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Depot fatty acid composition in immature green turtles (*Chelonia mydas*) residing at two near-shore foraging areas in the Hawaiian Islands

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Abstract

The lipid content and fatty acid composition of depot fat were determined for 58 immature green turtles (*Chelonia mydas*) residing at two near-shore foraging areas, Ahu-O-Laka, located in Kaneohe Bay on Oahu, and Kiholo Bay located on the island of Hawaii. Benthic flora at Kiholo was limited to a single algal species but included algae and seagrass at Ahu-O-Laka. Turtle straight carapace length ranged from 38.6 to 59.2 cm, suggesting that the sample set included new recruits to up to 12-year residents. Fatty acid data were analyzed using principal components analysis (PCA). PC1 accounted for over 50% of the variance. Turtles were generally delineated along PC1 by the length of time on benthic foraging grounds, with high (>0.75) negative loadings for the fatty acids 22:6n-3, 7M7H, t16:1n-10, 15:0, and 17:0 associated with relatively new recruits (suggesting a pelagic dietary source for these fatty acids) and high positive loadings for 12:0 and 14:0 associated with long-term residents. PC2 separated turtles primarily by capture location, with high positive loadings for 18:2n-6 and 18:3n-3 [the primary seagrass polyunsaturated fatty acids] associated with the Ahu-O-Laka turtles. Fatty acid profiles of turtles from both locations differed substantially from those of their benthic diets, suggesting considerable modification of dietary fatty acids and de novo biosynthesis.

Keywords: Algae; *Chelonia mydas*; Diet; Fatty acids; Gas chromatography; Lipid; Marine turtle; Seagrass

1. Introduction

Green turtles (*Chelonia mydas*) are listed as threatened or endangered throughout their range. In an effort to establish sound management practices, numerous studies have investigated potential factors affecting the growth, health, and reproduction of these animals (Hirth, 1997; Miller, 1997; Chaloupka and Musick, 1997; George, 1997). Although nutrition is recognized as having a pivotal role in the productivity of individuals and populations, large gaps remain in the understanding of the foraging ecology of sea turtles. Most available data on foraging habits of the green turtle have come from studies examining the mouth contents and stomach lavage, and stomach-crop, intestine, and fecal pellets of dead turtles (Hirth, 1997; Russell and Balazs, 2000). During early life stages, green turtles are pelagic, spending their "lost years" (Carr, 1980) on the high seas, and, based on limited data on stomach contents and plasma color, they are assumed to be largely carnivorous, feeding primarily on invertebrates occurring at or near the water's surface (Balazs, 1982; Bjorndal, 1997). As juveniles, they recruit to near-shore feeding grounds and shift to a benthic herbivorous diet. Among sea turtles, the herbivorous diet is unique to the green turtle (Bjorndal, 1997; Mortimer, 1982). In the western Atlantic, turtle grass (*Thalassia testudinum*) is the basic food of the green turtle (Bjorndal, 1982). In the mid-Pacific, seagrasses are sometimes scarce, and algae are a principal food source (Bjorndal, 1997).

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In recent years, numerous studies have focused on the use of lipids and fatty acids (FA) as biological markers and as indicators of diet in fish (Villerreal and Rosenblum, 1994; Seaborn et al., 2000) and in terrestrial (Reidinger et al., 1984) and marine (Iverson et al., 1997; Smith et al., 1997) carnivores. Fatty acid profiles are especially applicable to food-web studies of marine organisms because marine profiles are qualitatively very diverse, often having unique fatty acids, as well as distinctive groupings that can often be traced to a specific origin. The transfer of dietary fatty acids through the marine food web is well established. However, the degree to which an animal's depot fat profile reflects that of the diet could differ depending on the species and its environment. In monogastric animals, fatty acids remain essentially unaltered during digestion, while in ruminants, intestinal bacteria may alter fatty acids during the digestion process, resulting in a higher proportion of saturated and monoenoic fatty acids and the production of short- and odd-chained fatty acids (Meyer et al., 1998).

As an adaptive mechanism for utilizing a plant-based diet, the green turtle has the capability for bacterial fermentation in the hindgut for the digestion of cellulose (Bjorndal, 1979), and, like ruminants, green turtles may alter dietary fat prior to deposition. Joseph et al. (1985) reported that depot fatty acid profiles of wild Caribbean green turtles bore no resemblance to those of their herbivorous diet, while the fatty acid profile of a single captive green turtle was indistinguishable from that of the herring that it was being fed. They further observed that while profiles of captive green turtles reared at the Cayman Island Turtle Farm generally resembled those of wild green turtles, the depot fat of these captive green turtles exhibited elevated levels of 18:2n-6, indicating the incorporation of substantial levels of this fatty acid from their vegetable oil-based synthetic diet. These findings led the authors to conclude that the low-fat diet of the wild green turtle had substantially less effect on the fatty acid composition of the depot fat as compared with captive animals, which are fed regularly on a diet higher in fat content. These authors also suggested that the higher levels of 22:6n-3 in the depot fat of a single Hawaiian green turtle, as compared with Caribbean green turtles, may be due to the inclusion of algae in the diet of the Hawaiian turtle. Ackman et al. (1992) examined depot fatty acid profiles of 13 green turtles from the Hawaiian Islands and Johnston Atoll mid-Pacific region and found highly variable compositions, especially in the C20 and C22 polyunsaturated fatty acids (PUFAs). Although the authors did not associate individual turtles with specific foraging areas, they suggested that the variability in the profiles of these turtles may be due to dietary differences.

In this study, we examined the depot fatty acid composition of immature green turtles residing in two geographically distinct locations within the Hawaiian archipelago. The habitat characteristics of these two locations differed, resulting in differences in the types of flora available to turtles residing in the two locations. Our objectives were to examine the effect of the transition from a pelagic to a benthic diet on the fatty acid profile of turtle depot fat and to examine possible differences between the two locations. The two study sites were of particular interest because fibropapillomatosis (FP), a neoplastic disease that is characterized by the presence of epithelial fibropapillomas and internal fibromas, is prevalent in turtles at one site, while turtles at the other appeared to be free of the disease (Work et al., 2001).

2. Materials and methods

2.1. Sample collection

Immature turtles were caught at both Kiholo Bay, a small bay on the west coast of the Island of Hawaii (19.8669°N, 155.9275°W), and Ahu-O-Laka (AOL), a submerged sandbar in the middle of Kaneohe Bay (21.4836°N, 157.8361°W) on Oahu, during September 1994. Kiholo turtles feed by day on the red algal turf, *Pterocladiella capillacea* (Russell and Balazs, 2000), attached to the rocky lava substrate at the opening of the bay. AOL turtles feed primarily on *Halophila hawaiiensis*, a seagrass that grows abundantly in certain sandy substrates. They also have the opportunity to feed on algae such as *Hypnea*, *Acanthophora*, and *Ulva* (Russell and Balazs, 2000). The turtles were hand captured by diving from a slow-moving boat or by using large-mesh tangle nets carefully tended to prevent injury from forced submergence. Field data, including straight carapace length (SCL) and tumor score (0=no visible tumors; 1=no tumors ≥1 cm in diameter; 2=tumors ≥1 cm but ≤4 cm; 3=tumors ≥4 cm; Work and Balazs, 1999), were recorded at the time of capture. Biopsies were taken from the fat pocket located just dorsal to the insertion of the right hind flipper. The area was deadened with Lidocaine and rinsed with Betadine before a small incision was made with a scalpel, and the fat teased out using the scalpel and forceps. The amount of depot fat collected from each animal ranged from 0.5 to 4 g, depending on the available fat at the biopsy site. The incision was closed with one or two stitches, and the turtle returned to the water. Fat samples were placed in cryovials, held on ice, and frozen shortly thereafter. Samples of the primary algal (*P. capillacea*) and seagrass species (*H. hawaiiensis*) were also collected. The diet samples were placed in polyethylene bags, held on ice, and frozen shortly thereafter. All samples were packed with dry ice and shipped to the Center for Coastal Environmental Health and Biomolecular Research at Charleston, SC, where they were stored at −40 °C.

