

Using fatty acids to study marine mammal foraging: The evidence from an extensive and growing literature

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In his letter of criticism of our two recent papers on foraging habits of Arctic marine mammals (Thiemann *et al.* 2007, 2008), Grahl-Nielsen (2008) questions our interpretations and methods in using blubber fatty acids (FAs) as indicators of diet. The objections raised by Grahl-Nielsen are not new: they are the same arguments he has made for nearly a decade (Grahl-Nielsen 1999) and which have been refuted or addressed elsewhere in the literature (references below). Although FA analysis as an ecological tool has seen dramatic advancements in recent years, Grahl-Nielsen continues to ignore the substantial and growing body of scientific evidence that supports its use. It is unfortunate that we must repeat what has been thoroughly addressed and reviewed by a number of authors, including Budge *et al.* (2006) in this journal. However, because Grahl-Nielsen's comments are so flawed, and because we have been given the opportunity to respond, we endeavor to address his specific objections as succinctly as possible.

Grahl-Nielsen's letter has two main contentions: the first is that because other factors besides diet (*e.g.*, population, age, sex, condition, *etc.*) may influence the FA composition of marine mammal blubber, FA profiles cannot be used as an indicator of diet; concurrent with this argument, he states that only two captive experimental studies have ever been conducted and that the effect of dietary FAs on blubber FAs has never been established. The other main contention of the letter is that when FA profiles are used as an indicator of diet, only the inner portion of blubber should

be considered. In addition to being mutually exclusive and contradictory to one another, both of Grahl-Nielsen's main points are factually incorrect and ignore or misrepresent decades of published research. As Dalsgaard *et al.* (2003) wrote in their comprehensive review, "Fatty acids have been used as qualitative markers to trace or confirm predator-prey relationships in the marine environment for more than thirty years." To suggest that, among all animal taxa, marine mammals are the single exception in this regard, is to dismiss basic facts about the pathways of FA metabolism shared among animals and is wholly without support from the scientific literature.

POINT 1

Currently, it has not been scientifically established how dietary FAs affect the FA composition of the blubber. Surprisingly, few investigations have been conducted where blubber and prey have been analysed together.

This claim is simply not correct. An extensive scientific literature exists that demonstrates and quantifies the relationship between a consumer's FA stores and the FA composition of its diet. Marine mammals have the same basic metabolic pathways as other higher vertebrates and the predictable incorporation of ingested FAs into adipose tissue and blubber has been experimentally demonstrated through controlled feeding studies of seals (Kirsch *et al.* 2000, Iverson *et al.* 2003, 2004; Budge *et al.* 2004, Cooper 2004, Tollit *et al.* 2006, Cooper *et al.* 2006, Nordstrom *et al.* 2008), terrestrial and aquatic carnivores (*e.g.*, Reidinger *et al.* 1985, Rouvinen and Kiiskinen 1989, Rouvinen *et al.* 1992, Iverson *et al.* 2004, Cooper *et al.* 2006), and seabirds (Iverson *et al.* 2007), as well as fish and invertebrates (reviewed in Dalsgaard *et al.* 2003). In addition to these controlled experiments, many other studies have analyzed the FA composition of the blubber of free-ranging marine mammals and their prey "together" (note that some studies report predator and prey data in separate papers although they were collected "together," that is, spatially and temporally "from the same waters," which is clearly explained in the papers themselves; *e.g.*, Ackman and Eaton 1966, Iverson *et al.* 1995, 1997, 2004; Dahl *et al.* 2000, Bradshaw *et al.* 2003, Cooper 2004, Falk-Petersen *et al.* 2004, Beck *et al.* 2007*a, b*; Budge *et al.* 2008, Iverson 2008, Tucker *et al.* 2008). This has also been done for prey and adipose tissue FAs in free-ranging marine and terrestrial carnivores and seabirds (*e.g.*, Raclot *et al.* 1998, Iverson *et al.* 2006, 2007; Thiemann *et al.*, in press). Taken together, these studies of captive and free-ranging animals clearly confirm that diet is the primary factor determining adipose tissue and blubber FA composition in higher monogastric predators. The single laboratory experiment that does not confirm this (Grahl-Nielsen and Mjaavatten 1991) was invalid because the only samples obtained from seals were 2 mg ("a small drop") of skin-associated blubber (not general "outer layer" blubber, but a micro-sample taken directly under the skin). Such samples have been shown to produce strongly biased results (Thiemann *et al.* 2004).

