J. Mar. Biol. Ass. U.K. (2007), **87**, 223–230
Printed in the United Kingdom
doi: 10.1017/S0025315407054537

Using blubber biopsies to provide ecological information about bottlenose dolphins (*Tursiops truncatus*) around the Azores

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Fatty acid profiles were used to investigate aspects of bottlenose dolphin populations around the Azores archipelago. Biopsy samples were obtained from 70 dolphins during the period 2002–2004. No statistically significant differences in profiles were found between different island groups, between sexes or between year of sampling. Thus no evidence was seen for island group fidelity, in contrast to bottlenose dolphins found around similar island groups such as the Hawaiian archipelago or the Bahamas. The findings are consistent with concurrent genetic and photo-identification studies on dolphins in the Azores.

INTRODUCTION

Cetacean populations are often structured into smaller units that influence key demographic and evolutionary processes. Therefore, characterizing population structure provides ecological and evolutionary data and important information for management and conservation (Parsons et al., 2006). Population structure is usually determined by analysing variations in microsatellites or mtDNA. Since capturing wild cetaceans is difficult, tissue samples for DNA analysis are usually obtained using a biopsy dart (Cockcroft, 1994). The biopsy sample typically consists of a portion of skin (the source of DNA) attached to a small piece of blubber which is often discarded. However, blubber has the potential to provide useful ecological data through the analysis of stable isotopes (Hooker et al., 2003), pollutants (Borrell et al., 2006) or fatty acid (FA) profiles (Walton & Pomeroy, 2003).

Bottlenose dolphins (*Tursiops truncatus*) are the most common dolphin species worldwide and inhabit both temperate and tropical waters (see review by Shane et al., 1986). In the western North Atlantic distinctive inshore and offshore forms exist which are genetically different (Hoelzel, 1998). Natoli et al. (2004) measured genetic diversity of bottlenose dolphins from the Black Sea to the eastern North Atlantic and found population boundaries coincided with transitions between habitat regions as characterized by ocean floor topography, surface salinity, productivity and temperature. Their study covered most of the circumference of the Atlantic but did not include the mid-Atlantic where population trends and status are virtually unknown. The Azores consist of an isolated archipelago of islands, divided into three main groups, located in the middle of the North Atlantic. The geographical distance between the island groups is within the travel range of bottlenose dolphins and there are no apparent topographical or oceanographic features that would prevent gene flow. However, based on studies from other similar regions one might expect the population to be divided into some distinct communities. Around Hawaii, a Pacific archipelago, bottlenose dolphins are island-associated and only make limited movements between islands or into offshore waters (Baird et al., 2002, 2003, 2006). Similarly, bottlenose dolphins at three sites separated by less than 250 km in the Bahamas showed evidence of subdivisions (Parsons et al., 2006). One of the major aims of the 3-year CETAMARH project from 2002–2004 was to examine the population structure and ecology of bottlenose dolphins around the Azores by assessing the relationship and extent of interactions between different population units through the use of several complementary techniques (genetics, photo-identification, stable-isotopes and FA profiles).

The lipid rich subcutaneous blubber of marine mammals provides insulation, buoyancy and is a store of energy. A wide variety of fatty acids are found in the lipid and many of these have to be derived from the diet since animals lack the ability to synthesize them. Thus, blubber FA profiles (the relative proportions of each FA by weight) are influenced by the FAs of dietary prey species; however, the profiles are not identical because of metabolic factors. The FA profile is not uniform across the whole depth of the blubber, the degree of variation differing between species (see Smith &

Table 1. *Sample sizes.*

Year	All regions			West region			Central region			East region		
	all	M	F	all	M	F	all	M	F	all	M	F
2002	33	20	11	0	0	0	16	14	2	17	8	9
2003	27	22	5	3	3	0	18	13	5	6	6	0
2004	10	6	4	5	4	1	5	2	3	0	0	0
Total	70	48	20	8	7	1	39	29	10	23	14	9

Table 2. *Fatty acid profiles of blubber biopsy samples characterized by sex, year and location.*

