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

Gregory W. Thiemann*, Karyn D. Rode, Joy A. Erlenbach, Suzanne M. Budge, Charles T. Robbins

G.W. Thiemann: Faculty of Environmental and Urban Change, York University, Toronto, ON Canada; ORCiD 0000-0002-1888-900X

K.D. Rode: US Geological Survey, Alaska Science Center, Anchorage, AK USA; ORCiD 0000-0002-3328-8202

J.A. Erlenbach: School of Biological Sciences, Washington State University, Pullman, WA USA; ORCiD 0000-0003-0347-3711. (Current address: Kodiak National Wildlife Refuge, 1390 Buskin River Rd, Kodiak, AK USA)

S.M. Budge: Process Engineering and Applied Science, Dalhousie University, Halifax, NS Canada; ORCiD 0000-0003-4984-7344

C.T. Robbins: School of the Environment and School of Biological Sciences, Washington State University, Pullman, WA USA; ORCiD 0000-0003-1207-7745

*Corresponding author. E-mail: thiemann@yorku.ca

RH: Fatty acid metabolism in brown bears

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.

Key words: Foraging ecology, brown bear, hibernation, polar bear, QFASA

Funding. Financial support was provided by Natural Sciences and Engineering Research Council (NSERC) Canada, U.S. Fish and Wildlife Service Marine Mammals Management program, US Geological Survey, Interagency Grizzly Bear Committee, FRI Research Grizzly Bear Program, USDA National Institute of Food and Agriculture (Hatch project WNP 00226), Raili Korkka Brown Bear Endowment, Nutritional Ecology Endowment, and Bear Research and Conservation Endowment at Washington State University.

Conflicts of interest/Competing interests. The authors confirm 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 U.S. Government.

Availability of data and material. 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.

Authors' 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.

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. 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,

87 after tissue-diet equilibration (Iverson et al. 2004). CCs can then be applied to either the
88 predator or prey FA data prior to running the QFASA model (Bromaghin et al. 2015).
89 Because metabolic patterns may be species-specific, and potentially influenced by the
90 gross composition of the diet (Rosen and Tollit 2012), a lack of accurate and appropriate
91 CCs can limit the utility of QFASA as an investigative tool. Bromaghin et al. (2017)
92 developed a model that allows for simultaneous estimation of both predator diet
93 composition and CCs using only predator and prey FA data, which may eliminate the
94 need for empirically derived CCs. However, a single set of CCs may not be appropriate
95 for all groups of predators in a population and a better understanding of the influence of
96 diet composition and nutritional status (i.e., whether an animal is gaining, losing, or
97 maintaining body mass) on CCs and diet estimates is needed to determine how estimated
98 CCs should be applied across groups of animals.
99 The temporal window of QFASA diet estimates also remains uncertain. Iverson et
100 al. (2004) assumed that the blubber of captive gray seals (*Halichoerus grypus*) reflected
101 diet consumed over the preceding 3-5 months. Budge et al. (2004) showed that
102 radiolabeled FA consumed in the diet were deposited in gray seal blubber within 12
103 hours. Adipose tissue thus represents an integration of recent and long-term diet and most
104 studies using QFASA assume that results reflect diet over a period of "weeks to months"
105 (Beck et al. 2005; Budge et al. 2006; Galicia et al. 2015; McKinney et al. 2017).
106 However, the ambiguity of this timeframe limits ecological insights.
107 The temporal window of QFASA estimates may also depend on the energy
108 balance of the individual. Although few studies have been conducted, the rate of FA
109 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 4 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 two years (Table 1). The Trial 1 diet consisted of dry dog food (Science Diet, Hill's Pet Nutrition, Inc., Topeka, KS) 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 3 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 was 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 -80ºC 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 with a flame ionization detector and a flexible fused silica column (30 m x 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 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

223 FAs that accounted for a mean of 98.9% (range: 98.2 to 99.5%) of total bear FA. The full
224 FA set was rescaled to sum to 100% across all diets and bears.
225 Calibration coefficients were calculated by dividing the percentage of a given FA
226 in each bear by the percentage of the same FA in the diet, averaged across all 4 bears
227 (Iverson et al. 2004). We used the final (i.e., day 90 or 120) FA value for each bear to
228 calculate CCs. Thus, we generated a separate set of CCs for each of the 3 experimental
229 diets. We also compared our results to two sets of CCs generated from studies of captive
230 mink; one set from mink fed a controlled diet supplemented with herring, seal oil or
231 poultry (n = 37, hereafter called "Mink (all)"), and another set using only those mink fed
232 herring or seal oil (n = 21, hereafter called "marine-fed mink"). Both mink sets have been
233 used previously to estimate the diets of polar bears (see Thiemann 2006 and Thiemann et
234 al. 2008 for details).
235 We used QFASA (Iverson et al. 2004) to generate diet estimates for each bear
236 every time they were sampled. Briefly, QFASA models the FA composition of a predator
237 as a linear mixture of potential prey signatures and estimates diet composition by
238 minimizing the distance between the observed and modeled predator, after applying CCs.
239 We used the Aitchison distance measure and generated estimates in the prey space (see
240 (Bromaghin et al. 2015). We could not use a single set of FA to estimate the diets of all
241 bears in the study because some FA were not present in one or more of the experimental
242 diets or the mink CC sets (Table 2). Therefore, diets of bears in Trial 1 were estimated
243 using the Full FA set, minus those FA < 0.1% in either the Trial 1 diet or Trial 1 bears,
244 yielding a set of 18 FA. For bears in Trials 2, 3, and 4, we used a modeling set of 22 FA
245 that was patterned after Florko et al.'s (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, The R Foundation for Statistical Computing, 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):

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

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 3 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, The R Foundation for Statistical Computing, 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) 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*

292 synthesis or mobilization. Once switched to the Trial 2 diet, bear FA profiles changed
293 rapidly in the direction of the new diet. The most rapid change occurred between day 1
294 and day 45, with more gradual change evident in subsequent samples. During Trial 3,
295 bear FA profiles again changed rapidly in response to the new diet, with large shifts in
296 FA occurring between day 1 and day 29, with more gradual changes thereafter and
297 evidence of stabilization after day 62 (Fig 2).
298 There was variability in FA patterns during hibernation (Trial 4). FA profiles were
299 generally stable during the first 3 weeks of hibernation, but beyond that some FA
300 increased (e.g., 18:1n-9), some decreased (e.g., 20:5n-3), while others remained stable
301 (e.g., 18:2n-6; see Table 3, Fig. 2). Of the 45 total FA, 20 decreased during hibernation
302 (mean change in % total FA: -0.39 ± 0.76, range: -2.92 to -0.01) and 25 increased (mean
303 change in % total FA: 0.31 ± 0.73, range: 0.003 to 3.66). The FA showing the largest
304 proportional changes (i.e., >52%) were only present in small amounts (i.e., < 1% of total
305 FA). Of the FA accounting for >1% of total FA, 20:5n-3 showed the greatest proportional
306 change, declining by 51.5% over 140 days of hibernation (Table 3).
307
308 *Calibration coefficients*
309 Trial 1 yielded CC values for 38 FA, a smaller number than Trial 2 (45 FA) or
310 Trial 3 (44 FA) because of the more limited diversity of FA in the Trial 1 diet. Trial 1
311 CCs were generally comparable with Trial 2 and 3, with some exceptions (Fig 3). CC
312 values from Trials 1 and 2 were virtually identical for 16:1n-9, 18:0, 18:1n-11, and 20:0.
313 In contrast, the calibration for i-17:0 generated from Trial 1 was 37 times higher than the
314 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.

