
18:2n-6	8.53	0.59	9.41	0.73	0.89	0.49	10.46	5.80
18:2n-4	0.19	0.01	0.17	0.01	− 0.02	0.01	− 10.10	3.38
18:3n-6	0.18	0.01	0.15	0.01	− 0.04	0.01	− 20.59	4.78
18:3n-4	0.46	0.05	0.42	0.03	− 0.04	0.04	− 8.26	8.44
18:3n-3	1.16	0.05	1.04	0.10	− 0.12	0.09	− 10.33	8.05
18:4n-3	0.79	0.06	0.51	0.05	− 0.29	0.08	− 35.70	8.55
18:4n-1	0.76	0.07	0.42	0.09	− 0.34	0.14	− 43.56	14.31
20:0	0.17	0.00	0.27	0.03	0.10	0.03	58.16	16.47
20:1n-11	1.35	0.17	1.87	0.22	0.52	0.19	38.95	17.14
20:1n-9	1.20	0.05	1.69	0.10	0.49	0.11	40.81	10.52
20:1n-7	0.19	0.00	0.29	0.02	0.10	0.02	50.48	13.41
20:2n-6	0.27	0.01	0.31	0.02	0.04	0.02	16.21	7.55
20:3n-6	0.18	0.01	0.20	0.02	0.02	0.02	9.96	10.21
20:4n-6	0.68	0.05	0.52	0.06	− 0.15	0.07	− 22.60	8.58
20:4n-3	0.83	0.06	0.71	0.04	− 0.13	0.07	− 14.86	6.76
20:5n-3	5.59	0.56	2.68	0.32	− 2.92	0.81	− 51.50	9.95
22:1n-11	0.79	0.09	1.10	0.11	0.31	0.06	39.21	9.32
22:1n-9	0.16	0.01	0.24	0.02	0.08	0.02	52.31	11.11
21:5n-3	0.38	0.02	0.38	0.03	0.00	0.02	1.02	5.58
22:4n-6	0.14	0.01	0.20	0.02	0.06	0.03	42.92	20.32
22:5n-6	0.18	0.01	0.25	0.05	0.07	0.04	36.25	21.52
22:5n-3	2.02	0.14	2.38	0.27	0.36	0.31	18.42	15.80
22:6n-3	6.42	0.34	6.66	1.16	0.24	1.03	3.63	15.60
24:1	0.07	0.01	0.13	0.02	0.06	0.02	79.99	25.93

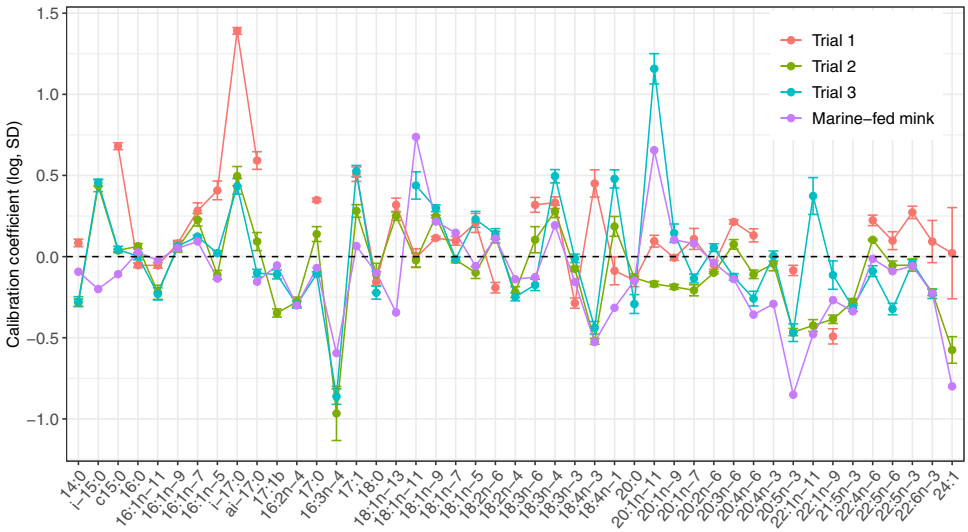

Fig. 3 Mean calibration coefficients (log scale, ± SD) calculated from four juvenile brown bears at the conclusion of three feeding trials. Also shown are calibration coefficients from captive mink fed a marine-based diet (mink data from Thiemann 2006; Thiemann et al. 2008)