	Females	Males	2002	2003	2004	West	Central	East
FAME	N=20	N=50	N=33	N=27	N=10	N=8	N=39	N=23
12	0.45 ±0.17	0.46 ±0.18	0.49 ±0.22	0.42 ±0.12	0.45 ±0.11	0.44 ±0.12	0.46 ±0.19	0.46 ±0.19
13	0.06 ±0.04	0.07 ±0.03	0.08 ±0.03	0.07 ±0.02	0.01 ±0.01	0.03 ±0.03	0.07 ±0.03	0.08 ±0.03
14	4.83 ±0.57	4.83 ±0.76	5.04 ±0.72	4.69 ±0.62	4.48 ±0.76	4.04 ±0.50	5.04 ±0.57	4.74 ±0.79
14:1n-9	0.80 ±0.32	0.84 ±0.46	0.83 ±0.47	0.87 ±0.44	0.73 ±0.23	0.94 ±0.39	0.84 ±0.48	0.77 ±0.34
14:1n-7	0.47 ±0.20	0.54 ±0.27	0.51 ±0.27	0.54 ±0.27	0.45 ±0.13	0.52 ±0.19	0.54 ±0.30	0.47 ±0.19
14:1n-5	1.74 ±0.69	1.92 ±0.78	1.82 ±0.74	1.98 ±0.83	1.72 ±0.58	1.91 ±0.82	1.90 ±0.77	1.80 ±0.72
iso15	0.60 ±0.17	0.53 ±0.16	0.54 ±0.16	0.55 ±0.17	0.58 ±0.19	0.51 ±0.18	0.57 ±0.17	0.52 ±0.15
anti15	0.27 ±0.14	0.30 ±0.09	0.31 ±0.11	0.29 ±0.10	0.22 ±0.07	0.25 ±0.08	0.28 ±0.09	0.33 ±0.13
15	0.62 ±0.06	0.62 ±0.08	0.65 ±0.07	0.60 ±0.06	0.59 ±0.09	0.55 ±0.09	0.63 ±0.07	0.64 ±0.07
15:1n-x	0.16 ±0.06	0.17 ±0.08	0.16 ±0.06	0.18 ±0.08	0.16 ±0.06	0.18 ±0.10	0.17 ±0.07	0.17 ±0.07
iso16	0.30 ±0.17	0.19 ±0.06	0.23 ±0.13	0.21 ±0.07	0.22 ±0.15	0.20 ±0.07	0.22 ±0.12	0.23 ±0.12
16	8.39 ±1.71	7.98 ±2.07	8.75 ±1.85	7.40 ±2.00	7.78 ±1.70	7.06 ±2.14	8.23 ±1.99	8.22 ±1.85
16:1n-11	0.36 ±0.04	0.37 ±0.04	0.38 ±0.04	0.35 ±0.04	0.38 ±0.05	0.39 ±0.06	0.36 ±0.04	0.38 ±0.04
16:1n-9	2.18 ±0.65	2.27 ±0.80	2.28 ±0.86	2.23 ±0.71	2.20 ±0.53	2.59 ±0.79	2.20 ±0.81	2.21 ±0.64
16:1n-7	16.97 ±3.63	16.02 ±3.77	16.45 ±4.06	16.12 ±3.65	16.23 ±3.06	16.70 ±3.65	16.32 ±3.77	16.10 ±3.84
16:1n-5	0.14 ±0.02	0.14 ±0.02	0.14 ±0.02	0.14 ±0.02	0.14 ±0.02	0.15 ±0.02	0.14 ±0.02	0.13 ±0.02
iso17	0.09 ±0.05	0.09 ±0.07	0.07 ±0.01	0.07 ±0.01	0.22 ±0.08	0.18 ±0.12	0.08 ±0.04	0.06 ±0.01
16:2n-6	0.06 ±0.02	0.07 ±0.03	0.05 ±0.02	0.07 ±0.03	0.08 ±0.03	0.08 ±0.04	0.06 ±0.02	0.06 ±0.03
anti17	0.15 ±0.07	0.13 ±0.04	0.13 ±0.06	0.12 ±0.03	0.18 ±0.04	0.16 ±0.06	0.13 ±0.04	0.14 ±0.06
16:2n-4	0.18 ±0.05	0.20 ±0.05	0.19 ±0.05	0.21 ±0.06	0.20 ±0.06	0.17 ±0.05	0.21 ±0.05	0.18 ±0.05
17	0.35 ±0.10	0.35 ±0.12	0.40 ±0.13	0.31 ±0.09	0.31 ±0.07	0.28 ±0.10	0.36 ±0.12	0.37 ±0.11
16:3n-4	0.05 ±0.01	0.05 ±0.02	0.05 ±0.01	0.05 ±0.01	0.03 ±0.01	0.04 ±0.02	0.05 ±0.01	0.05 ±0.02
17:1n-x	1.05 ±0.13	1.04 ±0.15	1.04 ±0.14	1.04 ±0.15	1.04 ±0.13	1.03 ±0.17	1.03 ±0.13	1.07 ±0.15
16:3n-1	0.20 ±0.06	0.18 ±0.05	0.19 ±0.02	0.18 ±0.04	0.22 ±0.11	0.20 ±0.10	0.19 ±0.05	0.18 ±0.03
iso18	0.25 ±0.11	0.26 ±0.10	0.25 ±0.10	0.30 ±0.12	0.18 ±0.03	0.26 ±0.13	0.25 ±0.10	0.26 ±0.10
16:4n-1	0.02 ±0.01	0.02 ±0.01	0.02 ±0.01	0.02 ±0.02	0.01 ±0.01	0.01 ±0.00	0.02 ±0.01	0.02 ±0.01
18	2.13 ±0.47	2.14 ±0.67	2.23 ±0.61	2.06 ±0.65	2.06 ±0.52	1.82 ±0.62	2.19 ±0.64	2.16 ±0.55
18:1n-11	0.94 ±0.61	0.87 ±0.49	0.83 ±0.41	0.82 ±0.43	1.30 ±0.85	1.65 ±0.88	0.89 ±0.36	0.63 ±0.34

