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Fatty Acid Profiles in Managed Care Green and Kemp's Ridley Turtles over Time

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Abstract: To understand fatty acid status and the potential impact of sample storage, dried blood spot (DBS) samples were collected from fourteen green turtles and ten Kemp's ridleys undergoing rehabilitation. Half the DBS samples from each animal species were collected in 2021 and sent for immediate analysis while the other half were collected in 2017 from different animals and stored at −80 °C for 4 years before analysis. The blood fatty acid profiles in both species during rehabilitation differed visually from prior wild turtle data. Essential fatty acids linoleic (18:2w6) and linolenic acid (18:3w3) were higher in green turtles than in Kemp's ridleys but both were approximately half of the percentage previously found in wild turtles. No difference in arachidonic acid between species in rehabilitation were found but percentages were approximately 10% of wild representatives. Sea turtle diets in rehabilitation management may need to be evaluated to ensure species-specific fatty acid balance. Twenty-eight of the 36 individual fatty acids tested were found and all seven fatty acid groupings were detected. When analyzed by storage time, 11 individual fatty acids and four fatty acid groups differed ($p = 0.05$). When compared by species, 14 individual fatty acids and three groups differed. Current data suggest DBS samples may be best utilized when analyzed immediately.

Keywords: fatty acids; nutrition; rehabilitation; sea turtles

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1. Introduction

All six species of Atlantic sea turtle are severely reduced from historic numbers due to over-exploitation, fishery interactions, tourism, and the collection of eggs [1,2]. Green sea turtles (*Chelonia mydas*) are classified by the International Union for Conservation of Nature (IUCN) as endangered while Kemp's ridley sea turtles *(Lepidochelys kempii*) are classified as critically endangered [1,2]. Therefore, research supporting the sustainability and conservation of these species is invaluable. Sea turtles can be found in managed situations such as rehabilitation centers, aquariums, and zoos [3–5]. Through the thorough study of diets and nutrient parameters in these managed situations, better conservation strategies may be implemented. A focal point of determining the effectiveness of managed animals' diets is understanding normal blood fatty acid profiles within species, as they may be indicators of nutritional, immune function and reproductive health status [5–7].

Lipid depot samples have long been the method of determining fatty acid status of sea turtles, however the invasive nature of this approach limits its application [5]. A strategy of blood analysis that has been more recently implemented in research efforts is the use of one drop of whole blood on a dried blood spot card (DBS) to provide a fatty acid profile [5,8–15].

The benefits of utilizing whole blood for analysis include the long-life span of red blood cells providing long term fatty acid status similar to major organs (unlike some body tissues) and the collection and storage on DBS cards being more convenient for field work as less processing is required [8,9,16–18]. Blood spot card fatty acid profiles are a relatively novel method of analysis, and thus previous research into wild and managed animal nutrition using this method is limited. However, the nutritional data provided has significant practical importance for animal health [10–15].

The current study examined: (1) whole blood fatty acid profiles from green and Kemp's ridley turtles housed in a rehabilitation setting and compared them to prior free-ranging data and (2) compared fresh whole blood card fatty acid samples to comparative card samples that were stored in a −80 °C freezer for 4 years to determine if frozen turtle blood spot cards are a stable form of storage over time.

2. Materials and Methods

Fourteen green turtles and 10 Kemp's ridley turtles undergoing rehabilitation for various conditions at the Karen Beasley Sea Turtle Rescue and Rehabilitation Center, Surf City, NC 28445, USA, were used for this study. All the turtles were considered juveniles. The conditions leading to rescue and rehabilitation varied widely for the 24 turtles but included the most common reported species problems of chronic debilitation, fishhook ingestion, and head trauma [19]. All turtles included in this study were active and eating well. At the time that blood samples were taken, all of the sea turtles were near to their release dates although their time in captivity varied from the shortest interval of 73 days to the longest of 3863 days (Table 1). One Kemp's ridley turtle in both 2017 and 2021 had extended rehabilitation times compared to the other turtles. When these animals were not included, the longest re-hab time was 500 days. Weights and straight carapace lengths (nuchal notch to pygal notch) were recorded from each turtle within the study.

Table 1. Size data for green (2017; n = 7; 2021; n = 7) and Kemp's ridley (2017; n = 5; 2021; n = 5) turtles investigated for analysis of circulating fatty acid profile precentages in managed care with a comparison between whole blood cards analyzed immediately and stored (−80 °C) for 4 years.