2.2. Determination of total lipid content and fatty acid composition

Lipids were extracted from each sample with chloroform–methanol, as described by Folch et al. (1957). Total lipid weights were determined gravimetrically and used to

calculate the volume of extract needed to provide approximately 25 mg of lipids for derivatization. Following evaporation of the chloroform, fatty acid methyl esters (FAME) were prepared as described by Christopherson and Glass (1969). Briefly, the neat lipid was dissolved in 1 mL of isooctane. After the addition of 200 μL of 2 N methanolic KOH, the sample was vortexed for 2 min and allowed to stand for 15 min. The isooctane layer, containing the esters, was dried over anhydrous sodium sulfate and transferred to a 2 mL vial for instrumental analyses.

Samples were analyzed using a Hewlett-Packard 5890 or 6890 gas chromatograph (GC) equipped with flame ionization detector. Separation was achieved on a 30 m×0.25 mm 50% cyanopropylphenyl-50% methyl fused-silica capillary column (DB225, J&W Scientific, Folsom, CA). The initial oven temperature of 170 °C was increased at a rate of 1 °C/min to 220 °C. Helium was the carrier gas, with a flow rate of 1.5 mL/min; nitrogen was the auxiliary gas. Injections were in the split mode with a split ratio of 1:60. The mass spectra of FAME from representative samples were obtained using a Hewlett-Packard 5890A GC interfaced with a 5970 Series mass selective detector or a Hewlett-Packard 6890A GC interfaced with a 5973 Series mass selective detector. GC parameters were as described above. Mass spectra were recorded at an ionization energy of 70 eV.

Fatty acids were identified by comparing their relative retention times with those of known primary (Nu Chek Prep, Elysian, MN) and secondary (menhaden and cod liver oils) standards, argentation thin-layer chromatography, and by GC-Mass Spectrometry. Double-bond positions for methylene-interrupted polyenoic esters were assigned based on knowledge of the elution order of positional isomers and by the comparison of ion intensities at m/e=108, 150, and 192 in their mass spectra, as described by Fellenberg et al. (1987). Correction factors (Craske and Bannon, 1988) were used to convert area percent to weight percent of fatty acids. The shorthand notation used for fatty acids specifies the number of carbon atoms and the number of double bonds, followed by the position of the terminal double bond relative to the hydrocarbon end of the molecule. For example, 18:2n-6 denotes a fatty acid that contains 18 carbon atoms with two double bonds and is a member of the n-6 family of fatty acids.

2.3. Data analysis

Analysis of variance (ANOVA) tested for differences in mean SCL and lipid content between locations and between AOL turtles with and without tumors. Fatty acid data were examined using principal components analysis (PCA). This allowed us to determine a reduced set of uncorrelated linear combinations, or principal components (PC), while retaining the combined information from all fatty acids. Fatty acids that did not exceed 1.0% in more than one turtle were excluded. Prior to PCA analysis, percentages for the remaining 26 fatty acids were log-ratio transformed to account for the compositional nature of the data (Aebischer et al., 1993). Transformed values were calculated as suggested by Budge et al. (2002) according to the following equation:

$$x_{\text{trans}} = \ln(x_i/c_i)$$

where x_i is the weight percent of a given fatty acid, c_i is the weight percent of the reference fatty acid (18:0), and x_{trans} is the transformed fatty acid value. In PCA, the PCs are sorted so that each accounts for a progressively smaller percentage of variance within the data set. Ideally, a large percentage of the variance is explained by a small number of PCs, and relationships may be assessed by inspection of two- or three-dimensional plots. We used two-dimensional plots of PC scores for individual turtles to examine natural animal groupings that could be related to length of time on benthic feeding grounds or capture location. We then examined the variable loadings for each of the PCs to determine which fatty acids influenced these groupings. Statistical analyses were performed using JMP 5.0 (SAS Institute, Cary, NC).

3. Results

To examine the effect of the transition from a primarily carnivorous pelagic diet to a primarily herbivorous benthic diet, we used straight carapace length (SCL) to place animals into three residency status groups (RS1, RS2, and RS3) within each location (Table 1). In the Hawaiian Islands, green turtles generally recruit to inshore benthic foraging areas at a size of approximately 35 cm SCL (Balazs, 1980; Zug et al., 2001). Tagging and recapture studies indicate that Hawaiian green turtles generally forage in the same coastal areas for many years and at some locations throughout their lives (Balazs, 1982). Mean growth rates from tagging and recapture studies are 2.0±1.5 cm/year for Kaneohe Bay turtles (Balazs et al., 2000a) and 1.7±1.1 cm/year for Kiholo Bay turtles (Balazs et al., 2000b). Using these growth rates, we estimated that the turtles in our study included new recruits to residents of up to 12 years. Although individual turtles do not recruit at exactly 35 cm, nor do they all have identical growth rates, RS1 should include mostly newer recruits, RS3 the turtles with the longest time on benthic foraging grounds, and RS2 the transitional animals.

Table 1
Classification of Hawaiian green turtles based on straight carapace length (SCL), capture location and tumor status

Residency Status (RS)	1	2	3
SCL	(38–43 cm)	(44–49 cm)	(≥50 cm)
Kiholo			
No tumors	7	8	9
Ahu-O-Laka			
No tumors	9	11	2
Tumors	0	5	7

Table 2
Median percentages and ranges of fatty acids found in depot fat of immature green turtles (*Chelonia mydas*) collected at two locations in the Hawaiian Islands