18:1n-9	32.87 ±2.63	31.50 ±3.04	31.87 ±3.21	31.39 ±2.85	33.32 ±2.19	34.19 ±2.52	31.08 ±2.73	32.48 ±3.11
18:1n-7	2.85 ±0.24	2.73 ±0.20	2.78 ±0.22	2.75 ±0.24	2.74 ±0.17	2.66 ±0.12	2.77 ±0.24	2.79 ±0.20
18:2d5,7	0.03 ±0.01	0.03 ±0.01	0.03 ±0.01	0.03 ±0.01	0.02 ±0.01	0.02 ±0.01	0.03 ±0.01	0.03 ±0.01
18:1n-5	0.13 ±0.02	0.13 ±0.02	0.14 ±0.02	0.13 ±0.01	0.14 ±0.02	0.15 ±0.02	0.13 ±0.02	0.13 ±0.01
18:2n-6	1.31 ±0.22	1.43 ±0.18	1.30 ±0.19	1.50 ±0.15	1.42 ±0.21	1.36 ±0.22	1.45 ±0.19	1.33 ±0.19
18:2n-4	0.04 ±0.01	0.05 ±0.02	0.04 ±0.02	0.07 ±0.02	0.04 ±0.01	0.04 ±0.01	0.05 ±0.02	0.05 ±0.02
18:3n-6	0.05 ±0.03	0.05 ±0.03	0.04 ±0.01	0.05 ±0.01	0.11 ±0.04	0.07 ±0.04	0.06 ±0.03	0.04 ±0.01
18:3n-4	0.12 ±0.02	0.12 ±0.02	0.12 ±0.02	0.13 ±0.02	0.11 ±0.01	0.11 ±0.02	0.12 ±0.02	0.13 ±0.03
18:3n-3	0.42 ±0.10	0.46 ±0.10	0.43 ±0.10	0.49 ±0.07	0.42 ±0.11	0.41 ±0.13	0.46 ±0.10	0.44 ±0.09
18:3n-1	0.27 ±0.09	0.24 ±0.07	0.28 ±0.08	0.20 ±0.06	0.28 ±0.04	0.26 ±0.06	0.24 ±0.08	0.27 ±0.07
18:4n-3	0.22 ±0.09	0.26 ±0.09	0.23 ±0.10	0.29 ±0.08	0.21 ±0.07	0.20 ±0.08	0.27 ±0.09	0.23 ±0.08
18:4n-1	0.03 ±0.02	0.03 ±0.01	0.03 ±0.01	0.03 ±0.01	0.01 ±0.00	0.01 ±0.01	0.03 ±0.01	0.03 ±0.01
20	0.14 ±0.04	0.14 ±0.05	0.15 ±0.05	0.14 ±0.04	0.13 ±0.04	0.11 ±0.03	0.14 ±0.05	0.15 ±0.04
20:1n-11	0.70 ±0.43	0.69 ±0.40	0.69 ±0.39	0.66 ±0.34	0.81 ±0.61	1.04 ±0.67	0.70 ±0.38	0.55 ±0.27
20:1n-9	2.30 ±0.90	2.26 ±0.89	2.27 ±0.87	2.17 ±0.77	2.56 ±1.22	2.97 ±1.26	2.19 ±0.84	2.18 ±0.73
20:1n-7	0.15 ±0.04	0.16 ±0.06	0.15 ±0.05	0.15 ±0.05	0.17 ±0.07	0.17 ±0.08	0.16 ±0.05	0.15 ±0.04
20:2n-6	0.19 ±0.05	0.20 ±0.05	0.18 ±0.05	0.21 ±0.05	0.21 ±0.04	0.18 ±0.04	0.19 ±0.05	0.20 ±0.06
20:3n-6	0.09 ±0.03	0.10 ±0.02	0.08 ±0.02	0.11 ±0.02	0.11 ±0.03	0.09 ±0.03	0.10 ±0.03	0.09 ±0.02
20:4n-6	0.95 ±0.38	0.95 ±0.23	0.85 ±0.18	1.07 ±0.33	0.96 ±0.29	0.93 ±0.22	0.99 ±0.34	0.90 ±0.15
20:3n-3	0.08 ±0.02	0.09 ±0.02	0.08 ±0.02	0.10 ±0.02	0.08 ±0.01	0.07 ±0.01	0.08 ±0.02	0.09 ±0.03
20:4n-3	0.41 ±0.13	0.46 ±0.12	0.40 ±0.14	0.49 ±0.09	0.45 ±0.12	0.42 ±0.14	0.46 ±0.11	0.41 ±0.14
20:5n-3	1.71 ±0.64	1.75 ±0.57	1.65 ±0.62	1.88 ±0.57	1.66 ±0.49	1.61 ±0.55	1.82 ±0.63	1.64 ±0.53
22:1n-11	0.84 ±0.71	0.92 ±0.64	0.88 ±0.74	0.88 ±0.52	1.03 ±0.76	1.26 ±0.80	1.00 ±0.70	0.60 ±0.41
22:1n-9	0.22 ±0.13	0.25 ±0.14	0.24 ±0.16	0.24 ±0.13	0.24 ±0.12	0.27 ±0.13	0.24 ±0.15	0.23 ±0.12
21:5n-3	0.07 ±0.04	0.09 ±0.04	0.08 ±0.04	0.10 ±0.04	0.08 ±0.03	0.07 ±0.03	0.09 ±0.04	0.08 ±0.04
22:4n-6	0.23 ±0.12	0.29 ±0.13	0.26 ±0.14	0.31 ±0.13	0.26 ±0.11	0.21 ±0.09	0.28 ±0.12	0.30 ±0.16
22:5n-6	0.35 ±0.18	0.47 ±0.20	0.39 ±0.20	0.50 ±0.20	0.41 ±0.17	0.33 ±0.14	0.43 ±0.18	0.48 ±0.24
22:4n-3	0.04 ±0.02	0.04 ±0.02	0.04 ±0.02	0.04 ±0.01	0.04 ±0.01	0.03 ±0.01	0.04 ±0.01	0.04 ±0.02
24	0.03 ±0.01	0.03 ±0.02	0.03 ±0.01	0.03 ±0.02	0.01 ±0.01	0.01 ±0.01	0.03 ±0.02	0.03 ±0.01
22:5n-3	1.88 ±1.06	2.39 ±1.05	2.13 ±1.24	2.45 ±0.94	2.09 ±0.78	1.68 ±0.72	2.29 ±0.93	2.38 ±1.34
22:6n-3	7.45 ±3.68	8.99 ±3.34	8.06 ±3.98	9.52 ±2.95	7.51 ±2.60	6.57 ±2.80	8.60 ±3.08	9.14 ±4.17
24:1	0.05 ±0.09	0.04 ±0.08	0.01 ±0.01	0.02 ±0.01	0.22 ±0.10	0.13 ±0.13	0.04 ±0.08	0.01 ±0.01
Total	100	100	100	100	100	100	100	100