Contrary to Grahl-Nielsen's claim, many of the above studies also specifically address other factors that can *directly* influence FA deposition. These factors include the effects of dietary lipid levels on FA metabolism, peroxisomal chain-shortening of long-chain monounsaturates, and possible FA-specific mobilization rates. Differences in blubber FA composition associated with factors such as population, age, sex, condition, temperature, and geographical location are confounded by underlying and overriding dietary differences; only in the outermost blubber (or head fats) of some cetaceans do metabolic influences potentially dominate over dietary influences on FA composition (*e.g.*, Koopman *et al.* 1996, 2003, 2006; Budge *et al.* 2006). Even in ectothermic fish where temperature may have a somewhat greater influence on FAs, it has been clearly demonstrated that dietary FAs are by far the greatest determinant of predator FA composition (reviewed in Dalsgaard *et al.* 2003 and Iverson 2008). The overwhelming conclusion from all of these studies is that blubber and adipose tissue can be used to infer aspects of, and differences in, predator diets.

Why Grahl-Nielsen raises the issue of calibration coefficients is unclear, as neither he, nor the two papers he criticized in his comment, deal with quantitative estimates of diet. For clarification, we remind readers that calibration coefficients (Iverson *et al.* 2004) are not relevant to the use of FAs in a qualitative manner to infer dietary differences among individuals or groups of predators (as was done in Thiemann *et al.* 2007, 2008). However, if Grahl-Nielsen wishes to address the issue of quantitative estimates, then he is also incorrect in stating that "it is an impossible task" to identify FA-specific patterns of metabolism. Estimates of calibration coefficients based on such patterns have now been established for five species of phocid and otariid seals, as well as mink (*Mustela vison*) and seabirds (reviewed in Iverson 2008). In general, all are remarkably similar, although some differences among taxa may exist. We are not aware of a single study of predator FAs that has implied that the quantitative estimation of predator diets using FAs is simple and without complication. Nevertheless, quantitative techniques and the use of calibration coefficients have been successfully employed and verified (*e.g.*, Cooper 2004, Iverson *et al.* 2003, 2004, 2007; Cooper *et al.* 2005, Tollit *et al.* 2006, Nordstrom *et al.* 2008) and continued research will surely improve our understanding and application of FAs and calibration coefficients to estimating predator diets.

POINT 2

Because "the blubber of all marine mammals is stratified, . . . When information about diet is sought, sampling of the whole blubber column . . . is not appropriate."

Aside from the fact that Grahl-Nielsen's statement here appears to acknowledge that blubber FAs *can* be used to make inferences about diet (despite the opposite claim in Point 1), Point 2 misrepresents the degree to which FAs are stratified in the blubber layer and ignores a number of studies that have examined the anatomical distribution of FAs in marine mammals. FA "stratification" does occur across the

depth of blubber and has long been acknowledged by previous investigators (*e.g.*, Koopman *et al.* 1996, Iverson 2002, Thiemann *et al.* 2004, Arnould *et al.* 2005). However, the absolute *degree* to which FAs are stratified—and the implication for dietary interpretation—varies widely among taxa.

In the cetaceans investigated to date, there is a distinct stratification of blubber tissue into inner, middle, and outer layers based on the size, shape, and metabolic characteristics of adipocytes, as well as on the lipid and collagen content of the tissue (*e.g.*, Lockyer *et al.* 1984, 1985). Commensurate with this, in some cetaceans, particularly the smaller odontocetes, stratification of FAs in blubber can be quite pronounced. Thus, a primary recommendation has always been that investigators sample the inner layer of cetacean blubber where dietary influence is most extensive and predictable (reviewed in Iverson *et al.* 2004 and Budge *et al.* 2006). However, in the larger cetaceans, FA stratification appears far less pronounced, with substantial dietary influence observed in the outer layers (*e.g.*, Hooker *et al.* 2001). Nevertheless, the recommendation has remained that the inner layers should be targeted in these species, at least until details of species-specific stratification are quantified.

In pinnipeds, the morphological structure of the blubber appears to be relatively uniform throughout its depth and not “stratified” *per se*, although information is not available on its histological characteristics. Instead, there exists a gradation in FA composition from the inner to outer portions (*e.g.*, Arnould *et al.* 2005, fig. 1 in Strandberg *et al.* 2008). Several carefully controlled experiments with captive animals have indicated that this reflects a somewhat more rapid turnover in the inner area compared to the outer. These controlled feeding studies have demonstrated that inner-portion and whole-depth samples of blubber yield similar estimates of calibration coefficients and diet composition, but that the inner-portion reflects relatively more recent diet while the whole-depth sample integrates a slightly longer-term diet (Cooper 2004, Tollit *et al.* 2006, D. Tollit and S. J. Iverson, unpublished data). Further, radio-labeled FAs fed in the diet of captive gray seals (*Halichoerus grypus*) are deposited in both the inner and outer portions of blubber within 12 h of ingestion (Budge *et al.* 2004, Cooper 2004). Clearly, the outer portion of pinniped blubber is readily influenced by diet and analyzing the entire blubber depth provides valuable dietary information.