Location	Kiholo						AOL					
Residency status (RS)	RS1 (*N*=7)		RS2 (*N*=8)		RS3 (*N*=9)		RS1 (*N*=9)		RS2 (*N*=16)		RS3 (*N*=9)	
SCL (cm) mean±S.E.	41.5±0.50		46.9±0.70		55.2±1.5		41.7±0.60		45.7±0.30		54.8±1.0	
Fatty acid	Wt.% of fatty acids											
10:0	0.08	0.07–0.18	0.10	0.06–0.17	0.11	0.06–0.18	0.10	0.08–0.19	0.10	<0.01–0.19	0.12	<0.01–0.16
12:0[a]	5.55	4.68– 11.78	7.40	4.36– 13.85	12.87	4.92–15.02	5.55	3.99–11.47	7.00	4.84–13.60	10.15	6.14–14.29
13:0	0.05	0.04–0.07	0.06	0.04–0.07	0.06	0.04–0.07	0.06	0.05–0.07	0.06	0.04–0.07	0.06	0.03–0.08
14:0[a]	5.97	5.03–9.90	7.95	5.04–11.43	10.47	6.64–12.42	5.69	4.92–8.34	6.40	4.97–9.46	8.09	5.93–9.65
15:0[a]	0.53	0.28–0.62	0.43	0.15–0.68	0.23	0.18–0.65	0.65	0.29–1.00	0.52	0.24–0.81	0.36	0.19–0.67
16:0[a]	13.29	12.80–17.18	14.02	9.76–20.45	17.35	9.62–18.33	14.33	12.41–16.90	14.56	13.25–16.89	15.83	14.74–17.38
17:0[a]	0.61	0.25–0.72	0.42	0.14–0.67	0.25	0.15–0.62	0.78	0.31–1.06	0.66	0.23–0.82	0.52	0.32–0.74
18:0[a]	5.42	4.84–6.20	4.99	4.42–5.87	5.45	4.29–6.01	5.63	5.06–6.35	5.50	4.76–6.17	5.70	5.03–6.46
19:0	0.41	0.18–0.48	0.36	<0.01–0.57	0.09	<0.01–0.71	0.42	0.12–0.66	0.36	0.20–0.52	0.22	0.12–0.49
20:0[a]	0.92	0.31–1.10	0.72	0.10–1.84	0.28	0.09–1.71	0.97	0.24–1.53	0.62	0.29–1.05	0.43	0.17–0.89
21:0	0.20	<0.01–0.27	0.21	<0.01–0.41	0.03	<0.01–0.25	0.17	<0.01–0.31	0.11	<0.01–0.25	0.10	<0.01–0.21
22:0	0.16	0.09–0.24	0.16	0.05–0.37	0.07	<0.01–0.40	0.14	0.06–0.22	0.11	0.06–0.24	0.07	<0.01–0.15
24:0	0.09	<0.01–0.25	0.05	<0.01–0.21	0.06	<0.01–0.27	0.09	<0.01–0.14	0.07	<0.01–0.22	<0.01	<0.01–0.11
12:1n–7?	0.05	0.02–0.09	0.07	0.02–0.12	0.10	0.02–0.14	0.03	0.02–0.07	0.06	0.02–0.13	0.07	0.04–0.16
14:1n–7	0.04	<0.01–0.07	0.05	0.03–0.06	0.06	0.02–0.07	0.03	<0.01–0.04	0.04	<0.01–0.08	0.04	<0.01–0.07
14:1n–5	0.37	0.28–0.61	0.48	0.30–0.69	0.62	0.38–0.74	0.33	0.18–0.40	0.35	0.23–0.64	0.43	0.33–0.59
C16:1	0.07	0.03–0.09	0.05	<0.01–0.12	0.02	<0.01–0.12	0.06	0.01–0.07	0.05	<0.01–0.08	0.03	<0.01–0.06
t16:1n-10[a]	0.89	0.26–1.40	0.50	0.03–1.01	0.12	<0.01–0.95	1.51	0.22–1.90	1.09	0.21–1.47	0.41	0.14–1.28
16:1n-9	0.20	<0.01–0.46	0.37	0.16–0.59	0.53	<0.01–0.65	0.17	<0.01–0.46	0.28	0.18–0.76	0.47	0.18–0.69
16:1n-7[a]	5.09	4.46–7.19	5.62	3.11–7.69	6.38	2.61–7.29	4.56	3.49–6.35	4.99	4.20–6.56	5.17	4.33–6.37
16:1n-5	0.08	0.07–0.11	0.11	0.05–0.16	0.11	0.06–0.17	0.07	0.06–0.15	0.09	0.06–0.20	0.11	0.05–0.20
17:1n-8[a]	0.47	0.23–0.64	0.36	0.16–0.69	0.27	0.14–0.46	0.62	0.41–0.85	0.54	0.22–0.69	0.36	<0.01–0.64
18:1n-9[a]	20.50	18.85–23.39	20.82	14.84–27.85	24.42	15.64–26.67	20.90	15.72–23.53	21.62	18.86–27.62	23.89	21.55–25.83
18:1n-7[a]	2.76	2.52–3.07	2.73	1.81–3.66	3.44	1.98–3.70	2.85	2.38–3.11	3.04	2.53–3.75	3.22	2.81–3.62
18:1n-5+18:2n-9	0.16	0.13–0.17	0.17	0.11–0.23	0.22	0.15–0.27	0.15	0.13–0.23	0.17	0.15–0.24	0.18	0.15–0.28
19:1n-10	0.08	<0.01–0.13	0.10	<0.01–0.22	0.04	<0.01–0.26	0.13	<0.01–0.39	0.11	<0.01–1.65	0.06	<0.01–0.14
19:1n-8/18:4n-3	0.27	0.11–0.36	0.14	0.05–0.32	0.10	0.07–0.18	0.30	0.07–0.45	0.26	0.06–0.50	0.16	0.04–0.32
20:1n-11+13	0.16	0.09–0.30	0.15	0.07–0.36	0.11	<0.01–0.40	0.15	0.08–0.42	0.21	0.09–0.33	0.15	0.07–0.33
20:1n-9[a]	1.21	0.70–1.48	1.20	0.49–1.72	0.67	0.37–2.42	1.09	0.51–1.87	1.20	0.71–1.79	0.89	0.56–1.91
20:1n-7	0.29	<0.01–0.32	0.29	0.07–0.38	0.13	<0.01–0.35	0.25	<0.01–0.59	0.27	0.15–0.44	0.15	<0.01–0.32
20:1n-5/20:2	<0.01	<0.01–0.17	0.12	0.00–0.28	0.21	<0.01–0.23	0.04	<0.01–0.11	0.07	<0.01–0.25	0.09	<0.01–0.21
22:1n-11+13	0.12	<0.01–0.29	0.13	<0.01–0.41	0.09	<0.01–0.32	0.11	<0.01–0.32	0.16	<0.01–0.28	0.10	<0.01–0.14
22:1n-9[a]	0.82	0.30–0.94	0.69	0.11–1.66	0.24	0.08–1.69	0.59	0.17–1.26	0.55	0.27–0.86	0.38	0.15–0.81
22:1n-7	0.15	<0.01–0.29	0.16	<0.01–0.82	0.11	<0.01–0.71	0.25	<0.01–0.50	0.11	<0.01–0.29	<0.01	<0.01–0.28