FAME, fatty acid methyl ester.

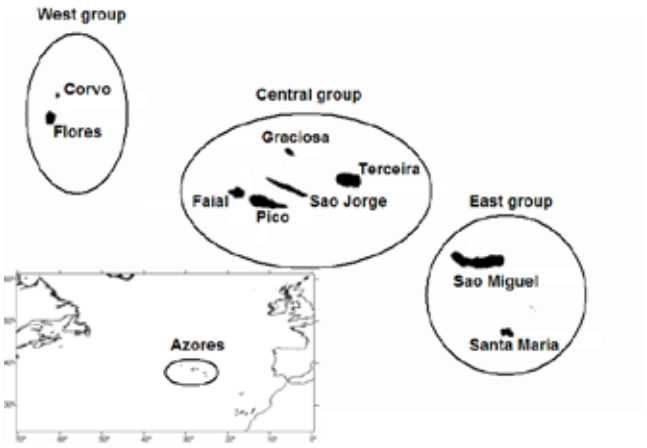

Figure 1. Map of the Azores archipelago.

Worthy, 2006). The consensus view is that the inner regions (nearer the muscle) have more similarity to the diet than the outer regions (nearer the skin). The turnover times of FAs in marine mammal blubber are not known, but in human adipose tissue their half-life is in the order of six months to two years (Beynon et al., 1980; Strawford et al., 2004) and it is likely that the outer blubber layers change more slowly than the inner layers.