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).

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 (Fig 5 and 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 (d =

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 from day 1 and day 29. 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).

383 *QFASA estimates during hibernation*

384 Trial 4 began when bears entered hibernation, following a 4-week interim period
385 after Trial 3. We used the Trial 3 diet as a benchmark to detect changes in sums of
386 differences because it was the last known diet consumed prior to hibernation. Trial 4 diet
387 estimates generated using Trial 3 CC showed only slight changes over time, although
388 more change was evident when we used marine-fed mink CC (Fig 5). Using marine-fed
389 mink CCs, the estimated contribution of anchovy oil declined 14.7% (from 71.3% to
390 56.6%) over the 140-day period, whereas estimated dog food and salmon oil
391 contributions increased 3.0% and 11.7%, respectively (Fig 5). These patterns were
392 reflected in sums of differences, which were not affected by sampling day when Trial 3
393 CC were used ($F_{4,12} = 1.568$, $p = 0.245$) but did change over time when we used marine-
394 fed mink CC ($F_{4,12} = 10.270$, $p < 0.001$; Fig 6). In the latter case, accuracy declined
395 significantly on the final sampling day, with differences between day 318 and all 4 earlier
396 samples (Tukey contrasts $p < 0.040$; Fig S3).

397

398 **Discussion**

399 Information on diet composition is fundamental to understanding animal ecology.
400 Recent and emerging methods of predator diet estimation, including FA and stable
401 isotope analyses, are premised on a predictable and quantifiable relationship between the
402 biochemical composition of a predator's tissue and that of its composite diet. However,
403 these biochemical relationships may be complex and are poorly understood in many taxa.
404 Our results directly address this knowledge gap and provide a response to calls in the

405 literature for additional experimental studies to improve the accuracy and utility of
406 nutrient-tracking approaches to estimating predator diets (e.g., Bowen and Iverson 2013).
407 This study provides direct estimates of the timeframe represented by FA-based
408 diet estimates in a terrestrial carnivore. Ambiguity about the timeframe of FA turnover
409 has limited the ecological interpretation of QFASA diet estimates. Thus, our results will
410 improve the utility of QFASA as an ecological tool. Our controlled diets were designed to
411 mimic the lipid content and composition of polar bear diets to the degree possible, so our
412 results are most relevant to polar bears, but are also applicable to wild brown bears
413 feeding on marine-based foods (e.g., spawning salmon).
414 The Trial 1 diet was compositionally simple and largely consistent with the
415 maintenance diet the bears received after weaning and prior to the start of the experiment.
416 Consequently, the bears' FA profiles did not noticeably change over the course of Trial 1.
417 QFASA diet estimates were similarly consistent during Trial 1 and estimates were highly
418 accurate, regardless of sampling day or the CC used. The high accuracy was likely
419 influenced by the simple "prey library" (Budge et al. 2006; Bromaghin 2017) of only
420 three potential foods. The clear difference between the compositionally simple dog food
421 (i.e., dominated by ca. six FA) and the two more complex fish oils (Fig. 1) presumably
422 reduced the potential for confounding prey types. However, the similarity of the two fish
423 oils may have impaired diet estimates, as discussed below.
424 When bears were started on a new diet (i.e., Trial 2 and 3), their FA profiles
425 abruptly shifted in the direction of the new diet. Some FAs were more variable across
426 individual bears, as reflected in differences in SD (Table 2), but individual variability was
427 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., 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

474 isomers as their peaks may overlap with each other and with the adjacent 18:1n-9 in
475 chromatographic analysis. The CC value for 20:1n-11 derived from marine-fed mink
476 (4.52) was substantially higher than the value derived from Trial 1 (1.25 ± 0.10) or Trial
477 2 (0.68 ± 0.03), but lower than Trial 3 (14.63 ± 3.17), suggesting the metabolism of this
478 FA is especially sensitive to diet and it thus may not be a useful dietary tracer. Indeed,
479 Bromaghin et al. (2015) found that modelled predator values using mink (all) CCs for this
480 FA were outside the range of prey values (i.e., the FA could not be modelled realistically)
481 and other studies have identified 20:1n-11 as an unreliable dietary indicator (Galicia et al.
482 2015; Goetsch et al. 2018). It is unclear why the marine-fed mink CC value for 20:5n-3
483 (0.14) was lower than either Trial 2 (0.34 ± 0.02) or Trial 3 (0.34 ± 0.04), but this FA
484 may be especially metabolically active. It showed the largest change in concentration
485 during hibernation of any FA > 1%.
486 That the marine-fed mink CCs performed well is also consistent with the results
487 of Bromaghin et al. (2017) who found that CCs derived mathematically from Chukchi
488 Sea polar bear and prey data were similar to those derived from the mink feeding trial
489 (see their Fig 8). Our results thus add to growing evidence that diet-specific variation can
490 be more important than species-specific differences in estimating CCs. Thus, whenever
491 possible, deriving CCs on diets similar to the predator of interest, even if in a model
492 species, may aid in producing the most accurate diet estimates. Estimation of CCs
493 mathematically, as proposed by Bromaghin et al. (2017), provides an alternative
494 approach for diet estimation from FA in which direct estimate of CCs are not required,
495 but feeding trials can continue to be useful in determining when separate CCs need to be
496 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 d. Bowen and Iverson (2013) cite unpublished data that QFASA diet estimates for captive juvenile Steller sea lions (*Eumetopias jubatus*) were most accurate between 56 d and 84 d. 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 (Fig 5 and 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.

519 This is also the first study to examine progressive changes in the FA profiles of
520 fasting carnivores. While estimating the diets of hibernating animals may not be
521 ecologically insightful, our results are relevant to non-hibernating, fasting carnivores.
522 Polar bears are able to go prolonged periods without food while maintaining activity; a
523 state that has previously been characterized as "walking hibernation" (Nelson et al.
524 1983). More recent studies have suggested this metabolic state is equivalent to fasting in
525 other mammals (Robbins et al. 2012; Whiteman et al. 2015), but it remains a common
526 occurrence in polar bears, especially during the ice-free season when ice-associated seals
527 are largely unavailable (Derocher et al. 1990; Atkinson and Ramsay 1995; Atkinson et al.
528 1996). Polar bears may also fast during winter and during the spring breeding season,
529 when adult males are focused on securing mates (Ramsay et al. 1991; Cherry et al. 2009;
530 Rode et al. 2018). Pregnant female polar bears may fast for up to 8 months, including the
531 ice-free period and subsequent maternity denning (Ramsay and Stirling 1986; Atkinson
532 and Ramsay 1995). Our results suggest that diet estimates generated from fasting animals
533 may have to be interpreted cautiously and may be particularly sensitive to inaccurate
534 CCs. When idealized CCs were used, QFASA diet estimates remained highly accurate
535 during the entire fasting period (Fig 6b). However, using marine-fed mink CCs, accuracy
536 declined significantly after 74 days of fasting. It seems unlikely that wild polar bears
537 would undergo such a prolonged fast on the sea ice, but it may be increasingly common
538 during the ice free-period (Molnár et al. 2020). It is possible that fasting, non-hibernating
539 polar bears would mobilize energy reserves, and alter FA stores, more rapidly than
540 hibernating bears because of higher energetic demands (Whiteman et al. 2015). Studies of

541 free-ranging polar bears in which animals are sampled on shore in summer and fall or

542 immediately after denning will need to take this into account.

