THE USE OF FATTY ACID SIGNATURE ANALYSIS TO ASSESS FORAGING IN DELPHINIDS AND AS A FORENSIC TOOL IN MARINE MAMMALS

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Abstract:

Recent literature regarding marine mammals includes an increasing number of studies using fatty acid signature analysis (FASA) to assess foraging. The use of FASA has been encouraged because other, traditional methods may be biased. With more research being conducted on the topic, the need for comparative methodology and proper data interpretation also increases. As such, different chemical methods used in different laboratories may provide varying degrees of completeness and confidence. In addition, for opportunistic foragers in tropical and subtropical environments (*e.g*., bottlenose dolphins and Hawaiian monk seals) factors such as diversity and inter-annual variation in both prey and their corresponding fatty acid components can make FASA daunting to interpret meaningfully. In this paper, we review the following in terms of potential applicability for *Sotalia* research: a) costs and benefits of different chemical approaches; b) difficulties in using FASA to assess foraging in a closely related species, *Tursiops truncatus*, with similar distribution in bays and estuaries; and c) evidence to date that FASA can provide a powerful biomarker of exposure to biotoxins and, perhaps, other factors that affect marine mammal morbidity and mortality. FASA may be particularly applicable to *Sotalia,* as exposure to toxins and other body burdens may differ throughout the more pristine or polluted areas of its coastal distribution, and provide a means for population characterization. We conclude by suggesting that, where multiple methods exist to address particular hypotheses, scientists and managers carefully weigh the costs (including financial) and benefits of particular approaches to ensure that studies are conducted efficiently and cost-effectively.

Studies of foraging ecology of marine mammals have employed a variety of methods, most of which introduce some bias (Bowen and Siniff, 1999). Scientists have therefore searched for alternative, and better methods, and in the past decade, feeding preferences in some marine mammals have been assessed by comparing fatty acid profiles of the blubber of the consumer with those of its potential prey (*e.g*. Iverson *et al*., 1997b; Smith *et al.*, 1997; Lea *et al*., 2002). Iverson *et al*. (2004) further indicated that foraging could be quantified using this approach. On the other hand, fatty acid signature analysis may not clarify foraging preferences in some instances. For example, Grahl-Nielsen *et al*. (2004) found that fatty acids in polar bears (*Ursus maritimus*) do not match those of their primary prey (three species of phocids). The extent to which fatty acid signature analysis may be useful as a tool by which to assess foraging remains somewhat controversial (*i.e*. Grahl-Nielsen, 1999; Grahl-Nielsen *et al*., 2000).

In addition to assessments of marine mammal foraging ecology, the technique of FASA has also been applied specifically to studies of milk and transference of fatty acids from lactating female marine mammals to their offspring (Iverson, 1993; Iverson *et al*., 1995, 1997a; Brown *et al.*, 1999; Grahl-Nielsen *et al*., 2000). A more novel application of FASA has been its use as a forensic tool to help assess effects of stress or disease in marine mammals (Wetzel and Reynolds, 2004). Studies of other species have suggested that changes in fatty acid constituents may be associated in marine organisms (not including marine mammals) with environmental change, stage of larval development, or exposure to contaminants (Grahl-Nielsen and Barnung, 1985). In addition, chemical changes, including those in mammalian fatty acid constituents, in perinodal adipose tissue may affect activity of lymph nodes (Pond, 2003; Mattacks *et al*., 2004). In humans, alterations in fatty acid profiles have been linked with various metabolic disorders and other diseases, or stress conditions (Korf *et al*., 1989; Siguel and Lerman 1994, 1996). Fatty acid derivatives may serve as mediators and indicators of oxidative stress associated with factors such as inflammation, diabetes, and neuro-endocrine regulation (Hammock *et al*., 2000). Thus, the potential for using changes in fatty acids as a clinical diagnostic marker (*i.e*. biomarker) of stress or disease in marine mammals seems high.

Analytical differences:

Different laboratories conducting FASA have employed different analytical chemistry and even different statistics (*e.g*. compare papers by Iverson and colleagues with those by Grahl-Nielsen and associates). Wetzel and Reynolds (2004) noted advantages of using a chemical analysis protocol for marine mammal samples that allows structural confirmation of the identity of fatty acids and have subsequently incorporated that technique in all their analyses of fatty acids. Thus, there is not a single, generally used approach to FASA. Inter-laboratory comparisons may provide guidance in this regard.

Challenges to use of FASA for assessing foraging: Case study--bottlenose dolphins, *Tursiops truncatus*

Given the amount of research on certain topics conducted in bottlenose dolphins, it is surprising that little has been done to assess fatty acids in dolphin blubber and dolphin prey as a means to clarify foraging. Samuel and Worthy (2004) assessed factors that could affect the principal components (most abundant 16) of blubber fatty acids of stranded dolphins off Texas and Louisiana. Although fatty acid profiles did not vary among nine body locations fatty acids did vary (not necessarily at a statistically significant level) with blubber depth, season, gender, and reproductive status. Worthy et al. (2005) suggested that Indian River, Florida, dolphins also exhibit such differences in fatty acid profiles, indicative of variable feeding strategies.