Differences in FA profiles can indicate that differences or changes in diet have occurred. Walton & Pomeroy (2003) using full-depth blubber samples could clearly classify two colonies of grey seals as different ecological stocks, even though no differentiation was detected by mtDNA analysis. Also a significant change in diet was indicated at one of the sites, but not at the other, over a 3-y period. Similarly Møller et al. (2003) found regional differences in the FA profiles from hunted minke whales *Balaenoptera acutorostrata* across the North Atlantic. Olsen & Grahl-Nielsen (2003) compared the inner and outer regions of blubber in minke whales from the Norwegian and North Seas and found that both layers could be used for population differentiation. Recently, Herman et al. (2005) used FA profiles of biopsied outer region blubber to differentiate between resident and transient killer whales *Orcinus orca* from the same regions of the North Pacific.

In this present study, the FA profiles of blubber biopsy samples from 70 dolphins found in waters around the Azores were compared and differences in profile between males and females, year of sampling and geographical location were tested.

MATERIALS AND METHODS

Study area

The Azores (Figure 1) is an isolated archipelago extending more than 480 km from north-west to south-east across the northern mid-Atlantic ridge. It consists of nine volcanic islands, divided into three groups: western (Flores and Corvo), central (Graciosa, Terceira, São Jorge, Pico and Faial) and eastern (São Miguel and Santa Maria), which are separated by deep waters of >2000 m depth with scattered seamounts.

Sample Collection

Biopsy samples of approximately 0.5 cm diameter by 2 cm depth were collected by biopsy dart fired by a cross-bow.

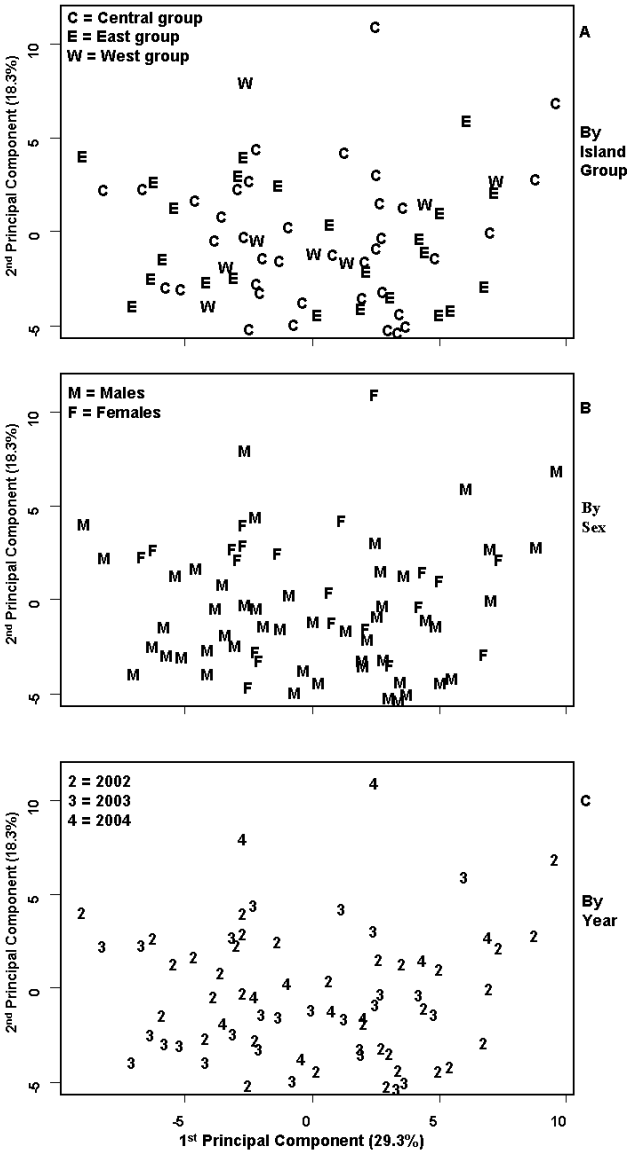

Figure 2. Principal components analysis of the fatty acid profiles of the blubber biopsy samples (N=70). (A) labels the samples by island group (C, central; E, east; W, west); (B) labels the samples by sex (M, males; F, females); (C) labels the samples by year of sampling.

The number of samples collected, categorized by sex, year and location are given in Table 1.