In the case of Thiemann *et al.* (2008), we utilized whole-depth blubber samples for the two cetacean species (beluga, *Delphinapterus leucas* and narwhal, *Monodon monoceros*) along with all the pinnipeds, because one of the stated objectives of our study was to establish a prey database for future studies of polar bears (*Ursus maritimus*; Thiemann *et al.* 2008, p. 93, Thiemann *et al.*, in press), which consume the whole blubber layer of marine mammals. Although we have therefore included some outer blubber effects in the cetaceans, because we did not use any FAs that arise strictly from endogenous sources in our analyses, our data still clearly showed that dietary FAs were the driving force behind differences in blubber FA composition among the several taxonomically distinct groups (Thiemann *et al.* 2008, p. 103).

In summary, there is no empirical or theoretical evidence to support Grahl-Nielsen's claims that: (1) the FAs in the diet of a marine mammal have an unknown

and unknowable impact on its blubber FA composition, and (2) all marine mammal blubber is so stratified that whole-depth biopsies cannot provide information on diet. The large body of original literature reviewed here clearly demonstrates that FA signature analysis is a useful and reliable tool for studying foraging, both qualitatively and quantitatively, in a variety of consumer species. We expect this technique will continue to be studied, applied, and refined by researchers around the world.

LITERATURE CITED

- ACKMAN, R. G., AND C. A. EATON. 1966. Lipids of the fin whale (*Balaenoptera physalus*) from North Atlantic waters. III. Occurrence of eicosenoic and docosenoic fatty acids in the zooplankton *Meganctiphanes norvegica* (M. Sars) and their effect on whale oil composition. *Canadian Journal of Biochemistry* 44:1561–1566.
- ARNOULD, J. P. Y., M. M. NELSON, P. D. NICHOLS AND W. H. OOSTHUIZEN. 2005. Variation in the fatty acid composition of blubber in Cape fur seals (*Arctocephalus pusillus pusillus*) and the implications for dietary interpretation. *Journal of Comparative Physiology B* 175:285–295.
- BECK, C. A., S. J. IVERSON, W. D. BOWEN AND W. BLANCHARD. 2007a. Sex differences in grey seal diet reflect seasonal variation in foraging behaviour and reproductive expenditure: Evidence from quantitative fatty acid signature analysis. *Journal of Animal Ecology* 76:490–502.
- BECK, C. A., L. D. REA, S. J. IVERSON, J. M. KENNISH, K. W. PITCHER AND B. S. FADELY. 2007b. Blubber fatty acid profiles reveal regional, seasonal, age-class and sex differences in the diet of young Steller sea lions in Alaska. *Marine Ecology Progress Series* 338:269–280.
- BRADSHAW, C. J. A., M. A. HINDELL, N. J. BEST, M. L. PHILLIPDS, G. WILSON AND P. D. NICHOLS. 2003. You are what you eat: Describing the foraging ecology of southern elephant seals (*Mirounga leonina*) using blubber fatty acids. *Proceedings of the Royal Society London B* 270:1283–1292.
- BUDGE, S. M., M. H. COOPER AND S. J. IVERSON. 2004. Demonstration of the deposition and modification of dietary fatty acids in pinniped blubber using radiolabelled precursors. *Physiological and Biochemical Zoology* 77:682–687.
- BUDGE, S. M., S. J. IVERSON AND H. N. KOOPMAN. 2006. Studying trophic ecology in marine ecosystems using fatty acids: A primer on analysis and interpretation. *Marine Mammal Science* 22:759–801.
- BUDGE, S. M., M. J. WOOLLER, A. M. SPRINGER, S. J. IVERSON, C. P. MCROY AND G. J. DIVOKY. 2008. Tracing carbon flow in an arctic marine food web using fatty acid-stable isotope analysis. *Oecologia* 157:117–129.
- COOPER, M. H. 2004. Fatty acid metabolism in marine carnivores: Implications for quantitative estimation of predator diets. Ph.D. thesis, Dalhousie University, Halifax, NS. 228 pp.
- COOPER, M. H., S. J. IVERSON AND H. HERAS. 2005. Dynamics of blood chylomicron fatty acids in a marine carnivore: Implications for lipid metabolism and quantitative estimation of predator diets. *Journal of Comparative Physiology B* 175:133–145.
- COOPER, M. H., S. J. IVERSON AND K. ROUVINEN-WATT. 2006. Metabolism of dietary cetoleic acid (22:1n-11) in mink (*Mustela vison*) and gray seals (*Halichoerus grypus*) studied using radiolabeled fatty acids. *Physiological and Biochemical Zoology* 79:820–829.
- DAHL, T. M., C. LYDERSEN, K. KOVACS, S. FALK-PETERSEN, J. SARGENT, I. GJERTZ AND B. GULLIKSEN. 2000. Fatty acid composition of the blubber of white whales (*Delphinapterus leucas*). *Polar Biology* 23:401–409.
- DALSGAARD, J., M. ST. JOHN, G. KATTNER, D. MÜLLER-NAVARRA AND W. HAGEN. 2003. Fatty acid trophic markers in the pelagic marine environment. *Advances in Marine Biology* 46:225–340.