QFASA estimates across sampling day

We also used sums of differences to examine how the accuracy of QFASA diet estimates changed as a function of sampling date, including diet estimates from hibernating bears (Trial 4; see next section). Because marine-fed mink CCs produced the second-most accurate estimates in Trials 2 and 3 (see above), we compared results across sampling day using two sets of CCs for each trial: (1) CCs generated from that same trial and (2) CCs from marine-fed mink (Fig. 5).

For bears in Trial 1, QFASA estimates showed relatively little variation across sampling day, regardless of which CC set was used (Figs. 5, 6). However, the accuracy of QFASA estimates from Trial 1 CCs varied across sampling date ($F_{3,9}=4.54$, $p=0.033$; Fig. 6a), whereas those from marine-fed mink CCs did not ($F_{3,9}=0.85$, $p=0.499$; Fig. 6c). For Trial 1 CCs, QFASA accuracy was lower (i.e., sum of differences was higher, Fig. S3) on day 45 than on day 75 (Tukey contrasts $p=0.006$) or day 89 ($p=0.017$). Sums of differences did not differ among any other sampling days ($p>0.05$; Fig. S3).

Bears in Trial 2 received their new diet immediately after Trial 1, and diet estimates responded with a rapid change between day 1 and day 45. More gradual change was evident beyond day 45, a pattern that was consistent across both CC sets (Fig. 5). Likewise, the accuracy of QFASA estimates improved by day 45 (Fig. 6), with sampling day having a significant effect on sums of differences for both Trial 2 CC ($F_{3,9}=323.7$, $p<0.001$) and marine-fed mink CC ($F_{3,9}=187.0$, $p<0.001$). For both CC sets, there was no difference in accuracy between day 75 and 95 (Tukey contrasts $p>0.50$). All other comparisons were significant ($p<0.05$; Fig S3).

The Trial 3 diet was given to the bears following winter hibernation and an interim recovery period during which they were fed dog food and allowed to graze on vegetation. Thus, diet estimates changed rapidly in response to the new dog food/anchovy oil diet (Fig. 5) and became more accurate (Fig. 6) as the trial progressed. Sampling day had a significant effect on sums of differences for both Trial 3 CC ($F_{6,18}=174.6$, $p<0.001$) and marine-fed mink CC ($F_{6,18}=61.57$, $p<0.001$; Fig. 6). The accuracy of day 1 diet estimates was significantly worse than all subsequent sampling days (Tukey contrasts $p<0.001$). Accuracy improved beyond day 29, with significant differences between day 29 and day 75 and beyond (Tukey contrasts $p<0.002$). Accuracy on day 62 was lower than day 120 ($p=0.039$) when using marine-fed mink CCs. No differences in accuracy were evident beyond day 75 with either set of CCs ($p>0.767$; Fig S3).

QFASA estimates during hibernation

Trial 4 began when bears entered hibernation, following a 4-week interim period after Trial 3. We used the Trial 3 diet as a benchmark to detect changes in sums of differences because it was the last known diet consumed prior to hibernation. Trial 4 diet estimates generated using Trial 3 CC showed only slight changes over time, although more change was evident when we used marine-fed mink CC (Fig. 5). Using marine-fed mink CCs, the estimated contribution of anchovy oil declined 14.7% (from 71.3 to 56.6%) over the 140-day period, whereas estimated dog food and salmon oil contributions increased 3.0 and 11.7%, respectively (Fig. 5). These patterns were reflected in sums of differences, which were not affected by sampling day when Trial 3 CC were used ($F_{4,12}=1.568$, $p=0.245$) but did change over time when we used marine-fed mink CC ($F_{4,12}=10.270$, $p<0.001$; Fig. 6). In the latter case, accuracy declined significantly