Lipid extraction and analysis

The blubber was separated from the skin and the lipid extracted by the method of Folch et al. (1957). Briefly, the samples were homogenized in 10 ml dichloromethane: methanol (2:1, vol/vol) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant and filtered. The organic phase was washed with 0.9% KCl, treated with anhydrous Na_2SO_4, dried under nitrogen, weighed and dissolved in toluene at a concentration of 100 mg/ml. An aliquot was converted to fatty acid methyl esters (FAMES) using acidified methanol (Henderson et al., 1994) and the purified FAMES were dissolved in hexane (10 mg/ml).

https://doi.org/10.1017/S0025315407054537 Published online by Cambridge University Press

Table 3. *Fatty acid profile distance measure (Dfap).*

Comparison	No. samples	Dfap	*P*	Significance
By year				
2002 versus 2003	33 & 27	0.020	0.138	n.s.
2002 versus 2004	33 & 10	0.013	0.322	n.s.
2003 versus 2004	26 & 10	0.028	0.190	n.s.
By sex				
males versus females	50 & 20	0.122	0.229	n.s.
By island group				
central versus east	39 & 23	0.007	0.651	n.s.
central versus west	39 & 8	0.059	0.017	SD
east versus west	23 & 8	0.051	0.073	n.s.
By island group/year				
central 2002 versus east 2002	16 & 18	–0.006	0.577	n.s.
central 2003 versus east 2003	18 & 5	–0.007	0.572	n.s.
central 2004 versus west 2004	5 & 5	0.100	0.182	n.s.
central 2003 versus west 2003	18 & 3	–0.149	0.605	n.s.

SD, significantly different; n.s., not significant.

The FAMES were analysed by gas-liquid chromatography using a Trace GC-2000 gas chromatograph (Thermoquest, CE Instruments) equipped with a flame-ionization detector and fitted with a DB23 fused silica capillary column (30 m×0.25 mm internal diameter, J&W Scientific). Hydrogen was employed as the carrier gas and the temperature programme was as described previously (Walton & Pomeroy, 2003). Separated components were identified by reference to authentic standards, equivalent chain length (ECL) values, fractionation of seal samples by silver-nitrate chromatography and by comparisons with samples run at other laboratories. Individual FAs are expressed as mass per cent of the total FAs characterized. As is customary, values are quoted to 2 decimal places, but this is for comparison purposes and this degree of accuracy is not implied (see Ackman et al., 1971).

Statistical treatment of results

Principal component analysis was performed using the open source statistical package R v. 2.0. The data were first standardized and normalized such that for each variable the mean was zero and the standard deviation was 1 (see Storr-Hansen & Spliid, 1993). Discriminant analysis was performed using SPSS v. 12 (SPSS Inc.).

RESULTS

The mean FA profiles of the samples categorized by sex, year and location are shown in Table 2. Sixty individual FAs were characterized of which 47 were present at less than 1% of the total by weight. The FA profiles were broadly similar and for all samples combined consisted of, by fatty acid category, 18.2% saturated (16.7% straight-chain and 1.5% branched chain); 63.5% monounsaturated (22.5% of chain length 16 or less, 41% of chain length >16); 18.3% polyunsaturated (13.9% n-3 series, 3.5% n-6 series. 0.9% others). Thus monounsaturated FAs predominated and indeed two of them 18:1n-9 and 16:1n-7 accounted for about 50% of the total fatty acids present.

The individual FA profiles were subjected to several multivariate statistical procedures. The purpose of principal component (PC) analysis is to reduce the large number of original correlated variables (FAs) to a small number of transformed uncorrelated variables (PCs) that retain as much of the information in the original variables and also explain as much of the sample variance as possible. The procedure does not require prior categorization of the data before analysis and it is not a test of statistical difference but rather a visual representation of the spread of the data points in which one can look for natural clusterings. Figure 2 shows the PC plot based on 61 FAs and 70 dolphins. The first PC accounted for 29.3% and the second PC 18.3% of the total variance. For clarity, the plot is shown three times with the individual animals labelled (A) by island group, (B) by sex and (C) by year of sampling. None of these plots contain clearly discernible clusters relating to island group, sex or year sampled.

A quantitative measure of the difference between FA profiles was obtained using the average inter-population difference between profiles called Dfap as described by Walton & Pomeroy (2003). This is an analogue of Gst (Palumbi et al., 1991) or the Phist measures of analysis of molecular variance (AMOVA) (Excoffier et al., 1992) but uses differences between FA profiles rather than DNA sequences. Dfap can take a value between 0 and 1 and represents the proportion of the total variance in the data due to inter-population differences. In practice, at least with DNA studies, the theoretical maximum of 1 is rarely approached and even values of 0.05 can indicate genetic differentiation (Wright, 1978). The statistical significance of the actual Dfap value was tested by Monte Carlo resampling of the dataset. The Dfap results are presented in Table 3. It is important to check for inter-year before pooling data from different years for

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Table 4. *Discriminant analysis jack-knifed classification tables.*