23:1	<0.01	<0.01-0.38	0.12	<0.01–0.51	<0.01	<0.01–0.64	0.04	<0.01–0.50	0.14	<0.01–0.46	<0.01	<0.01–0.50
24:1n-11+13	<0.01	<0.01–0.25	0.07	<0.01–0.34	<0.01	<0.01–0.16	<0.01	<0.01–0.20	0.08	<0.01–0.21	<0.01	<0.01–0.14
24:1n-9[a]	1.00	0.51–1.44	0.87	0.15–1.97	0.41	0.22–1.66	0.72	0.25–1.55	0.55	0.32–1.03	0.56	0.22–0.94
16:2n-7?	0.13	0.10–0.15	0.12	<0.01–0.19	0.03	<0.01–0.25	0.11	0.05–0.28	0.12	<0.01–0.20	0.06	<0.01–0.12
18:2n-7?/tt18:2n-6	<0.01	<0.01–0.16	0.09	<0.01–0.19	0.17	<0.01–0.22	<0.01	<0.01–0.07	0.06	<0.01–0.24	0.06	<0.01–0.18
18:2n-6[a]	0.58	0.32–0.66	0.44	0.31–0.81	0.61	0.27–1.24	0.83	0.56–2.18	1.01	0.56–1.75	1.66	0.24–2.24
C18:2	<0.01	<0.01–0.08	<0.01	<0.01–0.11	0.06	<0.01–0.19	<0.01	<0.01–0.41	0.10	<0.01–0.30	0.19	<0.01–0.30
20:2n-6	0.28	0.19–0.33	0.25	0.04–0.35	0.06	<0.01–0.51	0.28	0.15–0.72	0.31	0.09–0.69	0.18	0.10–0.27
22:2n-6	<0.01	<0.01–0.14	0.04	<0.01–0.12	<0.01	<0.01–0.12	<0.01	<0.01–0.07	<0.01	<0.01–0.12	<0.01	<0.01–0.04
18:3n-6	<0.01	<0.01–0.12	0.10	<0.01–0.16	0.13	<0.01–0.18	0.07	<0.01–0.13	0.09	<0.01–0.17	0.05	<0.01–0.12
18:3n-3[a]	0.12	<0.01–0.15	0.11	0.05–0.15	0.13	<0.01–1.16	0.18	0.13–3.88	0.97	0.13–2.78	1.86	0.07–3.41
20:3n-9	<0.01	<0.01–0.06	<0.01	<0.01–0.09	0.11	<0.01–0.17	<0.01	<0.01–0.03	<0.01	<0.01–0.06	<0.01	<0.01–0.07
20:3n-6	<0.01	<0.01–0.59	0.27	<0.01–0.47	0.52	0.09–1.05	0.12	<0.01–0.57	0.13	<0.01–0.21	0.29	<0.01–0.33
20:3n-4	<0.01	<0.01–0.17	0.04	<0.01–0.14	0.15	<0.01–0.29	<0.01	<0.01–0.10	<0.01	<0.01–0.19	<0.01	<0.01–0.16
20:3n-3/C21:1	<0.01	<0.01–0.08	<0.01	<0.01–1.00	<0.01	<0.01–0.29	0.12	<0.01–0.15	0.09	<0.01–0.14	0.05	<0.01–0.10
20:4n-6[a]	1.25	1.00–1.69	1.18	0.72–2.20	1.26	0.86–2.17	1.47	0.21–3.29	1.46	0.63–2.60	1.18	0.54–2.06
20:4n-3	0.25	0.08–0.27	0.15	0.08–0.30	0.16	<0.01–0.32	0.24	0.18–0.35	0.26	0.17–0.38	0.26	0.13–0.55
22:4n-6[a]	1.47	0.67–1.97	1.86	0.59–2.43	0.92	0.67–4.36	1.58	0.73 – 0.19	1.31	0.57–2.41	0.88	0.30–1.33
20:5n-3[a]	1.02	0.46–1.58	0.63	0.34–1.43	0.62	0.24–1.77	1.40	0.68–1.90	1.25	0.47–1.95	1.41	1.07–1.99
22:5n-6[a]	1.96	0.95–2.49	2.03	0.20– 4.24	0.39	0.15–3.43	1.97	0.46–6.65	1.56	0.34–2.56	0.72	0.45–1.12
22:5n-3[a]	2.74	2.00–3.12	3.15	1.51–4.50	2.74	1.59–4.67	2.76	1.91–3.69	2.43	1.71–3.55	2.73	1.60–3.94
24:5n-6	0.03	<0.01–0.11	0.07	<0.01–0.12	0.03	<0.01–0.12	0.04	<0.01–0.15	0.05	<0.01–0.07	0.03	<0.01–0.15
22:6n-3[a]	12.23	5.83–15.72	6.66	0.62–16.99	1.22	0.60–13.55	11.39	2.85–13.45	9.78	1.53–13.58	4.60	1.57–14.14
24:6n-3[a]	0.63	<0.01–1.51	0.83	0.16–2.01	0.17	<0.01–1.47	0.38	<0.01–1.12	0.39	<0.01–1.12	0.28	0.04–0.74
Phytanate+17:1	0.15	<0.01–0.31	0.13	<0.01–0.41	0.20	<0.01–0.63	0.07	<0.01–0.30	<0.01	<0.01–0.47	0.03	<0.01–0.30
Iso15:0	0.14	0.09–0.19	0.15	0.10–0.20	0.19	0.09–0.27	0.13	0.09–0.22	0.12	0.08–0.17	0.11	0.06–0.18
Aiso15:0	0.05	0.04–0.20	0.11	0.06–0.23	0.17	0.06–0.30	0.05	0.04–0.13	0.05	0.05–0.09	0.07	0.03–0.14
Iso16:0	0.17	0.08–0.20	0.11	<0.01–0.18	0.07	<0.01–0.18	0.18	0.07–0.24	0.15	0.05–0.24	0.10	0.04–0.23
Iso17:0	0.20	0.12–0.24	0.20	0.07–0.30	0.14	0.08–0.27	0.23	0.10–0.33	0.22	0.06–0.33	0.13	0.10–0.27
Aiso17:0	0.44	0.23–0.52	0.30	0.12–0.46	0.25	0.16–0.36	0.41	0.20–0.63	0.33	0.17–0.61	0.25	0.16–0.52
Iso18:0	0.30	0.16–0.37	0.25	0.03–0.36	0.05	<0.01–0.43	0.35	0.10–0.55	0.33	0.11–0.41	0.18	0.07–0.41
7M7H	1.35	0.45–1.48	0.84	0.13–1.68	0.32	0.07–1.66	1.33	0.32–1.77	1.01	0.30–1.54	0.53	0.30–1.44
Furan (F2)	0.21	<0.01–0.30	0.24	0.08–0.36	0.15	<0.01–0.24	0.13	<0.01–0.18	0.12	<0.01–0.28	<0.01	<0.01–0.12
Furan (F3)	<0.01	<0.01–0.12	<0.01	<0.01–0.15	<0.01	<0.01–0.23	<0.01	<0.01–0.09	0.03	<0.01–0.13	<0.01	<0.01–0.15
Furan (F4)	0.17	0.07–0.23	0.11	<0.01–0.30	0.05	<0.01–0.36	0.21	<0.01–0.30	0.18	0.06–0.23	0.08	<0.01–0.18
Furan (F5)	0.28	<0.01–0.37	0.27	0.04–0.55	0.04	<0.01–0.32	0.15	<0.01–0.44	0.22	<0.01–0.36	0.08	<0.01–0.26
Furan (F6)	0.71	0.40–0.91	0.68	0.10–1.57	0.21	0.13–1.74	0.66	0.34–0.96	0.62	0.23–0.83	0.29	<0.01–0.49
Furan (F9)	0.24	<0.01–0.47	0.31	<0.01–0.59	0.09	<0.01–0.54	0.12	<0.01–0.41	0.17	<0.01–0.39	0.06	<0.01–0.27
16:2 Conjugated	0.18	<0.01–0.27	0.10	<0.01–0.20	<0.01	<0.01–0.18	0.23	0.04–0.36	0.15	<0.01–0.38	0.09	<0.01–0.30
18:2 Conjugated	<0.01	<0.01–0.09	0.02	<0.01–0.09	0.07	<0.01–0.22	0.05	<0.01–0.16	0.07	<0.01–0.13	0.10	<0.01–0.22
UK280	0.93	0.39–1.17	0.70	0.06–1.22	0.17	0.07–1.34	0.96	0.20–1.76	0.87	0.25–1.14	0.47	0.14–0.88

Turtles are grouped by residency status within each location. Percentages are given to two decimal places for comparison of minor components.

[a] Fatty acids found at ≥1.0% in more than one animal. ? Assigned identity is questionable.

3.1. Lipid content

The total lipid content of the fatty tissue ranged from 3.5% to 47.0% for Kiholo turtles and 17.0% to 53.8% for AOL turtles. The distribution for lipid content within Kiholo turtles was unusual in that 45.8% of the animals (two of seven RS1 turtles, four of eight RS2 turtles, and five of nine RS3 turtles) had a lipid content of ≤10%, while that for the remaining animals was ≥25%. This unexpected finding suggested that the Kiholo turtles were composed of two distinct groups in terms of their lipid content and introduced an additional variable the could potentially influence fatty acid composition. We designated the group of Kiholo turtles with a lipid content of ≥25% to be "normal" and those with a lipid content ≤10% to be "low-lipid" animals. Total lipid percentages of AOL turtles were normally distributed within location. Excluding the low-lipid Kiholo turtles, the mean lipid content was not significantly different between locations [$F(1,47)=0.0193$, $P=0.890$]. Accounting for SCL, the presence of tumors had no significant effect on total lipid content for AOL turtles [ANOVA, $F(3,33)=2.605$, $P=0.117$]. No turtle sampled at AOL had a tumor score >2.

3.2. Turtle fatty acid composition

The median percentages and ranges for the 77 fatty acids identified in turtle depot fat lipids are given in Table 2. The 26 fatty acids found at levels ≥1.0% in at least two animals comprised 93% to 95% of the total for all animals. Generally, the most abundant fatty acid for all turtles was 18:1n-9. Percentages for total monoenes in individual turtles ranged from 27% to 41% and, in addition to 18:1n-9, included 14:1n-5, 16:1n-7, 18:1n-7, 20:1n-9, and 24:1n-9. Total saturates ranged from 27% to 51%, with 16:0 as the most abundant saturate; 18:0 ranged from 5% to 6%. A prominent feature of the data set was the high variability among all turtles in the percentages of 12:0, 14:0, and the PUFA, especially 22:6n-3. Percentages for 22:6n-3 for individual turtles ranged from 0.6% to 17.0% and were inversely correlated with 12:0 ($r=0.95$) and 14:0 ($r=0.93$).