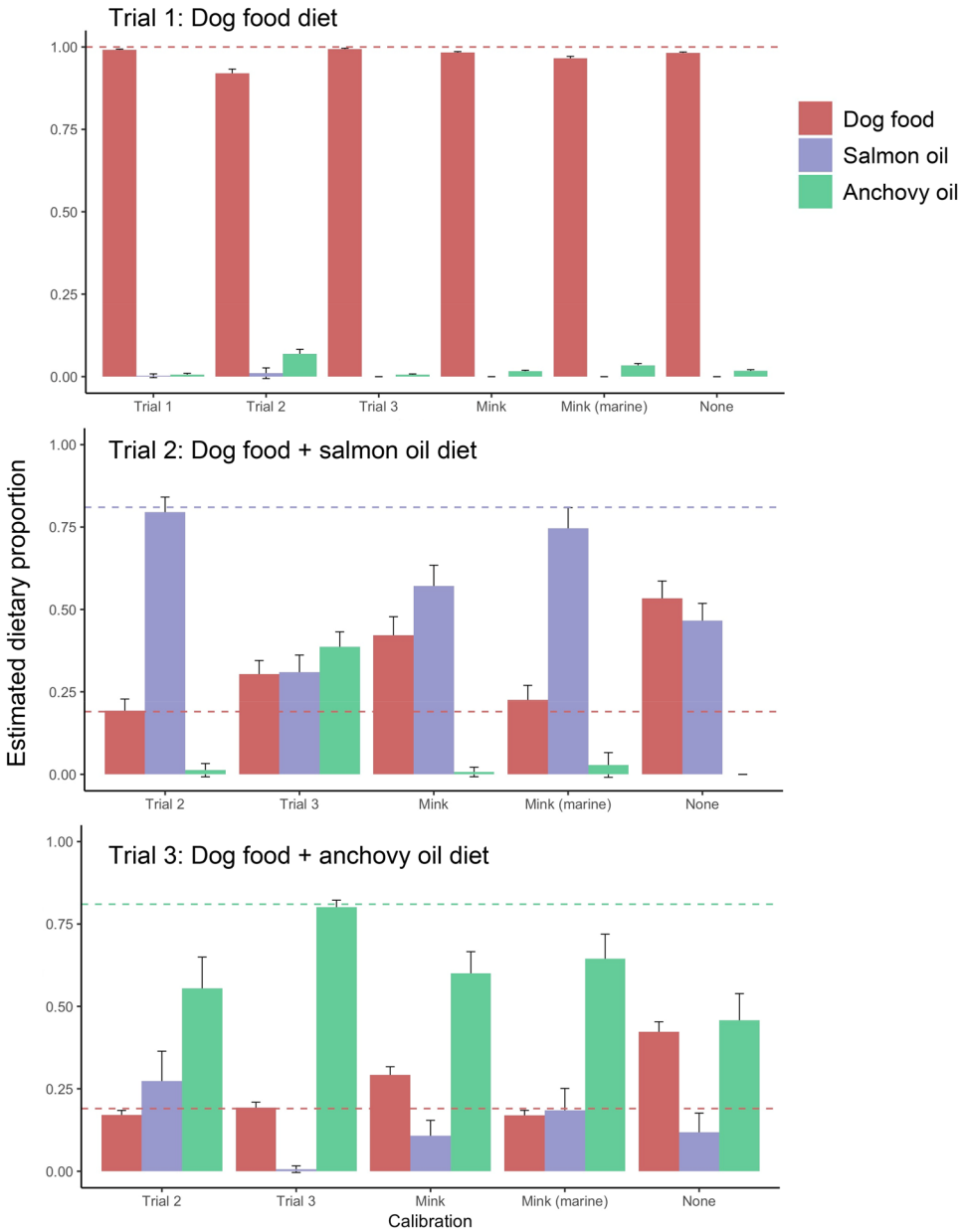

Fig. 4 Mean (±SD) diet estimates from QFASA for four brown bears sampled at the end of controlled feeding experiments. Horizontal dashed lines indicate true diet composition (see Table 1). Diet estimation used calibration coefficients generated from the feeding trials, from captive mink (Thiemann et al. 2008), or no calibration. Trial 1 calibration coefficients could not be applied to bears in Trial 2 or 3 because of the limited number of FA in Trial 1

on the final sampling day, with differences between day 318 and all 4 earlier samples (Tukey contrasts $p < 0.040$; Fig S3).

Discussion

Information on diet composition is fundamental to understanding animal ecology. Recent and emerging methods of predator diet estimation, including FA and stable isotope analyses, are premised on a predictable and quantifiable relationship between the biochemical composition of a predator's tissue and that of its composite diet. However, these biochemical relationships may be complex and are poorly understood in many taxa. Our results directly address this knowledge gap and provide a response to calls in the literature for additional experimental studies to improve the accuracy and utility of nutrient-tracking approaches to estimating predator diets (e.g., Bowen and Iverson 2013).

This study provides direct estimates of the timeframe represented by FA-based diet estimates in a terrestrial carnivore. Ambiguity about the timeframe of FA turnover has limited the ecological interpretation of QFASA diet estimates. Thus, our results will improve the utility of QFASA as an ecological tool. Our controlled diets were designed to mimic the lipid content and composition of polar bear diets to the degree possible, so our results are most relevant to