1) By sex			
	female	male	total
female	14	6	20
male	16	34	50
actual	20	50	70
overall percentage correct =			68.6

2) By year				
	2002	2003	2004	total
2002	17	14	2	33
2003	13	14	0	27
2004	2	1	7	10
actual	33	27	10	70
overall percentage correct =			54.3	

3) By place				
	west	central	east	total
west	4	3	1	8
central	7	19	13	39
east	7	2	14	23
actual	8	39	23	70
overall percentage correct =			52.9	

other comparisons since significant inter-annual differences in diet have previously been found in other marine mammal species (Tollit & Thompson, 1996; Walton & Pomeroy, 2003). No significant differences were seen between any of the inter-year comparisons, nor between males and females, nor between animals from different island groups sampled in the same year. When samples from all years were combined a difference was seen between the central and western island groups, but not central vs eastern nor western vs eastern. This may indicate that slight differences exist between at least the western and central groups.

Discriminant analysis shows how well two or more predefined groups of individuals are separated, given measurements of several variables. It provides linear functions of the variables that best separate the cases into the predefined groups. The results are presented in a jack-knifed classification matrix (Table 4), which classifies each sample without using that sample to calculate the group means. In all cases, when considering the samples by sex, year or location, the degree of correct classifications was better than what would be expected by chance, but misclassification rates were still fairly high, so no strong evidence was seen to allow the samples to be reliably classified by sex, year of sampling, or island group.

DISCUSSION

As samples were collected by biopsy dart, there was inevitably some variation in the body site sampled. This, however, is not likely to influence the results since Samuel & Worthy (2004) found FA profiles were indistinguishable when tested from nine different body sites of bottlenose dolphins. However, they did find variations within the blubber depth which they classified into three regions (outer, middle and inner) based on differences in structure and colour. In this and a later paper (Smith & Worthy, 2006) they found that on going from the inner to the outer regions, saturated FAs decreased from 22.9 to 11.2%, monounsaturated FAs increased from 53.4 to 71.8%, polyunsaturated FAs decreased from 21 to 12.3% and the n3/n6 ratio decreased from 5.5 to 3.5. This pattern is typical of studies on cetacean blubber where polyunsaturated FAs are richer in the inner regions but monounsaturated FAs are richer in the outer layers and this is thought to relate to better insulation at low temperatures (Pond, 1998).

In the present study the biopsy samples represent a portion of outer region blubber. For all samples combined the FA categories (saturated FA 16.7%, monounsaturated FA 63.5%, polyunsaturated FA 18.3%, and n3/n6 ratio of 4.0) are comparable with the North American dolphin samples (Smith & Worthy, 2006). A wide variety of FAs were present with 60 being identified but most were relatively minor constituents. Fourteen were present at amounts >1% and the six most abundant FAs (18:1n-9, 16:1n-7, 16:0, 22:6n-3 and 14:0) were the same as those found in the outer blubber by Smith & Worthy (2006) and were responsible for nearly 70% of the total in both studies. Thus the blubber compositions of Azores and North American dolphins are similar.

Many of the individual FAs found in blubber are derived from the diet and are deposited without modification. However, the overall profile of blubber FAs differs from the profile found in the diet because of selective metabolism, uptake and release of certain FAs. Following feeding trials the mathematical relationship between dietary and blubber FAs has been studied in several seal species (Iverson et al., 2004) and these workers proposed a method for obtaining quantitative information on the diets of marine mammals called QFASA. This method, which is still being developed, finds a best-fit of prey FA profiles which match the predator FA profile and requires a prey-library of FA profiles and a set of calibration coefficients (obtained from feeding experiments). The authors recommended that a complete cross-section of blubber or a portion of inner region be used and that the outer layer was probably unsuitable. Olsen & Grahl-Nielsen (2003), on comparing the FA profiles of minke whales with those of potential prey, concluded that the inner layer would be the more suited to possible quantitative studies but that the outer layer was suitable for investigating qualitative differences between groups of marine mammals. Thus the biopsy samples of outer region blubber collected in the present study material are unlikely to be suitable for QFASA. There are very few studies where FA profiles have been determined in biopsy samples but Hooker et al. (2001) found that the FA profiles of bottlenose whale *Hyperoodon ampullatus* biopsy blubber were consistent with those of its main prey the squid *Gonatus fabricii* while Herman et al. (2005) were able to differentiate between different ecotypes of killer whales.