FA profiles for animals from both locations included numerous less common fatty acids. The *trans*-16:1n-10 (identification and geometric configuration based on chromatographic behavior on polar and nonpolar columns and on mass spectra) was found in all animals at levels ranging from <0.01% to 1.9%. A branched-chain monoene, 7-methyl-7-hexadecaenoic acid (7M7H), was found in all turtles at levels similar to that of t16:1n-10. An unidentified fatty acid (UK280) with a molecular weight of 280 and eluting immediately after 18:2n-6 in the GC analysis was found in most of the animals. We have tentatively identified this component as 7-methyl-5,7-hexadecadieonic acid based on chemical and chromatographic behavior. Minor fatty acids included straight-chained saturates (10:0, 13:0, 19:0, 21:0, 22:0, and 24:0), as well as saturated branched-chained fatty acids. A 20-carbon (C) nonmethylene interrupted diene (NMID) was found at low levels, as were C16:2 and C18:2 conjugated fatty acids (double-bond positions not determined). Two C24 PUFAs were tentatively identified as 24:5n-6 and 24:6n-3 based on retention times and mass spectra of the methyl esters. Both were generally more abundant in the Kiholo turtles and appeared to be lower in larger turtles from both locations. The 20:3n-9 was found in trace (<0.05%) amounts in Kiholo-RS1, Kiholo-RS2, and AOL-RS3 turtles and at approximately 0.1% in Kiholo-RS3

Table 3
Fatty acid composition of primary dietary components available to turtles residing at Kiholo (*P. capillacea*) and AOL (*H. hawaiiensis*)

Fatty acid	*P. capillacea*	*H. hawaiiensis*
	Wt.% of fatty acids	
12:0	0.22	0.36
14:0	5.30	7.08
15:0	0.58	0.82
16:0	43.63	33.73
17:0	0.96	0.59
18:0	2.70	3.75
20:0	0.34	0.43
22:0	0.00	0.52
14:1n-5	0.38	0.18
16:1n-9	0.38	0.73
16:1n-7	7.26	6.24
16:1n-5	0.47	0.23
17:1n-8	0.24	0.19
18:1n-9	6.40	10.39
18:1n-7	2.90	3.11
18:1n-6?	0.05	0.06
18:1n-5+18:2n-9	0.07	0.22
20:1n-11+13	0.42	0.16
20:1n-9	0.21	0.32
20:1n-7	0.13	0.13
22:1n-11+13	0.00	0.15
22:1n-9	0.00	0.16
22:1n-7	0.00	0.07
16:2n-7?	0.22	0.68
16:2n-6?	0.14	0.08
16:2n-4	1.04	0.21
18:2n-6	2.74	9.20
20:2n-6	0.19	0.25
16:3n-3	0.11	0.18
18:3n-6	0.00	0.98
18:3n-3	1.66	7.76
20:3n-6	0.32	0.13
18:4n-3	0.91	1.82
20:4n-6	6.16	1.36
20:4n-3	0.14	0.18
22:4n-6	0.31	0.15
20:5n-3	6.69	2.37
22:5n-6	0.19	0.31
22:5n-3	0.41	0.22
22:6n-3	1.36	0.66
TMTD	0.18	0.00
Iso15:0	0.51	0.65
Aiso15:0	0.48	0.39
Iso16:0	0.13	0.19
Iso7:0	0.39	0.49
Iso17:1?	0.86	0.00
Phytanate	0.15	0.00

? Assigned identity is questionable.

turtles. Six furan (F) fatty acids (F2, F3, F4, F5, F6, and F9) were found in the profiles of most of the turtles. The abbreviated nomenclature for furan fatty acids is defined in terms of structure, GC, and mass spectral characteristics by Dembitsky and Rezanka (1996). The F6 was found at 0.1% to 1.7% of total fatty acids; only minor amounts were observed for the other furan fatty acids. The percentages of these fatty acids appeared to be higher in the smaller turtles from both locations.

3.3. Benthic dietary fatty acids

The 16:0 was the major fatty acid in *H. hawaiiensis* and *P. capillacea* at 33.7% and 43.6%, respectively (Table 3, Fig. 1). The primary differences between the two dietary items were in the percentages of the PUFAs. The percentage of 18:3n-3 in *H. hawaiiensis* was 4.7 times that found in *P. capillacea*, and 18:2n-6 was 3.3 times greater. The 20:4n-6 and 20:5n-3 were important *P. capillacea* PUFAs and exceeded the percentages found in *H. hawaiiensis* by 4.5 and 2.8 times, respectively. The percentage of 22:6n-3 found in *P. capillacea* (1.36%) was low, but twice that found in the *H. hawaiiensis*. Because we were interested in fatty acids consumed as a result of ingesting *H. hawaiiensis* and *P. capillacea*, we did not attempt to remove possible traces of adhering organisms prior to analysis, which, if present, could make a minor contribution to the profiles.

3.4. Turtle fatty acids vs. dietary fatty acids

Median percentages for fatty acids that were important in assessing dietary relationships are shown in Fig. 1A for RS1 and RS3 turtles from both locations for comparison with the fatty acid profiles of *H. hawaiiensis* and *P. capillacea* (Fig. 1B). Values for the RS2 turtles were generally intermediate between those for RS1 and RS3 turtles and were omitted from the figure for simplification. Profiles for the RS1 turtles would be expected to reflect the pelagic diet, while those for