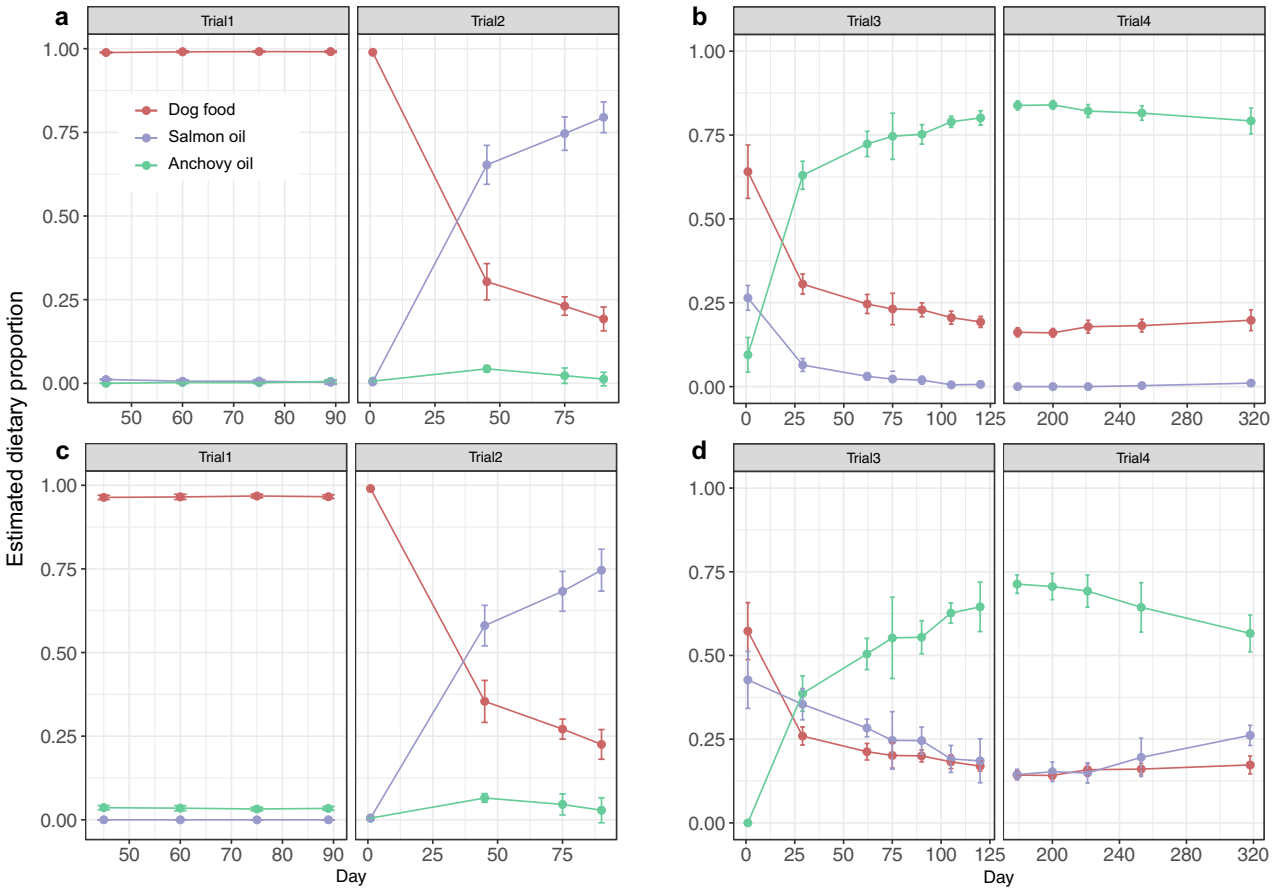

Fig. 5 Mean (±SD) diet estimates for four brown bears sampled intermittently during controlled feeding experiments. See Table 1 for diet composition. **a**, **b** Diet estimation used calibration coefficients generated from the same trial for trials 1–3; trial 4 used calibration coefficients from trial 3 (see text for details). **c**, **d** Diet estimation used calibration coefficients generated from captive mink fed a marine-based diet (Thiemann et al. 2008)

polar bears but are also applicable to wild brown bears feeding on marine-based foods (e.g., spawning salmon).

The Trial 1 diet was compositionally simple and largely consistent with the maintenance diet the bears received after weaning and prior to the start of the experiment. Consequently, the bears' FA profiles did not noticeably change over the course of Trial 1. QFASA diet estimates were similarly consistent during Trial 1 and estimates were highly accurate, regardless of sampling day or the CC used. The high accuracy was likely influenced by the simple "prey library" (Budge et al. 2006; Bromaghin 2017) of only three potential foods. The clear difference between the compositionally simple dog food (i.e., dominated by ca. six FA) and the two more complex fish oils (Fig. 1) presumably reduced the potential for confounding prey types. However, the similarity of the two fish oils may have impaired diet estimates, as discussed below.

When bears were started on a new diet (i.e., Trial 2 and 3), their FA profiles abruptly shifted in the direction of the new diet. Some FAs were more variable across individual bears, as reflected in differences in SD (Table 2), but individual variability was generally low. With only four bears in this study, our sample size was small (a common limitation of large-carnivore experiments); however, the limited individual variation suggests that a larger sample size would not have substantively altered our results. The relationship between bear and diet FA was variable across diets (Fig. 2), as reflected in differences in CC values across feeding trials (Fig. 3). For some FA (e.g., 18:2n-6, 22:1n-11; Fig. 2), bears had values that were higher or lower than their composite diet, depending on the feeding trial. For instance, 22:1n-11 had a Trial 2 CC value of 0.38 (i.e., the FA was higher in the diet than in the bears) but a Trial 3 CC value of 2.42 in (i.e., the FA was higher in the bears than in the diet; Table 2).