543

544 **References**

545 Anderson DB, Kauffman RG, Benevenga NJ (1972) Estimate of fatty acid turnover in
546 porcine adipose tissue. Lipids 7:488–489. https://doi.org/10.1007/BF02533166

547 Atkinson SN, Nelson RA, Ramsay MA (1996) Changes in the body composition of
548 fasting polar bears (*Ursus maritimus*): the effect of relative fatness on protein
549 conservation. Physiol Zool 69:304–316

550 Atkinson SN, Ramsay MA (1995) The effects of prolonged fasting of the body
551 composition and reproductive success of female polar bears (*Ursus maritimus*).
552 Funct Ecol 9:559. https://doi.org/10.2307/2390145

553 Barnas AF, Iles DT, Stechmann TJ, et al (2020) A phenological comparison of grizzly
554 (*Ursus arctos*) and polar bears (*Ursus maritimus*) as waterfowl nest predators in
555 Wapusk National Park. Polar Biol 43:457–465. https://doi.org/10.1007/s00300-
556 020-02647-w

557 Barnett A, Redd KS, Frusher SD, et al (2010) Non-lethal method to obtain stomach
558 samples from a large marine predator and the use of DNA analysis to improve
559 dietary information. J Exp Mar Biol Ecol 393:188–192.
560 https://doi.org/10.1016/j.jembe.2010.07.022

561 Beck CA, Iverson SJ, Bowen WD (2005) Blubber fatty acids of gray seals reveal sex
562 differences in the diet of a size-dimorphic marine carnivore. Can J Zool 83:377–
563 388. https://doi.org/10.1139/z05-021

564 Beck CA, Rea LD, Iverson SJ, et al (2007) Blubber fatty acid profiles reveal regional,
565 seasonal, age-class and sex differences in the diet of young Steller sea lions in
566 Alaska. Mar Ecol Prog Ser 338:269–280

567 Best RC (1985) Digestibility of ringed seals by the polar bear. Can J Zool 63:1033–1036

568 Bourque J, Atwood TC, Divoky GJ, et al (2020) Fatty acid-based diet estimates suggest
569 ringed seal remain the main prey of southern Beaufort Sea polar bears despite
570 recent use of onshore food resources. Ecol Evol. 10:2093-2103.
571 https://doi.org/10.1002/ece3.6043

572 Bowen WD, Iverson SJ (2013) Methods of estimating marine mammal diets: A review of
573 validation experiments and sources of bias and uncertainty. Mar Mammal Sci
574 29:719–754. https://doi.org/10.1111/j.1748-7692.2012.00604.x

Bromaghin JF (2017) qfasar: quantitative fatty acid signature analysis with R. Methods Ecol Evol 8:1158–1162. https://doi.org/10.1111/2041-210X.12740

Bromaghin JF, Budge SM, Thiemann GW, Rode KD (2017) Simultaneous estimation of diet composition and calibration coefficients with fatty acid signature data. Ecol Evol 7:6103–6113. https://doi.org/10.1002/ece3.3179

Bromaghin JF, Rode KD, Budge SM, Thiemann GW (2015) Distance measures and optimization spaces in quantitative fatty acid signature analysis. Ecol Evol 5:1249–1262. https://doi.org/10.1002/ece3.1429

Budge SM, Iverson SJ, Koopman HN (2006) Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Mar Mammal Sci 22:759–801. https://doi.org/10.1111/j.1748-7692.2006.00079.x

Budge SM, Penney SN, Lall SP, Trudel M (2012) Estimating diets of Atlantic salmon (*Salmo salar*) using fatty acid signature analyses; validation with controlled feeding studies. Can J Fish Aquat Sci 69:1033–1046. https://doi.org/10.1139/f2012-039

Budge SM, Townsend K, Lall SP, Bromaghin JF (2020) Dietary fat concentrations influence fatty acid assimilation patterns in Atlantic pollock (*Pollachius virens*). Philos Trans R Soc B Biol Sci 375:20190649. https://doi.org/10.1098/rstb.2019.0649

Cahill JA, Heintzman PD, Harris K, et al (2018) Genomic evidence of widespread admixture from polar bears into brown bears during the Last Ice Age. Mol Biol Evol 35:1120–1129. https://doi.org/10.1093/molbev/msy018

Cahill JA, Stirling I, Kistler L, et al (2015) Genomic evidence of geographically widespread effect of gene flow from polar bears into brown bears. Mol Ecol 24:1205–1217. https://doi.org/10.1111/mec.13038

Cherry SG, Derocher AE, Stirling I, Richardson ES (2009) Fasting physiology of polar bears in relation to environmental change and breeding behavior in the Beaufort Sea. Polar Biol 32:383–391. https://doi.org/10.1007/s00300-008-0530-0

Chevallier C, Gauthier G, Lai S, Berteaux D (2020) Pulsed food resources affect reproduction but not adult apparent survival in arctic foxes. Oecologia 193:557–569. https://doi.org/10.1007/s00442-020-04696-8

Derocher AE, Lunn NJ, Stirling I (2004) Polar bears in a warming climate. Integr Comp Biol 44:163–176

Derocher AE, Nelson RA, Stirling I, Ramsay MA (1990) Effects of fasting and feeding on serum urea and serum creatinine levels in polar bears. Mar Mammal Sci 6:196–203

611 Doupe JP, England JH, Furze M, Paetkau D (2007) Most northerly observation of a
612 grizzly bear (Ursus arctos) in Canada: Photographic and DNA evidence from
613 Melville Island, northwest territories. Arctic 60:271–276

614 Florant GL, Nuttle LC, Mullinex DE, Rintoul DA (1990) Plasma and white adipose tissue
615 lipid composition in marmots. Am J Physiol Regul Integr Comp Physiol
616 258:1123–1131

617 Foglia TA, Cartwright AL, Gyurik RJ, Philips JG (1994) Fatty acid turnover rates in the
618 adipose tissues of the growing chicken (*Gallus domesticus*). Lipids 29:497–502.
619 https://doi.org/10.1007/BF02578247

620 Fry B (2006) Stable isotope ecology. Springer, New York, NY

621 Fuller TK, Sievert PR (2001) Carnivore demography and the consequences of changes in
622 prey availability. In: Gittleman JL, Funk SM, Macdonald DW, Wayne RK (eds)
623 Carnivore Conservation. Cambridge University Press, New York, NY, pp 163–
624 178

625 Galicia MP, Thiemann GW, Dyck MG, et al (2016) Dietary habits of polar bears in Foxe
626 Basin, Canada: possible evidence of a trophic regime shift mediated by a new top
627 predator. Ecol Evol 6:6005–6018. https://doi.org/10.1002/ece3.2173

628 Galicia MP, Thiemann GW, Dyck MG, Ferguson SH (2015) Characterization of polar
629 bear (*Ursus maritimus*) diets in the Canadian High Arctic. Polar Biol 38:1983–
630 1992. https://doi.org/10.1007/s00300-015-1757-1

631 Goetsch C, Conners MG, Budge SM, et al (2018) Energy-rich mesopelagic fishes
632 revealed as a critical prey resource for a deep-diving predator using quantitative
633 fatty acid signature analysis. Front Mar Sci 5:.
634 https://doi.org/10.3389/fmars.2018.00430

635 Haynes TB, Schmutz JA, Bromaghin JF, et al (2015) Diet of yellow-billed loons (*Gavia*
636 *adamsii*) in Arctic lakes during the nesting season inferred from fatty acid
637 analysis. Polar Biol 38:1239–1247. https://doi.org/10.1007/s00300-015-1690-3