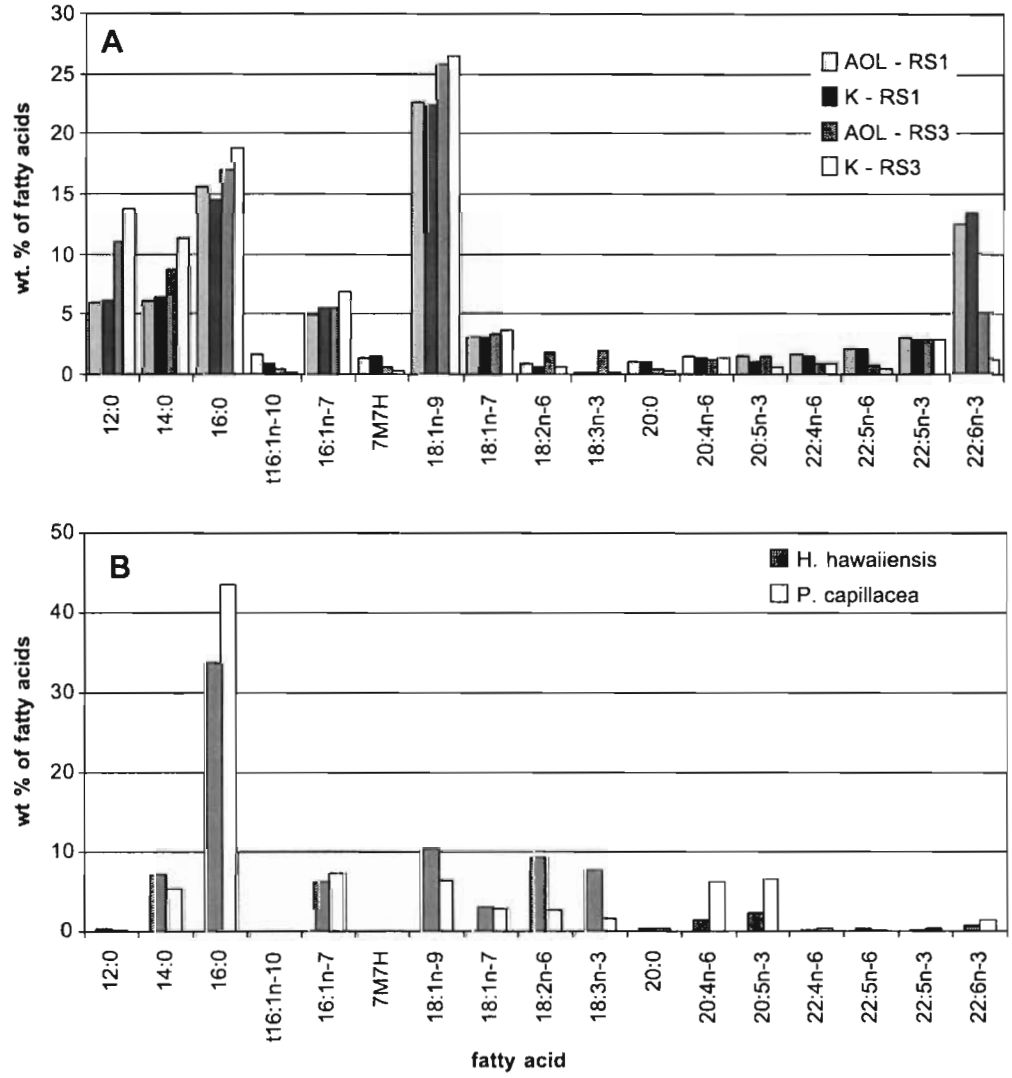

Fig. 1. Comparison of median percentages of selected fatty acids found in depot fat of RS1 and RS3 turtles from AOL and Kiholo (Panel A) and fatty acids of dietary items *H. hawaiiensis* and *P. capillacea* (Panel B).

the RS3 turtles should reflect relatively long-term feeding on their respective benthic diets. Regardless of capture location or time on benthic foraging grounds, turtles retained a characteristic profile that was quite different from those of their primary dietary components. While 16:0 was the predominant fatty acid in both dietary components (34–44%), levels in turtle depot fat were less than half that amount. The 18:1n-9 was the predominant fatty acid in turtle depot fat (>25%), but less than half that amount was found in the seagrass or algae. The median percentage of 12:0 in the RS3 turtles was approximately twice that of the RS1 turtles, but this fatty acid accounted for <0.5% in the benthic dietary components examined. Despite major differences between turtle and diet profiles, the benthic dietary effect was evident.

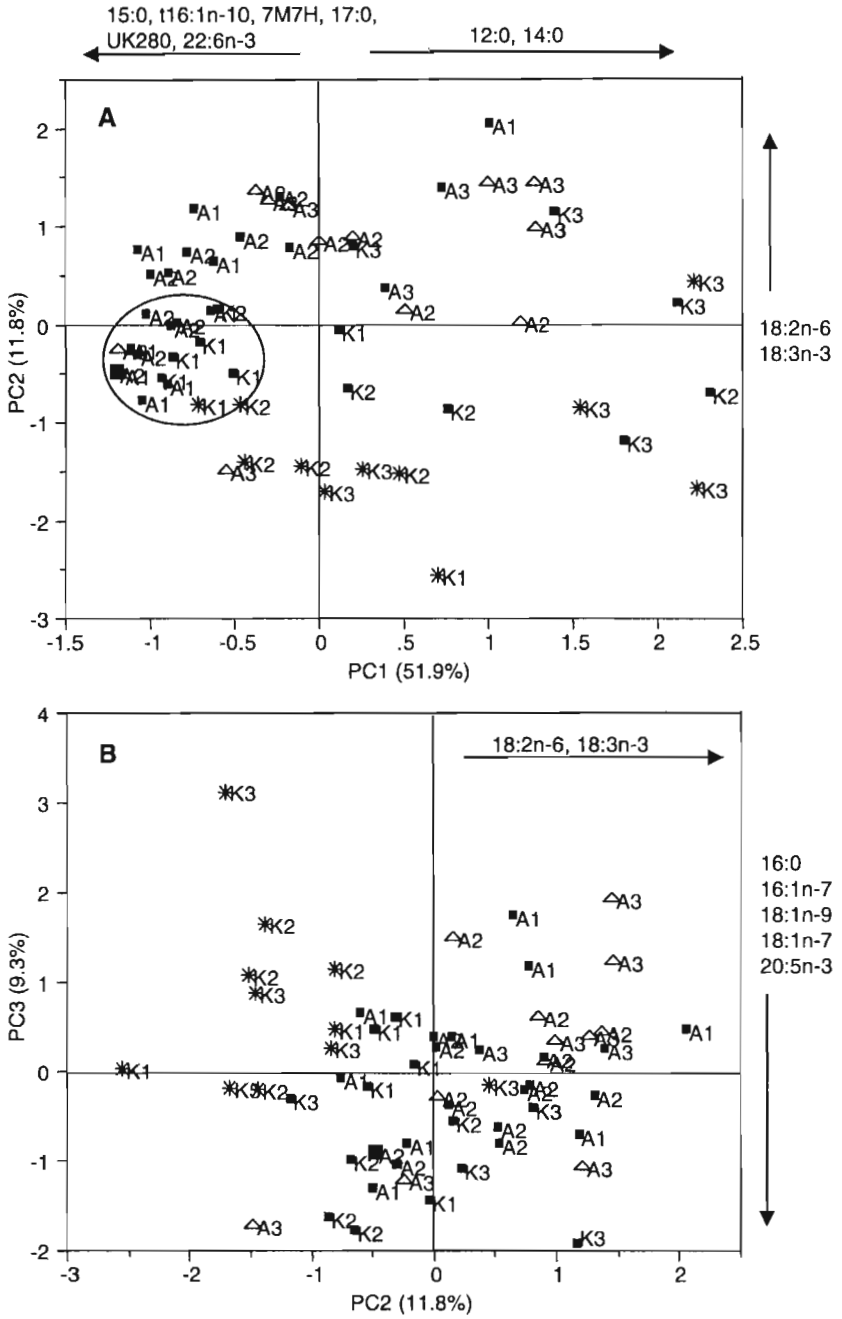

Fig. 2. PC1 vs. PC2 scores for all turtles (Panel A). The circle denotes a comparatively tight cluster including RS1 and RS2 animals from both capture locations, suggesting that relatively new recruits at both locations have similar fatty acid profiles. PC2 vs. PC3 scores for all turtles (Panel B); K=Kiholo turtles; *K denotes Kiholo turtles with a tissue lipid content of ≤10%; A=AOL turtles; ΔA denotes AOL turtles with tumors; 1, 2, and 3 denote residency status. Directional arrows parallel to each PC axis indicate an increase in the absolute loadings for the listed fatty acids influencing that PC in a positive or negative direction.

Compared with the RS3 Kiholo and the RS1 turtles from both locations, we observed higher levels of 18:2n-6 and 18:3n-3 (the major PUFAs in *H. hawaiiensis*) in the profiles of the RS3 AOL turtles. Likewise, the lower median percentages of 7M7H, t16:1n-10, 22:4n-6, 22:5n-6, and 22:6n-3 in the RS3 turtles at both locations were consistent with the low levels of these fatty acids in *H. hawaiiensis* and *P. capillacea*. However, the levels of 22:5n-3 appeared unchanged in turtles residing at both locations.

3.5. Principal component analysis

The first four PCs cumulatively accounted for 82% of total variance (51.9%, 11.8%, 9.3%, and 8.4%, respectively). To identify factors represented by sets of variables, we conducted an orthogonal rotation that focused on making as many values in each column of the factor coefficient table as close to zero as possible (varimax). We obtained two-dimensional plots using rotated scores for PC1 vs. PC2 (Fig. 2A) and PC2 vs. PC3 (Fig. 2B), with individual animals coded to indicate capture location, residency status, low lipid content (≤10%), and tumor status. Animals were generally distributed along PC1 by residency status, with most of the RS1 turtles having the highest negative scores and most of the RS3 turtles the highest positive scores. PC1 had high negative loadings for 22:6n-3 (−0.947), 17:0 (−0.938), t16:1n-10 (−0.931), UK280 (−0.922), 7M7H (−0.908), and 15:0 (−0.892), which were generally higher in smaller turtles, and high positive loadings for 12:0 (0.821) and 14:0 (0.851), which were generally higher in larger turtles (Fig. 2A; Table 2).