In Trials 2 and 3, CCs had an important effect on QFASA diet estimates (Fig. 4) and our study adds to existing evidence that CCs are to some extent diet-specific. For instance, the diets of bears in Trial 2 were not accurately estimated using CCs from Trial 3 (Fig. 4), even though they were the same bears with some common dietary components (i.e.,

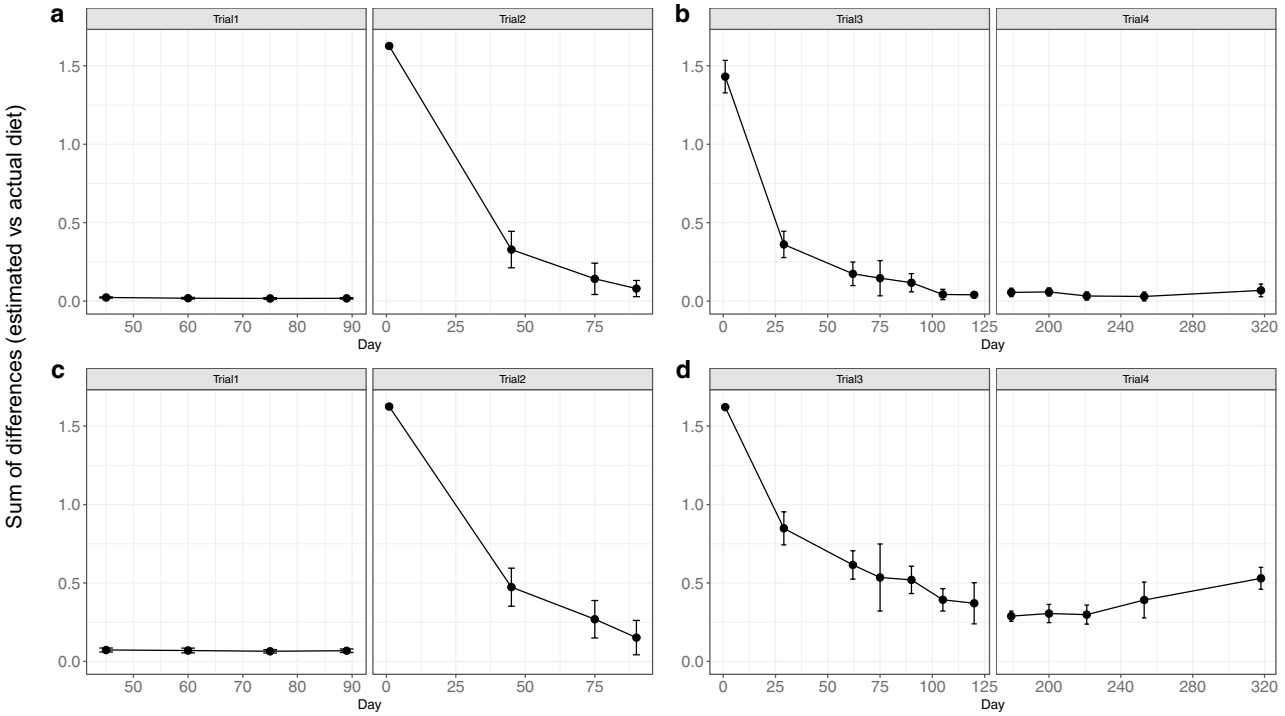

Fig. 6 Mean (±SD) sum of differences between estimated and actual diets for four brown bears sampled intermittently during controlled feeding experiments. See Table 1 for diet composition. **a**, **b** Diet estimation used calibration coefficients generated from the same trial for trials 1–3; trial 4 used calibration coefficients from trial 3 (see text for details). **c**, **d** Diet estimation used calibration coefficients generated from captive mink fed a marine-based diet (Thiemann et al. 2008)

dog food was present in both diets). In fact, Trial 3 CCs produced the worst estimates of Trial 2 diet among the five CC sets we compared. Differences in CC values for some FA in Trials 2 and 3 (e.g., 22:1n-11, see above) could have contributed to the reduced accuracy of diet estimates compared to the marine-fed mink CCs. The similarity of the fish oil components in the Trial 2 and Trial 3 diets may also have contributed to the poor performance of Trial 3 CCs in estimating the diets of Trial 2 bears. The dog food component of the diet was relatively accurately estimated by all CC sets aside from mink (all) and none. The Trial 3 CC set had difficulty resolving the two types of fish oil, which suggests the anchovy oil used in Trial 3 predisposed the Trial 3 CCs to estimate that dietary component. Similarly, the Trial 2 CCs led to misallocation of Trial 3 diets to salmon oil.

