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Stratification and intra- and inter-specific differences in fatty acid composition of common dolphin (*Delphinus* sp.) blubber: Implications for dietary analysis

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Abstract

Sixty-five fatty acids were quantified in the blubber of common dolphins (*Delphinus delphis*, *D. capensis*) incidentally caught off the coast of southern California. Dolphins were grouped by sex, reproductive status and species, and a blubber sample was collected at a mid-lateral site located caudal to the trailing edge of the dorsal fin. Samples were divided horizontally into inner, middle and outer layers and gradients in fatty acid content (mass percent) were observed across the depth of the blubber. Levels of monounsaturated fatty acids were greatest in the outer layer, whereas levels of saturated and polyunsaturated fatty acids were greatest in the inner layer. Degree of stratification was greatest in sexually mature dolphins. Blubber of sexually immature, but physically mature, male dolphins was also highly stratified, suggesting that this difference may be attributed to differences in diet. Classification and regression tree analysis resulted in the fewest misclassifications when dolphins were grouped by species, possibly indicating that these closely related animals forage on different prey species. Dietary-derived fatty acids were typically selected as splitting criteria in classification and regression tree analyses, suggesting that the observed differences in fatty acid composition between the various groups of dolphins may be attributed to differences in diet.

Keywords: Blubber; Cetacean; Diet; Dolphin; Fatty acid; Feeding habits; Layer; Stratification

1. Introduction

Blubber is a biochemically dynamic tissue, in which fatty acid (FA) composition is potentially influenced by diet. Marine mammals rely on their blubber layer for thermoregulation, streamlining, buoyancy, and energy storage (Parry, 1949; Worthy and Edwards, 1990; Pabst et al., 1999), and researchers have begun to take advantage of its potential as a record of dietary intake (Iverson et al., 1997b; Walton et al., 2000; Hooker et al., 2001). In order to effectively use FA composition of blubber in diet analysis techniques, the biochemical structure of this tissue must be considered.

Biochemical stratification, or layering, has been observed in the blubber of Pacific walrus (*Odobenus rosmarus divergens*) (West et al., 1979b), polar bears (*Ursus maritimus*) (Grahl-Nielsen et al., 2003), and several species of phocid seals (Fredheim et al., 1995; Best et al., 2003). Other studies, however, have shown that phocid blubber is homogeneous and therefore not layered (Jangaard and Ke, 1968; Käkelä and Hyvärinen, 1993). In contrast, biochemical stratification has consistently been observed in baleen whale blubber (Ackman et al., 1965, 1975a,b; Lockyer et al., 1984; Olsen and Grahl-Nielsen, 2003). Biochemical layering has also been noted in the blubber of toothed whales such as bottlenose dolphins (*Tursiops truncatus*) (Shoda et al., 1993; Samuel and Worthy, 2004), harbor porpoise (*Phocoena phocoena*) (Koopman et al., 1996), killer whales (*Orcinus orca*) (Worthy et al., 2003; Krahn et al., 2004) and numerous other odontocetes (Koopman, 2001).

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While it is evident that stratification exists, the number of distinct layers within cetacean blubber remains unclear. Koopman et al. (1996) found differences in FA composition in porpoise blubber that was divided into inner and outer layers, and suggested the existence of a continuous gradient in composition. Ackman et al. (1965, 1975a) noted differences between inner and outer layers in mysticete whales. Evidence for the existence of three distinct layers in bottlenose dolphins has been suggested based on histological (Cowan and Worthy, 1991), toxicological (Shoda et al., 1993), and biochemical (Samuel and Worthy, 2004) evidence. This stratification has been attributed to different levels of metabolic activity within layers (Ackman et al., 1975a,b; Koopman et al., 1996; Koopman, 2001).

Differences in FA composition within and between species have been attributed to diet (West et al., 1979a; Borobia et al., 1995; Smith et al., 1996; Iverson et al., 1997a,b). Intraspecific differences have also been attributed to age (Koopman et al., 1996; Koopman, 2001), body site (Koopman et al., 1996), season (Samuel and Worthy, 2004), reproductive status (Samuel and Worthy, 2004) and starvation (Koopman, 2001). Interspecific differences have been attributed to phylogeny (Koopman, 2001) and thermal regime (Koopman, 2001; Worthy et al., 2003). With few exceptions (West et al., 1979a,b; Koopman, 2001; Samuel and Worthy, 2004), the possible influences of sex and reproductive status on FA composition within a single species have received little attention.