PC2 appeared to delineate turtles by capture location (Fig. 2A). Some of the RS1 and RS2 animals from both capture locations comprised a comparatively tight cluster (circled in Fig. 2A), suggesting that relatively new recruits at both locations have similar fatty acid profiles. Greater separation was seen by location for the larger turtles, with positive scores for most of the AOL turtles and negative scores for most of the Kiholo turtles. Positive scores for the AOL turtles were influenced by high positive loadings for 18:2n-6 (0.888) and 18:3n-3 (0.850), corresponding to higher percentages of these fatty acids in AOL turtles (Fig. 2A). The lowest PC2 scores were noted for the low-lipid Kiholo turtles.

The plot of PC2 vs. PC3 scores partitions most of the low-lipid Kiholo turtles in the upper left quadrant with negative PC2 scores and positive PC3 scores (Fig. 2B), suggesting that the fatty acids with high loadings for both PC2 and PC3 influenced this partitioning. PC3 had high negative loadings for 16:1n-7 (−0.884), 16:0 (−0.808), 18:1n-9 (−0.754), 18:1n-7 (−0.746), and 20:5n-3 (−0.663). When the median percentages of the fatty acids influencing the PC3 partitioning were compared within the Kiholo turtles, percentages for the "normal" turtles were consistently higher than those for low-lipid Kiholo turtles, respectively (16:1n-7, 6.9% vs. 5.1%; 16:0, 16.7% vs. 15.3%; 18:1n-9, 24.5% vs. 23.2%; 18:1n-7, 3.4% vs. 2.9%; 20:5n-3, 1.5% vs. 0.5%). There was no apparent partitioning of turtles with tumors in either plot. PC4 had the highest loadings for 20:4n-6 (0.676), 22:4n-6 (0.768), and 22:5n-3 (0.702) but did not appear to differentiate turtle groups corresponding to any of the variables considered (data not shown).

4. Discussion

4.1. Lipid content

Excluding the low-lipid Kiholo turtles, the mean percentages for total lipid found in the fatty tissue of the AOL (35.2%±1.6 S.E.) and the Kiholo (34.8%±2.0 S.E.) turtles were slightly lower than those found in subcarapace depot fat collected from a group of green turtles freshly butchered by indigenous fishermen in Daru, Papua New Guinea, which included multiple maturity stages from prepubescent to breeding males and females (Kwan, 1994). Using graphs presented by Kwan, we estimate the lipid content/weight of fatty tissue in the Daru turtles to be around 50% for animals in most of the reproductive and maturity groups. The Kwan data suggest that the low-lipid (≤10%) level found in the depot fat of many of the Kiholo turtles is unusual. It is possible that turtles in a rapid growth stage may have less stored fat. Although SCL did not differ between the two locations, the Kiholo turtles appeared to be "thinner", possibly suggesting that the low-lipid levels may be associated with poor health. Body weights were not taken. The reason for the substantial within-site difference in lipid content is unclear. All Kiholo samples were collected within a 2-day period using identical techniques. Sex of the animals was unknown, but it is unlikely that sex would have a substantial effect in immature turtles. Because *P. capillacea* is, by far, the most abundant food source available to turtles residing at Kiholo, all turtles have qualitatively similar diets. However, food quantity may be limited, and some turtles may be more efficient foragers.

In light of a previous assessment of the green turtle diets, we expected lower and more variable lipid content in the AOL than in the Kiholo turtles. Bjorndal (1982) compared breeding female Caribbean green turtles feeding on seagrass with those from a population of Surinam green turtles feeding primarily on algae and concluded that the Surinam turtles feeding on algae were able to deposit fat stores for a much greater energy output in a shorter period of time than were the Caribbean turtles feeding on seagrass. She attributed this difference to the higher fiber content and poorer nutritional quality of the seagrass. Whether the low level of lipid in fatty tissue is problematic for the Kiholo turtles is not known. Large fat stores may not be critical until animals reach breeding status. Kwan (1994) reported that subcarapace fat depths varied from <1.0 mm in immature turtles to about 2.0 cm in vitellogenic females and concluded

that the depth of the fatty layer was a more meaningful index of fat reserves than amount of lipid/tissue weight was.

4.2. Tumor effect

Tumors associated with fibropapillomatosis are considered benign, but the disease can be life threatening, as large tumors can interfere with locomotion, vision, swallowing, and breathing (Quackenbush et al., 1998; Aguirre et al., 2002), sometimes resulting in the inability to find and consume food. Bacteraemia and immunosupression have also been noted in Hawaiian green turtles with tumor scores ≥ 2 (Work et al., 2001; Work et al., 2003). The fact that we observed no apparent tumor effect may be due to the fact that all AOL turtles were feeding similarly or, more likely, the turtles examined in our study had low tumor scores (≤ 2). Brill et al. (1995) observed daily movements, habitat use, and submergence intervals of juvenile green turtles in the same geographic area as our study animals. Based on submergence intervals, there appeared to be no differences in the observed feeding schedules of tumored and non-tumored turtles.

4.3. Fatty acid composition

The fatty acid compositions for turtles in this study were similar in proportions of the saturated and monoenoic fatty acids to those reported for Caribbean green turtles (Joseph et al., 1985) and for green turtles collected at Johnston Atoll and the Hawaiian Islands (Ackman et al., 1992). The major fatty acids, 18:1n-9, 16:0, 16:1n-7, and 18:0, are commonly found in marine animals, including loggerhead (*Caretta caretta*; Ackman et al., 1971; Guitart et al., 1999) and leatherback turtles (*Dermochelys coriacea*; Ackman et al., 1971; Holland et al., 1990), and can be biosynthesized de novo, as well as acquired through diet. The levels of other relatively abundant saturates, 14:0 and especially 12:0, found in green turtles are high when compared with similar tissues from most other marine animals. C12:0 was found in only trace amounts in either of the benthic dietary items analyzed. Although we did not attempt an exhaustive examination of all potential items that might be included in turtle diets, the occurrence of substantial levels of this fatty acid is unusual, and we found no evidence in the literature to suggest a dietary source. Relatively high percentages of 12:0 have been previously reported in Caribbean green turtles (Joseph et al., 1985), Hawaiian green turtles (Ackman et al., 1992), and the leatherback turtle (Holland et al., 1990; Ackman et al., 1972). This fatty acid is likely biosynthesized by way of acetyl-CoA condensation from short-chain fatty acids that are products of cellulose digestion and microbial fermentation in the hindgut (Bjorndal, 1979). This process would provide temporary energy storage for the animal. Conjugated and branched-chain fatty acids found in our study animals are often associated with microbial fermentation, suggesting that this is an active process in these animals. Carbohydrates in jellyfish may be metabolized by leatherback turtles in a similar fashion as cellulose in the green turtle, giving a potential source of 12:0 in the leatherback (Joseph et al., 1985).

It was not surprising that the greatest variance in our data (PC1) appears to be related to turtle size, or more accurately, the transition from the pelagic to the benthic diet and possible metabolic changes associated with growth and maturation. The 12:0, 14:0, and 22:6n-3 fatty acids were clearly important in the delineation of turtles by residency status. As with our study, Ackman et al. (1992) found considerable individual variation in profiles among animals (especially in the percentages of 12:0, 14:0, and the PUFAs) and suggested that dietary differences may account for the variations. Although no data were given on the size, age, or diets for the turtles analyzed, one of us (GHB) confirmed that the turtles examined in the Ackman et al. study included both immature and adult turtles, suggesting that profile differences observed in their study may be attributed to the transition from pelagic to benthic feeding.