Given apparent differences among CC sets derived from different diets, the numerical (Fig. 3) and functional (Fig. 4) similarity between CCs derived from marine-fed mink (Iverson et al. 2004; Thiemann 2006; Thiemann et al. 2008) and the CCs derived from Trials 2 and 3 was surprising. Marine-fed mink CCs generated the second-most accurate estimates of diet for bears in both Trial 2 and 3. Marine-fed mink CCs also performed well in Trial 1, although better estimates were generated from mink (all) and no CCs. Estimating the diet composition of individual predators using CCs generated from that same diet and those same predators is obviously not feasible in wildlife research and is mathematically circular, i.e., the predator FA profile is used to calculate the CC, which is then applied to the predator FA profile. We used these idealized, same-trial CCs as a benchmark against which other CCs could be tested and, in that context, marine-fed mink CCs emerged as the top performer. This finding is encouraging in a couple of ways; first, it suggests that the marine-fed mink CCs that have been used in previous studies of polar bear diet composition perform as well as those generated from species more closely related to polar bears; second, it suggests that CC sets may have relatively broad applicability across taxa for similar (e.g., marine-based) diets.

Most of the values for marine-fed mink CCs were similar to, or within the range of, values derived in the current study, with a few exceptions, including 18:1n-13, 18:1n-11, 20:1n-11, and 20:5n-3. Of those, only 20:5n-3 was used in QFASA modelling. The two 18:1 isomers showed inverse trends (Fig. 3) as the marine-fed mink value for 18:1n-11 (5.47) was higher than Trial 2 (0.95 ± 0.09) or Trial 3 (2.78 ± 0.55), but the value for 18:1n-13 (0.45) was lower than Trial 2 (1.78 ± 0.11) or Trial 3 (1.64 ± 0.00). This pattern may reflect some degree of mis-identification, as it can be difficult to resolve these two isomers as their peaks

may overlap with each other and with the adjacent 18:1n-9 in chromatographic analysis. The CC value for 20:1n-11 derived from marine-fed mink (4.52) was substantially higher than the value derived from Trial 1 (1.25 ± 0.10) or Trial 2 (0.68 ± 0.03), but lower than Trial 3 (14.63 ± 3.17), suggesting the metabolism of this FA is especially sensitive to diet and it thus may not be a useful dietary tracer. Indeed, Bromaghin et al. (2015) found that modelled predator values using mink (all) CCs for this FA were outside the range of prey values (i.e., the FA could not be modelled realistically) and other studies have identified 20:1n-11 as an unreliable dietary indicator (Galicia et al. 2015; Goetsch et al. 2018). It is unclear why the marine-fed mink CC value for 20:5n-3 (0.14) was lower than either Trial 2 (0.34 ± 0.02) or Trial 3 (0.34 ± 0.04), but this FA may be especially metabolically active. It showed the largest change in concentration during hibernation of any FA > 1%.

The performance of the marine-fed mink CCs was also consistent with the results of Bromaghin et al. (2017) who found that CCs derived mathematically from Chukchi Sea polar bear and prey data were similar to those derived from the mink feeding trial (see their Fig. 8). Our results thus add to growing evidence that diet-specific variation can be more important than species-specific differences in estimating CCs. Thus, whenever possible, deriving CCs on diets similar to the predator of interest, even if in a model species, may aid in producing the most accurate diet estimates. Estimation of CCs mathematically, as proposed by Bromaghin et al. (2017), provides an alternative approach for diet estimation from FA in which direct estimate of CCs are not required, but feeding trials can continue to be useful in determining when separate CCs need to be generated for different groups of predators.