638 Hill VL, Florant GL (1999) Patterns of fatty acid composition in free-ranging yellow-
639 bellied marmots (*Marmota flaviventris*) and their diet. Can J Zool 77:1494–1503

640 Iverson SJ, Field C, Bowen WD, Blanchard W (2004) Quantitative fatty acid signature
641 analysis: a new method of estimating predator diets. Ecol Monogr 74:211–235

642 Iverson SJ, Stirling I, Lang SLC (2006) Spatial and temporal variation in the diets of
643 polar bears across the Canadian Arctic: indicators of changes in prey populations
644 and environment. In: Boyd IL, Wanless S, Camphuysen CJ (eds) Top predators in
645 marine ecosystems. Cambridge University Press, New York, NY, pp 98–117

646 Kirsch PE, Iverson SJ, Bowen WD (2000) Effect of a low-fat diet on body composition
647 and blubber fatty acids of captive juvenile harp seals (*Phoca groenlandica*).
648 Physiol Biochem Zool 73:45–59

649 Klare U, Kamler JF, Macdonald DW (2011) A comparison and critique of different scat-
650 analysis methods for determining carnivore diet: Comparison of scat-analysis
651 methods. Mammal Rev 41:294–312. https://doi.org/10.1111/j.1365-
652 2907.2011.00183.x

653 Lunn NJ, Servanty S, Regehr EV, et al (2016) Demography of an apex predator at the
654 edge of its range: impacts of changing sea ice on polar bears in Hudson Bay. Ecol
655 Appl 26:1302–1320. https://doi.org/10.1890/15-1256

656 McKinney MA, Atwood TC, Iverson SJ, Peacock E (2017) Temporal complexity of
657 southern Beaufort Sea polar bear diets during a period of increasing land use.
658 Ecosphere 8:e01633. https://doi.org/10.1002/ecs2.1633

659 McKinney MA, Iverson SJ, Fisk AT, et al (2013) Global change effects on the long-term
660 feeding ecology and contaminant exposures of East Greenland polar bears. Glob
661 Change Biol 19:2360–2372. https://doi.org/10.1111/gcb.12241

662 Meynier L, Morel PCH, Chilvers BL, et al (2010) Quantitative fatty acid signature
663 analysis on New Zealand sea lions: model sensitivity and diet estimates. J
664 Mammal 91:1484–1495. https://doi.org/10.1644/09-MAMM-A-299.1

665 Miller S, Schliebe S, Proffitt K (2006) Demographics and behavior of polar bears feeding
666 on bowhead whale carcasses at Barter and Cross Islands, Alaska, 2002–2004. US
667 Fish and Wildlife Service, Anchorage, AK USA

668 Molnár PK, Bitz CM, Holland MM, et al (2020) Fasting season length sets temporal
669 limits for global polar bear persistence. Nat Clim Change 10:732–738.
670 https://doi.org/10.1038/s41558-020-0818-9

671 Nelson RA, Folk GE Jr, Pfeiffer EW, et al (1983) Behavior, biochemistry, and
672 hibernation in black, grizzly, and polar bears. Int Conf Bear Res Manag 5:284–
673 290

674 Nordstrom CA, Wilson LJ, Iverson SJ, Tollit DJ (2008) Evaluating quantitative fatty acid
675 signature analysis (QFASA) using harbour seals *Phoca vitulina richardsi* in
676 captive feeding studies. Mar Ecol Prog Ser 360:245–263

677 Northrup JM, Pitt J, Muhly TB, et al (2012) Vehicle traffic shapes grizzly bear behaviour
678 on a multiple-use landscape. J Appl Ecol 49:1159–1167.
679 https://doi.org/10.1111/j.1365-2664.2012.02180.x

680 Pagano AM, Durner GM, Rode KD, et al (2018) High-energy, high-fat lifestyle
681 challenges an Arctic apex predator, the polar bear. Science 359:568–572.
682 https://doi.org/10.1126/science.aan8677

683 Parrish FA, Abernathy K, Marshall GJ, Buhleier BM (2002) Hawaiian monk seals
684 (*Monachus schauinslandi*) foraging in deep-water coral beds. Mar Mammal Sci
685 18:244–258. https://doi.org/10.1111/j.1748-7692.2002.tb01031.x

686 Peterson RO, Thomas NJ, Thurber JM, et al (1998) Population limitation and the wolves
687 of Isle Royale. J Mammal 79:828. https://doi.org/10.2307/1383091

688 Pilfold NW, Derocher AE, Stirling I, Richardson E (2015) Multi-temporal factors
689 influence predation for polar bears in a changing climate. Oikos 124:1098–1107.
690 https://doi.org/10.1111/oik.02000

691 Pongracz JD, Paetkau D, Branigan M, Richardson E (2017) Recent hybridization
692 between a polar bear and grizzly bears in the Canadian Arctic. Arctic 70:151.
693 https://doi.org/10.14430/arctic4643

694 Raclot T (2003) Selective mobilization of fatty acids from adipose tissue triacylglycerols.
695 Prog Lipid Res 42:257–288. https://doi.org/10.1016/S0169-7827(02)00066-8

696 Ramsay MA, Nelson RA, Stirling I (1991) Seasonal changes in the ratio of serum urea to
697 creatinine in feeding and fasting polar bears. Can J Zool 69:298–302

698 Ramsay MA, Stirling I (1986) On the mating system of polar bears. Can J Zool 64:2142–
699 2151

700 Regehr EV, Lunn NJ, Amstrup SC, Stirling I (2007) Effects of earlier sea ice breakup on
701 survival and population size of polar bears in Western Hudson Bay. J Wildl
702 Manag 71:2673–2683. https://doi.org/10.2193/2006-180

703 Robbins CT, Lopez-Alfaro C, Rode KD, et al (2012) Hibernation and seasonal fasting in
704 bears: the energetic costs and consequences for polar bears. J Mammal 93:1493–
705 1503. https://doi.org/10.1644/11-MAMM-A-406.1

706 Rode KD, Regehr EV, Douglas DC, et al (2014) Variation in the response of an Arctic
707 top predator experiencing habitat loss: feeding and reproductive ecology of two
708 polar bear populations. Glob Change Biol 20:76–88.
709 https://doi.org/10.1111/gcb.12339

710 Rode KD, Stricker CA, Erlenbach J, et al (2016) Isotopic incorporation and the effects of
711 fasting and dietary lipid content on isotopic discrimination in large carnivorous
712 mammals. Physiol Biochem Zool 89:182–197. https://doi.org/10.1086/686490

713 Rode KD, Wilson RR, Douglas DC, et al (2018) Spring fasting behavior in a marine apex
714 predator provides an index of ecosystem productivity. Glob Change Biol 24:410–
715 423. https://doi.org/10.1111/gcb.13933

716 Rosen DAS, Tollit DJ (2012) Effects of phylogeny and prey type on fatty acid calibration
717 coefficients in three pinniped species: implications for the QFASA dietary

718 quantification technique. Mar Ecol Prog Ser 467:263–276.
719 https://doi.org/10.3354/meps09934

720 Sierro A, Arlettaz R (1997) Barbastelle bats (*Barbastella* spp.) specialize in the predation
721 of moths: implications for foraging tactics and conservation. Acta Oecologica
722 18:91–106. https://doi.org/10.1016/S1146-609X(97)80067-7

723 Stirling I (1974) Midsummer observations on the behavior of wild polar bears (Ursus
724 maritimus). Can J Zool 52:1191–1198