Examination of FA stratification in marine mammal blubber is confounded by the type of ester prepared from lipid extracted from the tissue of interest. Methyl esters are commonly used to quantify medium- and long-chain FA in order to make dietary inferences (Smith et al., 1997; Walton et al., 2000; Iverson et al., 2004). These FAs have been used as "biomarkers" in diet studies as they are generally passed from the prey to predator adipose storage tissues without modification, whereas ingested short-chain FAs are generally oxidized as a source of energy (Pond, 1998). Unlike methyl esters, butyl esters allow for the quantification of short-chain fatty acids (Christie, 1972), and have been used in studies where levels of short-chain FA such as isovaleric acid (iso 5:0) are of interest (Koopman et al., 1996, 2003).

Common dolphins (*Delphinus* sp.) are relatively small, toothed whales (Suborder Odontoceti) that are easily identified at sea by their black or dark grey V-shaped saddle. Adult animals weigh an average of 80 kg, and are sexually dimorphic with males being larger than females. Common dolphins are distributed worldwide in temperate, tropical and subtropical oceans. They are found along most coasts over the continental shelf, and are commonly associated with prominent bathymetric features such as seamounts and ridges (Evans, 1994). The distribution of common dolphins has also been correlated with the distribution of preferred prey species (Young and Cockcroft, 1994), water temperature, and possible competitive exclusion by spinner (*Stenella longirostris*) and spotted (*S. attenuata*) dolphins (Evans, 1975). *Delphinus* sp. forage on numerous species of fish, cephalopods and crustaceans throughout the water column (Fitch and Brownell, 1968; Jones, 1981; Evans, 1994; Silva, 1999). Stomach content analysis has indicated that the relative proportions of prey types vary with several factors including season and age of individual (Evans, 1975; Young and Cockcroft, 1994; Chou et al., 1995; Silva, 1999).

Prior to 1994, common dolphins off the southern coast of California were grouped into short- and long-beaked populations. These sympatric populations have not been observed to form mixed herds (Evans, 1975), and were later recognized as distinct species which could be differentiated based on aspects of color pattern, external morphology and skeletal characters (Heyning and Perrin, 1994). Rosel et al. (1994) examined mitochondrial DNA sequences and found no DNA haplotypes common to both forms, providing further support for the existence of two species.

These two species of common dolphins are typically parapatrically distributed, with *D. delphis* (short-beaked) found in pelagic waters, and *D. capensis* (long-beaked) in nearshore waters less than 100 fathoms deep (Evans, 1975). As noted by Heyning and Perrin (1994), these two species must be exploiting the environment differently as their distributions overlap off the southern coast of California. It is reasonable to expect that foraging strategies, and therefore diet, influenced by differences in body size and skull morphology, would differ between these two species. There is little published data supporting this hypothesis, however, as previous diet composition studies have failed to distinguish between or compare the diets of these two species.

As with other cetaceans, common dolphins spend much of their time away from shore and below the surface, making direct observation of dietary intake near impossible. Other dietary analysis techniques such as scat and stomach content analyses are of limited use with cetaceans as their scats are virtually impossible to collect, they do not haul-out, and stomach contents are typically only available from a small segment of the population such as stranded animals or those associating with and incidentally caught in fishing nets. Fatty acid analysis shows great promise for gaining insight into the diet of free-ranging cetaceans as only a small piece of blubber is required for analysis. However, in order to design appropriate sampling protocols for this type of work, factors that may influence the fatty acid composition of cetacean blubber must be considered. The overall aim of this research was to examine the fatty acid composition of common dolphin blubber in the context of making dietary inferences for these species. The first objective of this study was to determine if *Delphinus* blubber could be divided into biochemically distinct layers. The second objective was to examine the intraspecific differences in blubber FA composition with respect to sex and reproductive status. The final objective was to determine if *D. delphis* could be distinguished from *D. capensis* on the basis of blubber FA composition.

2. Materials and methods

2.1. Sample collection

Blubber samples ($n=84$) and accompanying biological data (species, sex, body size, reproductive condition) were made available by the Southwest Fisheries Science Center (SWFSC)