Our study is one of the few to examine changes in FA profiles over time in individual carnivores and thus provides important new insights into the temporal window of QFASA diet estimates. In a controlled feeding study of juvenile harbor seals (*Phoca vitulina*), Nordstrom et al. (2008) sampled individual seals three times over 42 days and estimated that blubber FA would have equilibrated with the diet at 50–65 days. Bowen and Iverson (2013) cite unpublished data that QFASA diet estimates for captive juvenile Steller sea lions (*Eumetopias jubatus*) were most accurate between 56 and 84 days. We found that bear FA profiles responded rapidly to a change in diet and QFASA estimates were reasonably accurate within about 30 days of a dietary switch (Figs. 5, 6). Bears came to maximally resemble their diets after about 90 days and sampling beyond 90 days provided no improvement in accuracy. Thus, for these growing brown bears on a relatively high-fat diet, the temporal window for QFASA estimates was essentially 90 days. Samples taken before that day still captured some pre-trial diet. This timeframe is longer than the estimates from captive pinnipeds (Kirsch et al. 2000; Nordstrom et al. 2008; Bowen and Iverson 2013), but corresponds well to the general "weeks-to-months" timeframe often cited in QFASA studies. Understanding the timeframe of diet estimates will also help in interpreting QFASA results in the context of seasonal food availability. This could be especially important for highly seasonal foragers like polar bears (Galicia et al. 2020). Although our estimates of turnover are for young, growing bears, rates of fat deposition and mobilization are more likely to be influenced by nutritional status than by age since fat stores are mobilized when dietary intake is insufficient to meet energetic needs, rather than as a function of metabolic rate which would vary with age or size.

This is also the first study to examine progressive changes in the FA profiles of fasting carnivores. While estimating the diets of hibernating animals may not be ecologically insightful, our results are relevant to non-hibernating, fasting carnivores. Polar bears are able to go prolonged periods without food while maintaining activity; a state that has previously been characterized as "walking hibernation" (Nelson et al. 1983). More recent studies have suggested this metabolic state is equivalent to fasting in other mammals (Robbins et al. 2012; Whiteman et al. 2015), but it remains a common occurrence in polar bears, especially during the ice-free season when ice-associated seals are largely unavailable (Derocher et al. 1990; Atkinson and Ramsay 1995; Atkinson et al. 1996). Polar bears may also fast during winter and during the spring breeding season, when adult males are focused on securing mates (Ramsay et al. 1991; Cherry et al. 2009; Rode et al. 2018). Pregnant female polar bears may fast for up to 8 months, including the ice-free period and subsequent maternity denning (Ramsay and Stirling 1986; Atkinson and Ramsay 1995). Our results suggest that diet estimates generated from fasting animals may have to be interpreted cautiously and may be particularly sensitive to inaccurate CCs. When idealized CCs were used, QFASA diet estimates remained highly accurate during the entire fasting period (Fig. 6b). However, using marine-fed mink CCs, accuracy declined significantly after 74 days of fasting. It seems unlikely that wild polar bears would undergo such a prolonged fast on the sea ice, but it may be increasingly common during the ice free-period (Molnár et al. 2020). It is possible that fasting, non-hibernating polar bears would mobilize energy reserves, and alter FA stores, more rapidly than hibernating bears because of higher energetic demands (Whiteman et al. 2015). Studies of free-ranging polar bears in which animals are sampled on shore in summer and fall or immediately after denning will need to take this into account.

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Author contributions GWT, KDR, and CTR conceived the work; GWT analyzed the data and wrote the initial manuscript; JAE and CTR conducted the experiments; SMB analyzed the samples. All authors contributed to writing and editing the final version.

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Availability of data and materials The datasets generated during the current study are available from the corresponding author on reasonable request.

Code availability (software application or custom code) All analyses were conducted in R, version 4.0.0.

Declarations

Conflict of interest The authors confirm that they have no conflicts or completing interests. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government.

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