725 Stirling I, Archibald WR (1977) Aspects of predation of seals by polar bears. J Fish Res
726 Board Can 34:1126–1129

727 Stirling I, McEwan EH (1975) The caloric value of whole ringed seals (*Phoca hispida*) in
728 relation to polar bear (*Ursus maritimus*) ecology and hunting behavior. Can J Zool
729 53:1021–1027

730 Thiemann GW (2006) Continental scale variation in polar bear (*Ursus maritimus*) diets
731 and the fatty acid signatures of their marine mammal prey. PhD dissertation,
732 Dalhousie University

733 Thiemann GW, Iverson SJ, Stirling I (2008) Polar bear diets and arctic marine food webs:
734 insights from fatty acid analysis. Ecol Monogr 78:591–613

735 Wang SW, Hollmen TE, Iverson SJ (2010) Validating quantitative fatty acid signature
736 analysis to estimate diets of spectacled and Steller's eiders (*Somateria fischeri* and
737 *Polysticta stelleri*). J Comp Physiol B 180:125–139

738 Welch AJ, Bedoya-Reina OC, Carretero-Paulet L, et al (2014) Polar bears exhibit
739 genome-wide signatures of bioenergetic adaptation to life in the Arctic
740 environment. Genome Biol Evol 6:433–450. https://doi.org/10.1093/gbe/evu025

741 Whiteman JP, Harlow HJ, Durner GM, et al (2015) Summer declines in activity and body
742 temperature offer polar bears limited energy savings. Science 349:295–298.
743 https://doi.org/10.1126/science.aaa8623

744

745

746

747

748

749

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 (d)
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

Table 2 FA composition of diets and bears and resulting calibration coefficients calculated from 3 controlled feeding studies of 4 juvenile brown bears

	Trial 1 - Dog food					Trial 2 - Dog food + salmon oil					Trial 3 - Dog food + 30% fish oil						
		Final Bear FA (%)		CC			Final Bear FA (%)		CC			Final Bear FA (%)		CC			
Fatty acid	Diet (%)	Mean	SD	Mean	SD	Diet (%)	Mean	SD	Mean	SD	Diet (%)	Mean	SD	Mean	SD	Mink CC	Mink (marine) CC
Saturated																	
14:0*	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*	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*	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*	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*	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*	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*	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*	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*	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*	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*	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*	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*	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
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*	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*	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*	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*	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*	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					

* denotes the 18 fatty acids used to generate QFASA estimates for bears in Trial 1
Bold type denotes the 22 fatty acids used to generate QFASA estimates for bears in Trial 2-4

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

	Start concentration (%)		Final Concentration (%)		Total individual change		Percent individual change (%)	
Fatty acid	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. 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 4 juvenile brown bears during 3 feeding trials, followed by hibernation (Trial 4). Pink circles indicate the FA composition of the experimental diets

Fig. 3 Mean calibration coefficients (log scale, ± SD) calculated from 4 juvenile brown bears at the conclusion of 3 feeding trials. Also shown are calibration coefficients from captive mink fed a marine-based diet (mink data from Thiemann 2006, Thiemann et al. 2008)

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

Fig. 5 Mean (± SD) diet estimates for four brown bears sampled intermittently during controlled feeding experiments. See Table 1 for diet composition. Panels A and 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). Panels C and D: Diet

23 estimation used calibration coefficients generated from captive mink fed a marine-based
24 diet (Thiemann et al. 2008)
25
26 **Fig. 6** Mean (± SD) sum of differences between estimated and actual diets for four brown
27 bears sampled intermittently during controlled feeding experiments. See Table 1 for diet
28 composition. Panels A and B: Diet estimation used calibration coefficients generated
29 from the same trial for trials 1-3; trial 4 used calibration coefficients from trial 3 (see text
30 for details). Panels C and D: Diet estimation used calibration coefficients generated from
31 captive mink fed a marine-based diet (Thiemann et al. 2008)
32
33 **Fig S1** Concentration (mass % of total FA) of all 45 FA in adipose tissue of 4 juvenile
34 brown bears over 3 feeding trials, followed by hibernation (Trial 4). Pink circles indicate
35 the FA composition of the experimental diets
36
37 **Fig S2** Sums of differences between actual and estimated diet composition across
38 different sets of calibration coefficients in 3 controlled feeding trials. Boxplots show
39 upper and lower quartiles and maximum, minimum, and median values
40
41 **Fig S3** Sums of differences between estimated and actual diets for four brown bears
42 sampled intermittently during controlled feeding experiments. See Table 1 for diet
43 composition. Diet estimation used calibration coefficients generated from the same trial
44 (top row) or from captive mink fed a marine-based diet (bottom row). Boxplots show
45 upper and lower quartiles and maximum, minimum, and median values

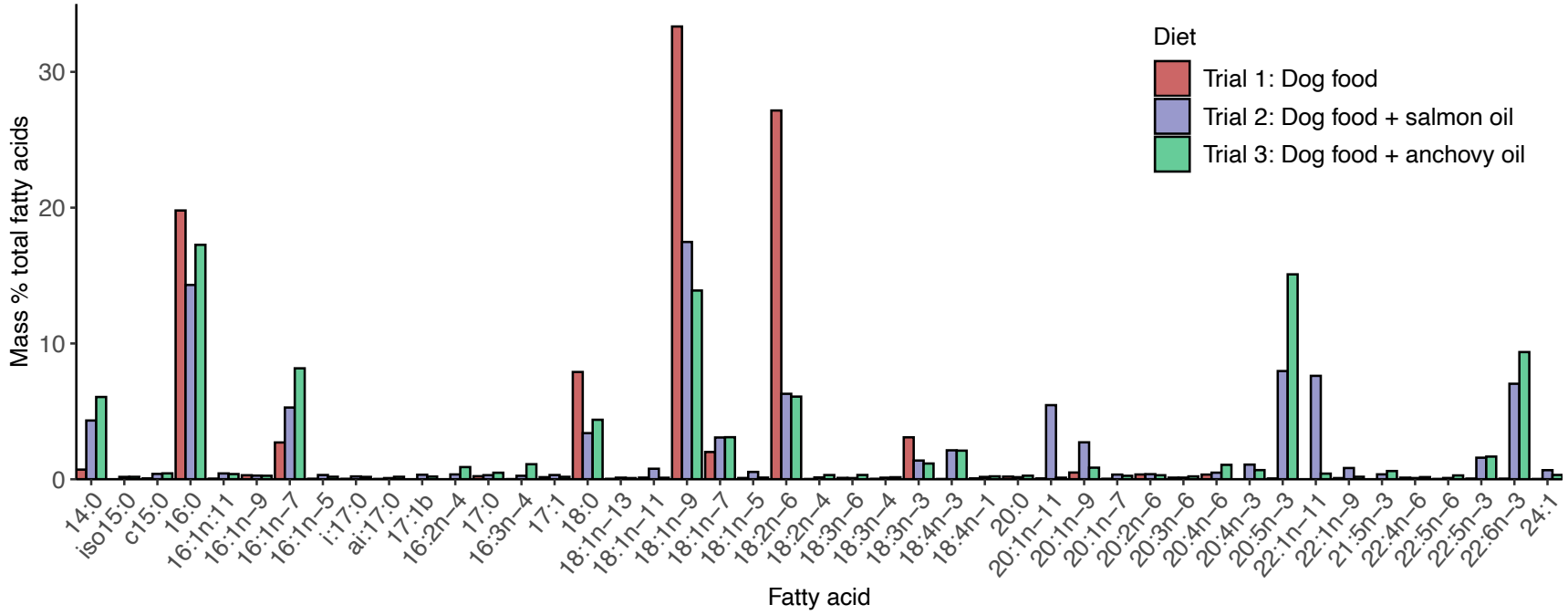
Diet
Trial 1: Dog food
Trial 2: Dog food + salmon oil
Trial 3: Dog food + anchovy oil
Mass % total fatty acids
0
10
20
30
Fatty acid
14:0
iso15:0
c15:0
16:0
16:1n-11
16:1n-9
16:1n-7
16:1n-5
i-17:0
ai-17:0
17:1b
16:2n-4
17:0
16:3n-4
17:1
18:0
18:1n-13
18:1n-11
18:1n-9
18:1n-7
18:1n-5
18:2n-6
18:2n-4
18:3n-6
18:3n-4
18:3n-3
18:4n-3
18:4n-1
20:0
20:1n-11
20:1n-9
20:1n-7
20:2n-6
20:3n-6
20:4n-6
20:4n-3
20:5n-3
22:1n-11
22:1n-9
21:5n-3
22:4n-6
22:5n-6
22:5n-3
22:6n-3
24:1