- FALK-PETERSEN, S., T. HAUG, K. T. NILSSEN, A. WOLD AND T. M. DAHL. 2004. Lipids and trophic linkages in harp seal (*Phoca groenlandica*) from the eastern Barents Sea. *Polar Research* 23:43–50.
- GRAHL-NIELSEN, O. 1999. Comment: Fatty acid signatures and classification trees: New tools for investigating the foraging ecology of seals. *Canadian Journal of Fisheries and Aquatic Sciences* 56:2219–2223.
- GRAHL-NIELSEN, O. 2008. Exploration of the foraging ecology of marine mammals by way of the fatty acid composition of their blubber. (in press) *Marine Mammal Science*.
- GRAHL-NIELSEN, O., AND O. MJAAVATTEN. 1991. Dietary influence on fatty acid composition of blubber fat of seals as determined by biopsy: A multivariate approach. *Marine Biology* 110:59–64.
- HOOKE, S. K., S. J. IVERSON, P. OSTROM AND S. C. SMITH. 2001. Diet of northern bottlenose whales inferred from fatty-acid and stable-isotope analyses of biopsy samples. *Canadian Journal of Zoology* 79:1442–1454.
- IVERSON, S. J. 2002. Blubber. Pages 107–112 in W.-F. Perrin, B. Würsig and H. G. M. Thewissen, eds. *Encyclopedia of marine mammals*. Academic Press, San Diego, CA.
- IVERSON, S. J. 2008. Tracing aquatic food webs using fatty acids: From qualitative indicators to quantitative determination. In M. T. Arts, M. T. Brett and M. Kainz, eds. *Lipids in aquatic ecosystems*. Springer-Verlag, New York, NY. in press.
- IVERSON, S. J., O. T. OFTEDAL, W. D. BOWEN, D. J. BONESS AND J. SAMPUGNA. 1995. Prenatal and postnatal transfer of fatty acids from mother to pup in the hooded seal. *Journal of Comparative Physiology B* 165:1–12.
- IVERSON, S. J., K. J. FROST AND L. L. LOWRY. 1997. Fatty acid signatures reveal fine scale structure of foraging distribution of harbor seals and their prey in Prince William Sound, Alaska. *Marine Ecology Progress Series* 151:255–271.
- IVERSON, S. J., B. S. STEWART AND P. K. YOCHER. 2003. Captive validation and calibration of fatty acid signatures in blubber as indicators of prey in Hawaiian monk seal diets. NOAA Technical Report, San Diego, CA. 22 pp.
- IVERSON, S. J., C. FIELD, W. D. BOWEN AND W. BLANCHARD. 2004. Quantitative fatty acid signature analysis: A new method of estimating predator diets. *Ecological Monographs* 74:211–235.
- IVERSON, S. J., I. STIRLING AND S. L. C. LANG. 2006. Spatial and temporal variation in the diets of polar bears across the Canadian arctic: Indicators of changes in prey populations and environment. Pages 98–117 in I. L. Boyd, S. W. Wanless and C. J. Camphuysen, eds. *Top predators in marine ecosystems*. Cambridge University Press, Cambridge, UK.
- IVERSON, S. J., A. M. SPRINGER AND A. S. KITAYSKY. 2007. Seabirds as indicators of food web structure and ecosystem variability: Qualitative and quantitative diet analyses using fatty acids. *Marine Ecology Progress Series* 352:235–244.
- KIRSCH, P. E., S. J. IVERSON AND W. D. BOWEN. 2000. Effect of a low-fat diet on body composition and blubber fatty acids of captive juvenile harp seals (*Phoca groenlandica*). *Physiological and Biochemical Zoology* 73:45–59.
- KOOPMAN, H. N., S. J. IVERSON AND D. E. GASKIN. 1996. Stratification and age-related differences in blubber fatty acids of the male harbour porpoise (*Phocoena phocoena*). *Journal of Comparative Physiology B* 165:628–639.
- KOOPMAN, H. N., S. J. IVERSON AND A. J. READ. 2003. High concentrations of isovaleric acid in the fats of odontocetes: Variation and patterns of accumulation in blubber vs. stability in the melon. *Journal of Comparative Physiology* 173:247–261.
- KOOPMAN, H. N., S. M. BUDGE, D. R. KETTEN AND S. J. IVERSON. 2006. The topographical distribution of lipids inside the mandibular fat bodies of odontocetes: Remarkable complexity and consistency. *IEEE Journal of Oceanic Engineering* 31:95–106.
- LOCKYER, C., L. C. MCCONNELL AND T. D. WALTERS. 1984. The biochemical composition of fin whale blubber. *Canadian Journal of Zoology* 62:2553–2562.
- LOCKYER, C., L. C. MCCONNELL AND T. D. WALTERS. 1985. Body condition in terms of anatomical and biochemical assessment of body fat in north Atlantic fin and sei whales. *Canadian Journal of Zoology* 63:2328–2338.