Wetzel *et al*. (in review) have assessed fatty acid profiles in the blubber of bottlenose dolphins and their prey. Blubber samples from bottlenose dolphin females and their two-to-three year old calves were collected during routine health examinations in Sarasota Bay, Florida. Statistical analyses considered all 102 identified fatty acids. The results coalesced around three primary themes: a) bottlenose dolphin blubber contains a high number of different fatty acids, relative to that of other marine mammals; b) inter-annual variability exists in fatty acid composition of these matrices, so a sampling in a single year is not necessarily representative of all years; and c) fatty acid constituents of the blubber of lactating females and their two-to-three year old calves were not significantly different. The most abundant fatty acids found in Sarasota Bay dolphins tended to be among the more common ones reported elsewhere (*e.g*. Koopman, 2001; Samuel and Worthy, 2004).

Wetzel and colleagues have also started to develop a fatty acid library of dolphin prey in the Sarasota Bay area. However, this effort is complicated by the high diversity of dolphin prey (Barros and Wells, 1998), high diversity of fatty acids in any particular prey (more than 200 in some fish consumed by dolphins), the fact that prey fatty acid profiles differ with life stage of the prey species, and that FA profiles in blubber may reflect exogenous inputs besides food (e.g., biotoxins). Similar issues may confound the use of FASA with Hawaiian monk seals, *Monachus schauinslandi*

(S. Iverson, pers. comm. 2005).

FASA as a forensic tool: Case study—Florida manatees (*Trichechus manatus*)

FASA is a promising biomarker by which scientists may be able to assess exposure to certain natural and anthropogenic stressors. Results of studies using Florida manatees assigned to four cause-of-death categories indicated that those animals exposed to or dying due to brevetoxin exposure demonstrate a statistically distinctive hepatic fatty acid profile (Wetzel et al, unpublished). Further, animals suffering long-term health stress have certain fatty acids not found in animals that die quickly. One manatee (of 15) that died due to watercraft collisions and two manatees (of 15) that died from cold stress presented the fatty acid profile associated with brevetoxin exposure even though other findings at necropsy suggested otherwise; this observation potentially links exposure of manatees to brevetoxin with vulnerability to boat collisions, the main cause of human-related mortality in free-ranging individuals (Marine Mammal Commission 2005). The application of FASA to questions associated with health provides an additional forensic tool to assist scientists and managers to understand cause of death or debilitation in manatees and serves as a model that could be subsequently applied to studies designed to better assess cause of death in other marine mammals.

In fact, other marine mammals succumb to effects of brevetoxin (e.g., Flewellen *et al*., 2005; Van Dolah, 2005). We are currently investigating whether these species may, like manatees, present distinctive hepatic fatty acid profiles after exposure to red tide.

Red tide is one type of harmful algal bloom or HAB (Van Dolah, 2005), and scientists consider effects of HABs to represent one of the most serious future conservation issues for marine mammals (Reynolds *et al.*, 2005). Preliminary results suggest that exposure to toxin resulting from another HAB (*e.g.* domoic acid) may alter hepatic fatty acid profiles in California sea lions, *Zalophus californianus* (Wetzel, Gulland, and Reynolds, unpublished) from areas of the coast where (like manatees in Florida with brevetoxin) they have suffered high mortality many years due to domoic acid exposure (Scholin *et al*., 2000). Thus, the use of hepatic fatty acid profiles may provide a widespread indicator for marine mammal exposures to HABs for which no alternative techniques currently exist.

Costs and benefits: Selecting the optimal research approach

FASA can provide useful insights with high resolution to particular questions. For some species, and for some questions, FASA may be ideal, whereas for others FASA may not provide clarity. As with any well-designed study, one must weigh a range of issues including the following, when designing an optimal approach for research:

- What exactly is the question that needs to be answered? FASA may or may not be the optimal tool to provide the answer to a particular question.
- What resources exist? FASA requires that freshly-collected tissues be archived, frozen, for later analysis. Do proper facilities exist for that? FASA is expensive; other approaches are less expensive, but may or may not provide adequate resolution.
- What level of resolution is required? If, for example, only a general assessment of prey selection is needed, other, less expensive techniques may provide adequate insight.
- Is the species of interest likely to lend itself to FASA? For some tropical species with diverse feeding habits, FASA may not easily be able to provide clarity.

FASA represents an exciting and novel approach that should be considered for use by scientists working with *Sotalia*. The use of FASA should, however, be governed by the factors such as those listed above.

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