Fig. 1 R1

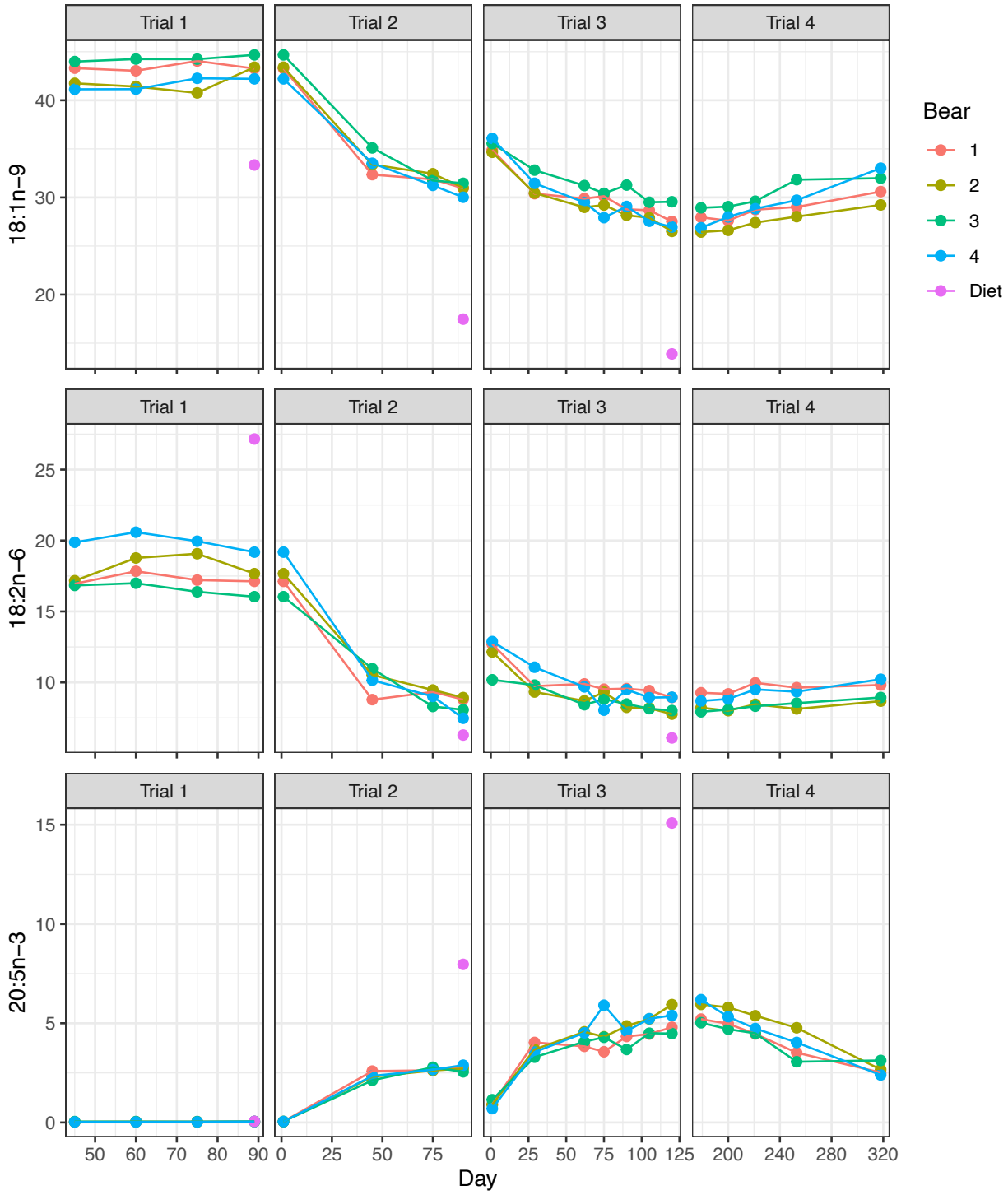
Trial 1
Trial 2
Trial 3
Trial 4
18:1n−9
18:2n−6
20:5n−3
Day
Bear
1
2
3
4
Diet