Differences in diet can lead to differences in blubber FA profiles. Foraging differences between male and female common dolphins have been observed in the wild (Young &

https://doi.org/10.1017/S0025315407054537 Published online by Cambridge University Press

Cockcroft, 1994; Chou et al., 1995). Smith & Worthy (2006) found that FA differences in the outer, middle and inner blubber layers of the common dolphin could distinguish between male and females, and contrary to expectations the outer layer showed the lowest misclassification rate. However, in North American bottlenose dolphins no statistically significant differences were seen between the sexes (Samuel & Worthy, 2004). In the present study no clear differences in profiles due to the sex of the animal were observed either by PCA or using the Dfap measure. Discriminant analysis produced about 70% correct classification for both males and females. This suggests that the sexes fed broadly on similar diets, with perhaps some minor differences, or alternatlively there might be some metabolic differences between the sexes leading to variations in profiles. This contrasts with the results on the common dolphin described above.

Likewise no clear differences were detected due to the year of sampling of the animals by any of the statistical procedures employed. The results indicate it is possible to pool samples from different years. No other studies have been reported where FAs have been used to compare diets between years in cetaceans but FA profiles in grey seals at two locations have been investigated over a 3-y period (Walton & Pomeroy, 2003). At one site, North Rona, no significant differences in FA profiles were detected over the 3-y period. However, at the other site, Isle of May, there were clear differences in profiles between 1996 and 1998. From this it was concluded that there were major dietary shifts at the Isle of May but not at North Rona. This finding was reinforced with results from a concurrent study on faecal analysis that showed a major shift in the prey species eaten. Thus in the Azores, based on outer blubber profiles, there is no indication of major dietary changes over the period 2002 to 2004.

No clear differences were detected due to the island group where animals were sampled using PC or discriminant analysis. Samples from the same year were not significantly different using the Dfap measure but when samples from all years were combined the difference between central and west became significant. In studies on other species, clustering due to location has been seen in PCA plots of grey seals from two locations in Scotland (Walton et al., 2000; Walton & Pomeroy, 2003), of minke whales from the Norwegian Sea and the North Sea (Olsen & Grahl-Nielsen 2003), and of minke whales from the North Sea and North Atlantic (Møller et al., 2003). Although there is a hint that minor differences between areas exist, overall the results suggest that the profiles from the three different island groups show little differentiation.

Although initially, based on studies of other bottlenose dolphin populations, it was thought that some degree of population structure or site fidelity to the island groups for bottlenose dolphins in the Azores would exist, FA profile analysis did not reveal any clear evidence to support this. Instead, the evidence suggests that there is extensive inter-island-group mixing of dolphin groups, such that any differences that would exist are not detectable. This contrasts with the situation in the Hawaiian archipelago where little or no inter-island travel by dolphins has been detected (Baird et al., 2002, 2003, 2006) and also with the northern Bahamas (Parsons et al., 2006).

Another possibility exists to explain the lack of finding population structure in that it is possible the dolphins are group-faithful but feed on very similar diets and hence no differences are detected. Also it may be that compared to the inner layers, the outer layers are relatively insensitive to dietary changes, but at present the rates of change of the different layers are not known. However, evidence arising from other studies of the CETAMARH project agrees with the conclusion about the lack of strong island group fidelity around the Azores. Neither mtDNA nor microsatellite analyses (Quérouil et al., unpublished data) revealed the existence of any sub-groups. In an observational and photo-identification study 966 individuals were identified but fewer than 8% were found in successive seasons and years in the same location (Silva et al., 2006). This indicates that relatively few individuals show site fidelity ('residents') while the vast majority of animals can be regarded as 'transients'. Some long distance movements were detected between groups of islands and the ranging pattern was independent of sex or age-class. Of the animals tentatively identified as 'residents' biopsy samples were only collected from five of them and it was not possible to test for possible differences between 'residents' and 'transients'. Association patterns also failed to separate the dolphins into distinct geographical communities.

The present study has shown that FA profile analysis of outer layer blubber biopsies can provide useful qualitative ecological information. The FA profiles of bottlenose dolphins of the Azores are similar to those found in dolphins off the Unites States coast. No clear differences in FA profiles were seen between sex, year of sampling or location. Thus it would seem that the dolphins in the Azores constitute a single and open population. These findings agree with the findings from concurrent studies which utilized photo-identification and genetic procedures.

This research was funded by the Portuguese Foundation for Science and Technology, under the CETAMARH project (POCTI/BSE/38991/01). DOP is R&D Unit #351 funded under the Pluriannual/Programmatic scheme of FCT–Lisbon and the Pluriannual scheme of DRCT–Azores. M.A.S. was supported by an FCT Ph.D. fellowship (SFRH/BD/8609/2002) and S.M.M. received a research grant from FCT, through the CETAMARH project. The work was conducted under license of the Environment Directorate of the Regional Government of the Azores.

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Submitted 25 May 2006. Accepted 14 September 2006.