Stratification and age-related differences in blubber fatty acids of the male harbour porpoise (*Phocoena phocoena*)

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**Abstract** Fatty acid composition of blubber was determined at four body sites of 19 male harbour porpoises. A total of 65 fatty acids were quantified in each sample. The array of fatty acids contained in harbour porpoise blubber was similar to those found in other marine mammals. While chemical composition of total blubber was uniform over the body, with the exception of the caudal peduncle, vertical stratification was evident between the deep (inner) and superficial (outer) blubber layers. Fatty acids with chain lengths shorter than 18 carbons were present in significantly greater amounts in the outer blubber layer, while the longer-chain unsaturated fatty acids were more prevalent in the inner layer. This distribution suggests that the inner blubber layer is more active metabolically than the outer layer in terms of lipid deposition and mobilization. Harbour porpoise blubber contained high levels (2–27%) of isovaleric acid in the outer blubber layer, and these levels were positively correlated with age.

**Key words** Blubber · Fatty acids · Isovaleric acid · Harbour porpoise, *Phocoena phocoena*

**Abbreviations** Caud caudal dorsal body site · GC gas chromatograph · FA fatty acid(s)

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**Introduction**

The blubber layer is the most important site of fat storage in marine mammals (Pond 1978). It functions as an insulator, buoyancy adjuster and body streamliner, and lipids in this tissue are mobilized in times of energetic need and replenished when food is in excess (Ryg et al. 1988). The biochemical composition of blubber can provide insights into the nutritive status, feeding habits, lipid turnover and general lipid metabolism of a given species (Ackman et al. 1975b, c; Aguilar and Borrell 1990; Lockyer 1991; Iverson 1993; Käkelä et al. 1993). In particular, the FA composition of this tissue can be used to examine patterns of deposition, maintenance, mobilization and replenishment (Ackman et al. 1965; West et al. 1979; Lockyer et al. 1984; Iverson et al. 1995).

Harbour porpoises (*Phocoena phocoena*) rely on a relatively thick blubber layer to provide insulation in temperate waters where the seasonal average temperatures range from 4 to 16°C (Worthy and Edwards 1990; Gaskin 1992). The diet of this species has been well documented (Smith and Gaskin 1974; Recchia and Read 1989; Smith and Read 1992) and a few studies have shown a relationship between blubber thickness and condition (Read 1990; Koopman 1994). To date only two studies have examined the FA composition of harbour porpoise blubber. In an early study, Lovern (1934) quantified ten FA in the blubber of one porpoise, and suggested that the composition of head blubber and body blubber differed. Lovern (1934) also noted the presence of isovaleric acid in both depots. Isovaleric acid is an unusual, very short-chain (C5) fatty acid, that has been reported in a few other
Materials and methods

Porpoises

Blubber samples were taken from 19 male harbour porpoises from the Bay of Fundy (n = 8) and the Gulf of Maine (n = 11). Porpoises from the Bay of Fundy were incidentally caught either in herring weirs (n = 6) or demersal gill nets (n = 2) during August and September 1993. Animals from the Gulf of Maine were all caught incidentally in the gill net fishery during October-December 1992 and 1993, and April 1992. All porpoise carcasses were robust and in good condition, and were necropsied or frozen within 24 h of death.

Porpoises were classified into three reproductive classes: calves [Gulf of Maine: n = 2, Bay of Fundy: n = 0; identified by incomplete eruption of teeth and presence of milk in the stomach (Read and Gaskin 1990)]; immature males (Gulf of Maine: n = 5, Bay of Fundy: n = 4; lack of spermatozoa in testes; testis mass < 100 g); and mature males [Gulf of Maine: n = 4, Bay of Fundy: n = 4; presence of spermatozoa in the testes; testis mass > 100 g (outlined in Hohn et al. 1985 and Read and Hohn, 1995)]. Ages of all porpoises were estimated from counts of dentinal growth layers in stained decalcified thin sections of teeth as recommended by the Oslo harbour porpoise age determination workshop (Bjorge et al. in press).

The sites of blubber sampling are shown in Fig. 1a. The complete blubber layer was removed from the carcass at these sites (blubber pieces about 10 cm x 10 cm) and frozen at −20 °C until further analysis.

To examine stratification in blubber, these frozen pieces were subsampled to obtain samples from the deep and superficial layers (see Fig. 1b). Due to the absence of definitive histological information on these layers, we decided to sample only the innermost and outermost layers to test for stratification. The *Delphinus capensis* and subdermal connective tissue sheath were removed and a sample of approximately 0.5 g was trimmed from the deep blubber surface (inner layer sample). The skin (epidermis and dermis) was also removed and a blubber sample of 0.5 g was taken (outer layer sample). The remaining blubber between these layers, corresponding to about 40% of the blubber depth at most sites, was not sampled.

Sample extraction and preparation

Blubber samples (approximately 0.5 g each) were weighed and placed in 9 ml of 2:1 chloroform:methanol with 0.005% butylated hydroxytoluene in Kimax test tubes with Teflon caps. Samples were mashed manually with a glass homogenizer until thin and transparent, then vortexed for 20 s, and allowed to soak for 2-3 days. Next, samples were extracted according to a modified Folch procedure (Folch et al. 1957; Iverson 1988). FA analysis was carried out on total lipid samples because harbour porpoise blubber is almost entirely triglyceride and contains little, if any, wax ester and only trace amounts of phospholipid (H.N. Koopman, unpublished data). FA butyl esters were used in place of methyl esters because many of the FA of interest had short chains (< C6) and hence would be exceptionally vulnerable to volatilization as methyl esters (Shantha and Napolitano 1992). Butyl esters were prepared by placing 50 mg of lipid with 1 ml hexane and 1 ml 10% BF3 in butanol in a Kimax tube. Tubes were incubated at 100 °C for 1 h, then cooled to room temperature. Water (3 ml) was added, the sample was vortexed and centrifuged at 480 g for 5 min. The hexane layer was removed and washed with 5 ml H2O and dried with sodium sulphate. Samples were flushed with N2 to prevent oxidation, and were capped tightly.

Fatty acid analysis

Samples were analyzed using temperature-programmed capillary gas-liquid chromatography on a Perkin-Elmer Autosystem GC fitted with a 30 m x 0.25 mm i.d. column, coated with 50% cyanopropyl polysiloxane (0.25 μm, DB-23). The injection and detector temperatures were held at 250 and 270 °C, respectively. The temperature program was developed by trial and error until all components were satisfactorily separated within the shortest time period as follows: 65 °C for 2 min, ramped at 20 °C·min⁻¹ to 165 °C, held for 0.4 min, ramped at 2 °C·min⁻¹ to 215 °C and held for 6.6 min, ramped at 5 °C·min⁻¹ to 240 °C and held for 1.0 min. The entire program took 45 min to complete.

Identification of FA was made from known standard mixtures (Nu Check Preparations, Elysian, Minn., USA) and from samples earlier identified using silver nitrate chromatography (Iverson 1988). GC response factors for each FA were used to calculate weight percentage and were based on modified theoretical response factors (Ackman 1991) that were adjusted according to values from known standard mixtures (Iverson 1988).

All FA components were converted to a weight percentage of the total array of FA plus unknowns and are named according to the IUPAC nomenclature of n carbons: m double bonds, where n-m denotes the position of the last double bond relative to the terminal methyl end of the FA.