Fig. 2 R1

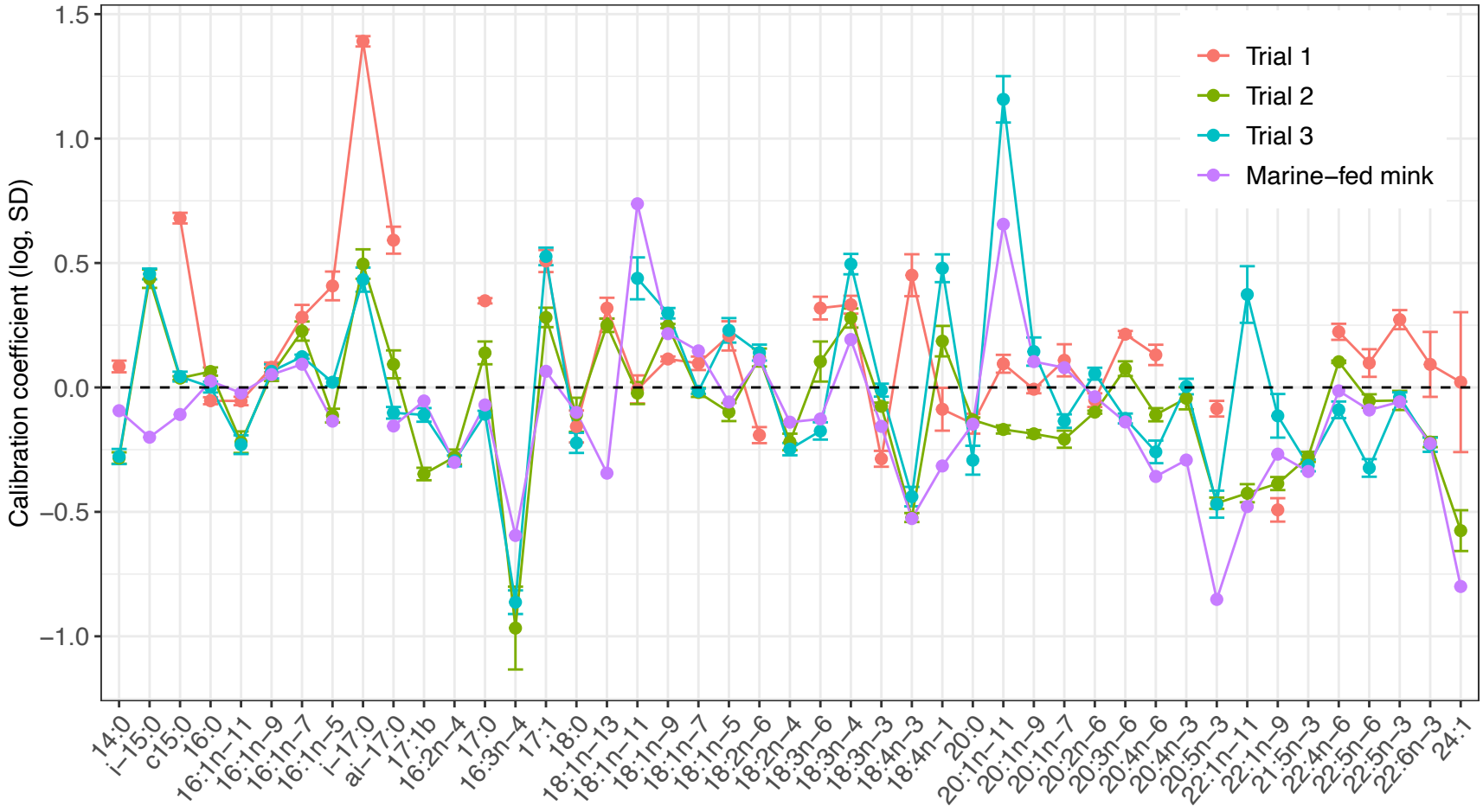
Calibration coefficient (log, SD)
1.5
1.0
0.5
0.0
−0.5
−1.0
Trial 1
Trial 2
Trial 3
Marine−fed mink
14:0
i−15:0
c15:0
16:0
16:1n−11
16:1n−9
16:1n−7
16:1n−5
i−17:0
ai−17:0
17:1b
16:2n−4
17:0
16:3n−4
17:1
18:0
18:1n−13
18:1n−11
18:1n−9
18:1n−7
18:1n−5
18:2n−6
18:2n−4
18:3n−6
18:3n−4
18:3n−3
18:4n−3
18:4n−1
20:0
20:1n−11
20:1n−9
20:1n−7
20:2n−6
20:3n−6
20:4n−6
20:4n−3
20:5n−3
22:1n−11
22:1n−9
21:5n−3
22:4n−6
22:5n−6
22:5n−3
22:6n−3
24:1

Fig. 3 R1

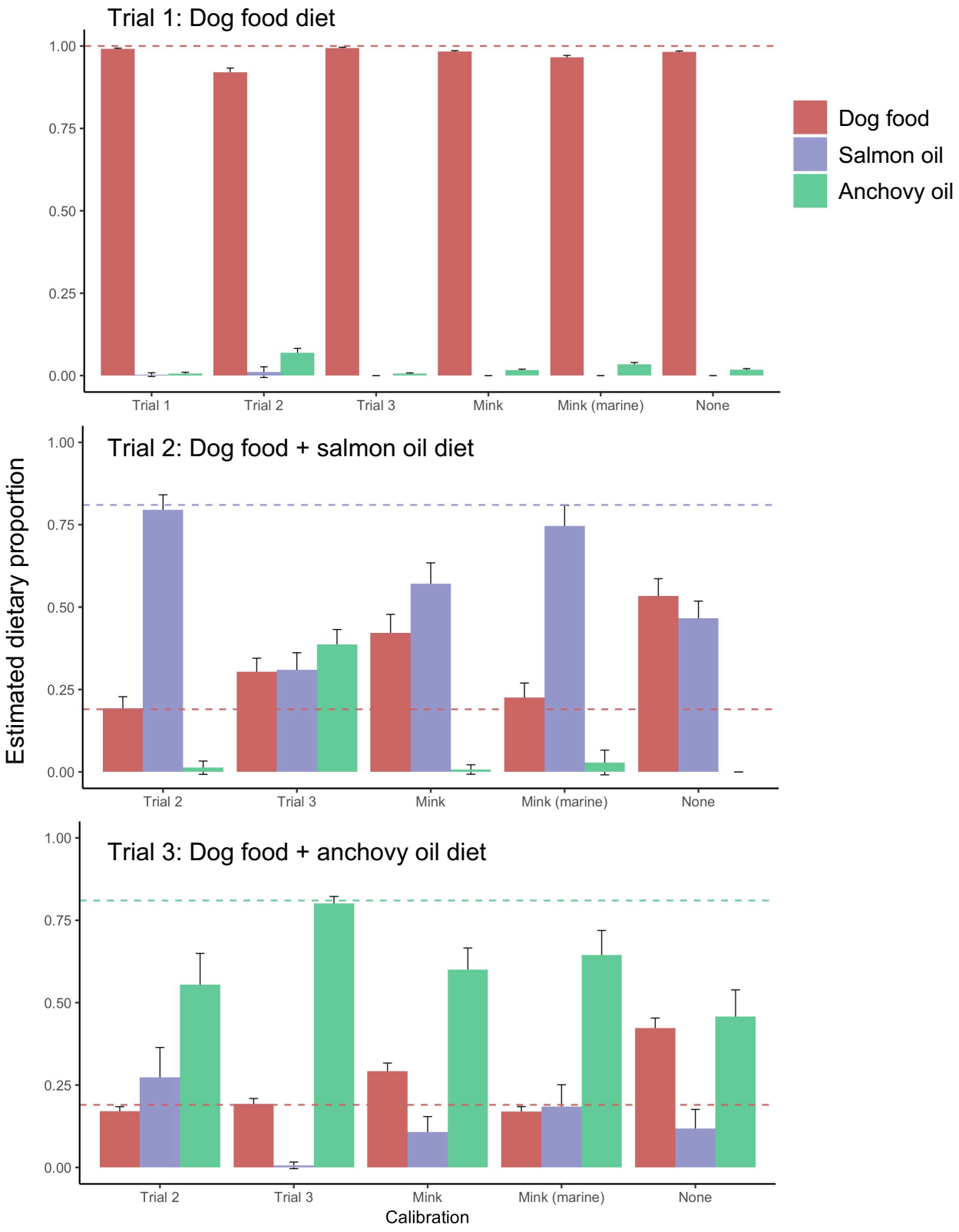
Trial 1: Dog food diet
Trial 2: Dog food + salmon oil diet
Trial 3: Dog food + anchovy oil diet
Estimated dietary proportion
1.00
0.75
0.50
0.25
0.00
Trial 1
Trial 2
Trial 3
Mink
Mink (marine)
None
Calibration
Dog food
Salmon oil
Anchovy oil