Although little is known about the requirement for functional lipids in most reptiles, the importance of 22:6n-3 in early development (of the retina and brain) in mammals (Salem et al., 1986) has been documented. The substantially higher levels of 22:6n-3 found in the depot fats of the smaller turtles suggests that the pelagic green turtle consumes dietary 22:6n-3 in excess of immediate needs and stores this excess in the depot fat. If the trend of diminishing 22:6n-3 continues throughout the life of a reproducing female, it may result in lower amounts for deposition in egg yolk that provides nutrition for the developing embryo. We found no published information on the relationship between depot fat composition and yolk lipid composition in marine turtles. We found 22:6n-3 levels of 0.12 to 0.35% in yolk lipids from eggs collected from four nests laid at French Frigate Shoals (Seaborn, unpublished data), the primary nesting area for Hawaiian green turtles (Balazs and Chaloupka, 2004). It is possible that the diet, high in long-chained fatty acids, consumed by the green turtle during the pelagic stage is critical to the early development of these animals.

Other fatty acids strongly contributing to RS delineation include t16:1n-10, 7M7H, and the component tentatively identified as 7-methyl-5,7-hexadecadienoic acid. The t16:1n-10 and 7M7H have been previously suggested as biomarkers of dietary fatty acids in marine species. The presence of these fatty acids in the leatherback turtles and the ocean sunfish (*Mola mola*) has been attributed to the inclusion of jellyfish in the diet (Hooper et al., 1974). The higher percentage of t16:1n-10 in the smaller turtles substantiates the belief that jellyfish or similar organisms are important in the diet of the pelagic green turtle. Published fatty acid compositions for several jellyfish species (Hooper and Ackman, 1972) suggest that they could be a source of 22:6n-3 and numerous branch-chained fatty acids as well. Green turtles are reported to consume jellyfish opportunisti-

cally at all life stages. Although we found no published references to 7-methyl-5,7-hexadecadienoic acid, we have identified traces of this fatty acid in a variety of marine species, including menhaden and tuna (Seaborn, unpublished), suggesting that the source of this fatty acid in the green turtle may be the pelagic diet.

Furan (F) fatty acids have not been previously reported in the profiles of green turtles but have been reported in crabs, sponges, and molluscs (Dembitsky and Rezanka, 1996), soft corals (Groweiss and Kashman, 1978), and fishes (Glass et al., 1975; Gunstone et al., 1978). Furans are a novel series of fatty acids with furanoid rings in the chain. Spiteller (1993) indicated that F-acids are produced by plants and in the marine environment. Because lower percentages of the major furan fatty acid (F6) were observed in the RS3 turtles, it seems likely the primary source is the pelagic diet.

The second greatest variance in the fatty acid data (PC2) was effective in separating turtles by location, with high loadings for 18:2n-6 and 18:3n-3 associated with the AOL turtles. Because these two fatty acids are the major PUFA found in *H. hawaiiensis* and are present at much lower levels in *P. capillacea*, it seems reasonable that the benthic diet is influential in the differentiation of animals from the two locations. While we did not analyze samples of algal species available at AOL, published compositions of similar species (Li et al., 2002) suggest that they may also be a source of 18:2n-6 and 18:3n-3. Although the algal biomass at AOL is approximately 100 times that of *H. hawaiiensis* (personal observation, GHB), 16 of the 35 AOL turtles had *H. hawaiiensis* in their mouths when captured. It appears that both algae and seagrass are important dietary components for Hawaiian green turtles. Variation within the RS3 animals from both locations is likely explained by individual differences or other factors not accounted for in this study.

The apparent differentiation of the low-lipid Kiholo turtles by PC2 and PC3 suggests a distinct difference in the depot fat composition for these animals. In animals with low lipid content, the ratio of adipocytes to connective tissue would likely decrease, and the lipid content of the connective tissue would make a greater contribution to the total depot fat sample. However, the percentage of lipid in connective tissue would be low compared with that contained in adipocytes. Therefore, even at 5% lipid, the fatty acid composition would likely represent that of the stored lipid rather than that in the connective tissue.

Although the differences are small between "normal" and low-lipid Kiholo turtles in the median percentages of individual fatty acids with negative PC3 loadings, collectively, these differences apparently distinguish the low-lipid Kiholo turtles. This fact emphasizes the advantage of using multivariate rather than univariate techniques for the evaluation of fatty acid data. With the exception of 20:5n-3, all of the fatty acids with high PC3 loadings are readily biosynthesized. Because 20:5n-3 is the major PUFA in algae, which is the primary foraging item for these animals, it appears that factors other than diet are influencing the fatty acid profiles of these animals.

Several minor fatty acids, not included in the PC analysis, were of interest in relating dietary fatty acids to depot fat profiles. Very long chain PUFA (>C24) have been reported in aquatic invertebrates, fish, and seals (Kakela et al., 1995). Fatty acids found in the marine environment are very diverse, and the presence of very long chain fatty acids in turtle depot fat is not surprising. The fact that the very long chain fatty acids appear to be lower in the largest turtles suggests a pelagic dietary origin. Nichols et al. (2003) found 24:6n-3 (9.3%) and 24:5n-6 (0.8%) in the jellyfish *Aurelia* sp. collected off the Western Australia coast. The 24:5n-6 and 24:6n-3 are also intermediates in the revised pathways for the biosynthesis of PUFA (20:4n-6→22:4n-6→24:4n-6→24:5n-6→22:5n-6 and 20:5n-3→22:5n-3→24:5n-3→24:6n-3→22:6n-3; Sprecher, 2000). Because the relatively high percentages of 22:6n-3 in the smaller turtles strongly suggests that the dietary intake exceeds the need, biosynthesis of this fatty acid in these smaller animals seems unlikely.

The detection of low levels of 20:3n-9 in a few of the larger turtles may indicate a partial essential fatty acid deficiency. In the absence of adequate dietary 18:2n-6, some vertebrates will attempt to satisfy their requirement for PUFAs by desaturation of 18:1n-9, resulting in the production of 20:3n-9. In previous studies, low levels of 20:3n-9 have been reported in the depot fat and oils of green turtles (Joseph et al., 1985; Ackman et al., 1992). Joseph et al. (1985) suggested that this could be due to periodic food deprivation for migratory turtles. However, the detection of 20:3n-9 in resident immature turtles in this study may imply that this may be a consequence of the herbivorous low-fat diet.

We believe that the trends observed among the groups of turtles examined in this study are of considerable biological relevance and provide a strong indication that fatty acid profiles could provide a complementary technique for the study of feeding ecology in marine turtles. The large number of both common and unusual fatty acids, typical of marine organisms, found in the depot lipid of the smaller turtles appears to reflect direct incorporation from the pelagic diet. This primarily carnivorous diet consumed during the pelagic stage may be critical to lifetime health and reproduction. The benthic diet consumed by the larger animals appears to exert a more indirect effect on the depot fat profile and reflects possible microbial fermentation with hydrogenation of dietary PUFA, cellulose digestion, and de novo synthesis. The variability in PC1 score patterns suggests that the change from the typical profile observed in the presumably early recruits to that of the approximately 12-year residents occurs at different rates in individual animals. The rate of change in fatty acid profiles may coincide with maturation rate. Some females (and presumably males) appear to mature at a small size and then reach a large size after many years of slow growth while others do

not mature until reaching a large size (Balazs, 1982). Individual differences in food preferences could be a factor in the AOL turtles. Although limited by food availability at a particular site, feeding discrimination has been observed. Based on the examination of stomach contents of nine turtles from the Masirah Channel, Ross (1985) determined that one turtle ate only algae, three ate only seagrass, two ate seagrass and a trace of algae, and three ate bulk amounts of both seagrass and algae. It is also likely that we have not considered all of the variables affecting the fatty acid profiles of these animals.

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