- NORDSTROM, C. A., L. J. WILSON, S. J. IVERSON AND D. J. TOLLIT. 2008. Evaluating quantitative fatty acid signature analysis (QFASA) using harbour seals (*Phoca vitulina richardii*) in captive feeding studies. *Marine Ecology Progress Series* 360:245–263.
- RACLOT, T., R. GROSCHOLAS AND Y. CHEREL. 1998. Fatty acid evidence for the importance of myctophid fishes in the diet of king penguins, *Aptenodytes patagonicus*. *Marine Biology* 132:523–533.
- REIDINGER, R. F., J. N. LABOWS, D. P. FELLOWS AND J. R. MASON. 1985. Fatty acid composition of adipose tissue as an indicator of diet: A preliminary assessment. *Journal of Wildlife Management* 49:170–177.
- ROUVINEN, K., AND T. KIISKINEN. 1989. Influence of dietary fat source on the body fat composition of mink (*Mustela vison*) and blue fox (*Alopex lagopus*). *Acta Agriculturae Scandinavica* 39:279–288.
- ROUVINEN, K., J. MAKELA, T. KIISKINEN AND S. NUMMELA. 1992. Accumulation of dietary fish fatty acids in the body fat reserves of some carnivorous fur-bearing animals. *Agricultural Science Finland* 1:483–489.
- STRANDBERG, U., A. KÄKELÄ, C. LYDERSEN, K. M. KOVACS, O. GRAHL-NIELSEN, H. HYVÄRINEN AND R. KÄKELÄ. 2008. Stratification, composition, and function of marine mammal blubber: The ecology of fatty acids in marine mammals. *Physiological and Biochemical Zoology* 81:473–485.
- THIEMANN, G. W., S. M. BUDGE AND S. J. IVERSON. 2004. Determining blubber fatty acid composition: A comparison of *in situ* direct and traditional methods. *Marine Mammal Science* 20:284–295.
- THIEMANN, G. W., S. J. IVERSON AND I. STIRLING. 2007. Variability in the blubber fatty acid composition of ringed seals (*Phoca hispida*) across the Canadian Arctic. *Marine Mammal Science* 23:241–261.
- THIEMANN, G. W., S. J. IVERSON AND I. STIRLING. 2008. Variation in blubber fatty acid composition among marine mammals in the Canadian Arctic. *Marine Mammal Science* 24:91–111.
- THIEMANN, G. W., S. J. IVERSON AND I. STIRLING. In press. Polar bear diets and arctic marine food webs: Insights from fatty acid analysis. *Ecological Monographs*.
- TOLLIT, D., S. HEASLIP, B. DEAGLE, S. IVERSON, R. JOY, D. ROSEN AND A. W. TRITES. 2006. Estimating diet composition in sea lions: Which technique to choose? Pages 293–308 in A. W. Trites, S. K. Atkinson, D. P. DeMaster, L. W. Fritz, T. S. Gelatt, L. D. Rea and K. M. Wynne, eds. *Sea lions of the world*. Alaska Sea Grant publication AK-SG-0601 Fairbanks, AK.
- TUCKER, S., W. D. BOWEN AND S. J. IVERSON. 2008. Convergence of diet estimates derived from fatty acids and stable isotopes within individual grey seals. *Marine Ecology Progress Series* 354:267–276.

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