Fig. 4 R1

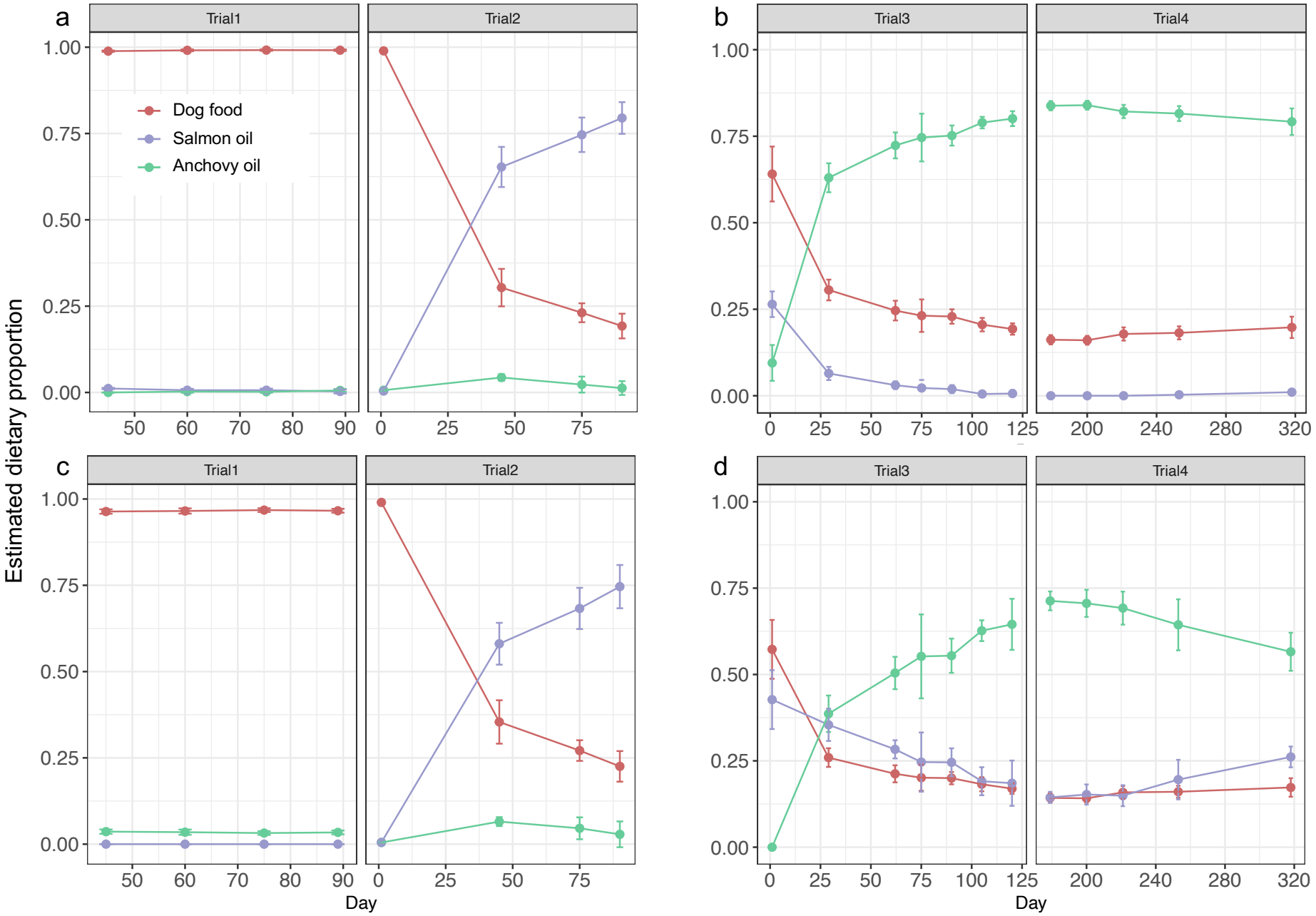
a
Trial1
Trial2
b
Trial3
Trial4
c
Trial1
Trial2
d
Trial3
Trial4
Dog food
Salmon oil
Anchovy oil
Estimated dietary proportion
1.00
0.75
0.50
0.25
0.00
50
60
70
80
90
0
25
50
75
100
125
200
240
280
320
Day

Fig. 5 R1

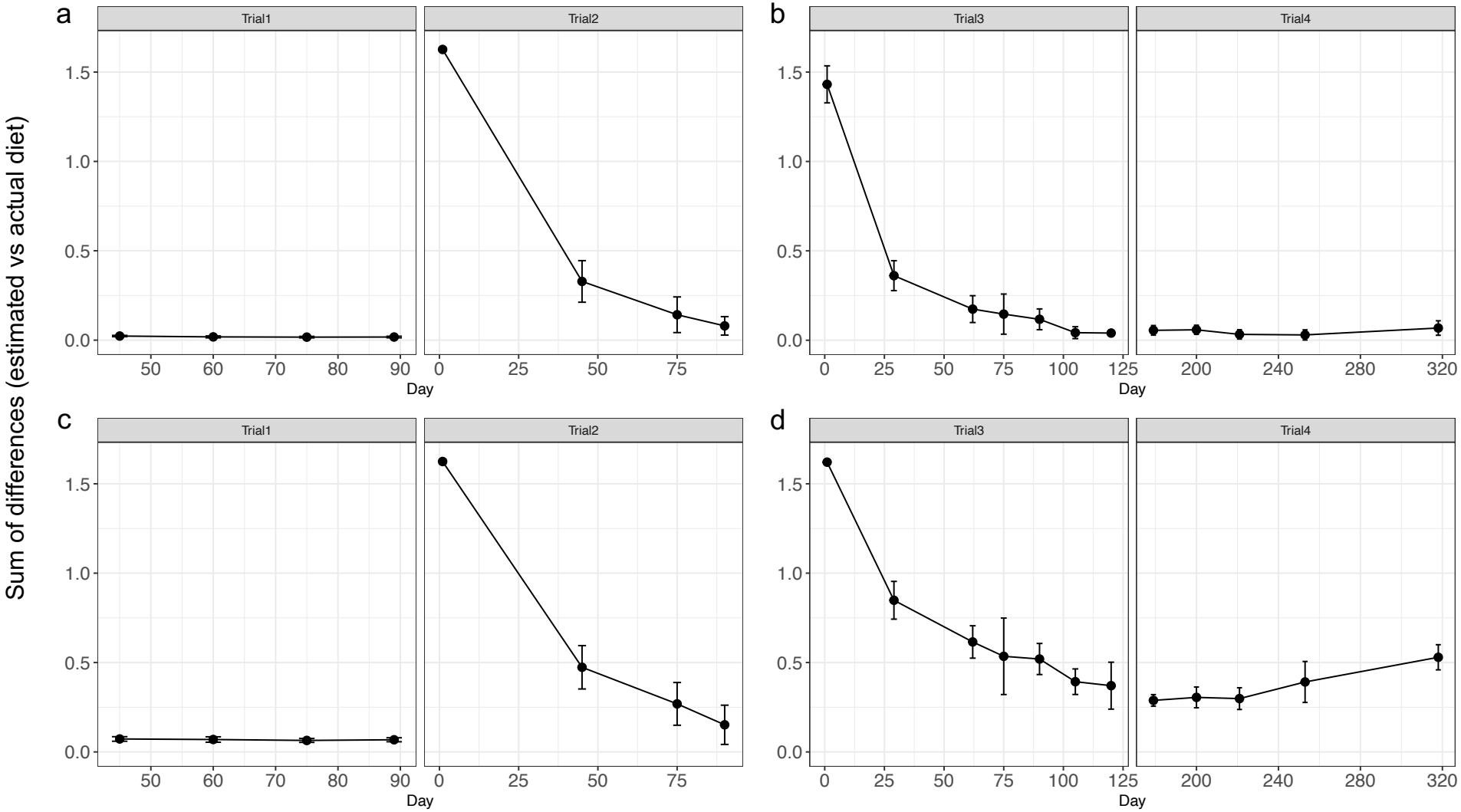
a
Trial1
Trial2
b
Trial3
Trial4
c
Trial1
Trial2
d
Trial3
Trial4
Sum of differences (estimated vs actual diet)
Day
Day
Day
Day

Fig. 6 R1