	Green Turtles (2017)		**Kemp's Ridleys (2017)**	
	Median	**Range**	**Median**	**Range**
Weight (kg)	3.6	2.7–5.1	3.8	3.0–29.8
SCL-N (cm) [1]	32.6	28.1–33.6	30.2	27.0–53.2
Days in Captivity Prior to Sampling	147	91–462	943	104–3862
	Green turtles (2021)		**Kemp's ridleys (2021)**	
	Median	**Range**	**Median**	**Range**
Weight (kg)	7.7	3.2–12.4	10.2	5.7–25.0
SCL-N (cm) [1]	38.5	27.2–42.7	38.8	31.9–54.5
Days in Captivity Prior to Sampling	**304**	**73–428**	**339**	**108–1164**

[1] Straight carpace length (nuchal notch to pygal notch).

Turtles were housed in individual variably sized plastic or fiberglass tanks containing filtered saltwater. Larger tanks were plumbed into a communal filtration system with multiple forms of mechanical, chemical and biological filtration and smaller tanks were on a daily dump and fill routine. Water temperatures ranged from 25–29 °C and salinity was maintained around 23% (within the United States Fish and Wildlife Service (US FWS) standards) and monitored daily by rehabilitation staff.

Heparinized whole blood was collected for pre-release health exams from the external jugular vein (dorsal cervical sinus) of each turtle. Blood (~160 µL) was applied to make two paper blood spot cards (PerkinElmer 226 Spot Saver RUO Card, PerkinElmer Health Sciences, Inc., Greenville, SC 29611, USA) for each animal. Each card was allowed to air dry. Of the 14 individual green sea turtles, seven had samples collected in 2017 and stored at −80 °C until analyses in 2021 and seven had samples collected in 2021 and immediately sent to the lab for analysis. Similarly, of the 10 individual Kemp's ridleys, five were collected in 2017 and

stored at −80 °C until analyses in 2021 and five were collected in 2021 and immediately sent to the lab for analysis.

Sea turtle capture, and sample collection, storage and transfer were authorized by the NC Wildlife Resources Permits 17ST42 (2017) and 21ST42 (2021) and NC State IACUC protocols 17–044–0 and 20-166-01. All animals were presumed healthy based upon physical examination, packed cell volume, and total solids during their pre-release exams.

Fatty acid analysis for all 24 animals was conducted on whole blood samples based on established methods (Lipid Technologies, Austin, MN 55912, USA; https://lipidlab.com/services/ [9]. Thirty-six individual fatty acids and 7 fatty acid groups were analyzed (Tables 2 and 3). Briefly, blood spots were transmethylated with acidified methanol, then fatty acid methyl esters analyzed by gas chromatography. NuChek Prep FAME std 490 (Nu-Chek Prep, Elysian, MN 56028, USA) was used to identify fatty acids, and because sample volume was not quantified, fatty acid profiles, rather than quantities, are reported.

Table 2. Total whole-blood individual and fatty acid grouping profiles (%, mean ± SEM) for juvenile green (GST; *Chelonia mydas)* and Kemp's ridley (KR; *Lepidochelys kempii*) turtles in rehabilitation (rehab) housing compared to published wild juvenile turtle data [15] [1].

		Rehab Sea Turtle Fatty Acid Profiles by Species		*p*-Value [#] by Species in Rehab	*Published Wild Sea Turtle Fatty Acid Profiles by Species*	
Individual Fatty Acids *						
Number	**Common Name**	**GST (*n* = 14)**	**KR (*n* = 10)**		**GST (*n* = 9)**	**KR (*n* = 8)**
14:0	Myristic acid	8.3 ± 0.58	10.1 ± 0.69	0.0674	*4.22 ± 0.50*	*4.98 ± 0.41*
14:1	Myristoleic acid	0.6 ± 0.14	1.0 ± 0.17	0.1569	*ND*	*ND*
15:0	Pentadecylic acid	0.5 ± 0.03	0.6 ± 0.04	0.3365	*ND*	*ND*
16:0	Palmitic acid	18.5 ± 0.69	21.1 ± 0.82	**0.0244**	*17.4 ± 0.59*	*16.9 ± 0.37*
16:1n7	Palmitoleic acid	15.5 ± 0.66	18.3 ± 0.78	**0.0132**	*5.4 ± 0.46*	*12.0 ± 0.56*
17:0	Margaric acid	ND	ND	-	*0.4 ± 0.12*	*0.9 ± 0.06*
17:1n7	Heptadecenoic acid	ND	ND	-	*0.8 ± 0.20*	*2.6 ± 0.25*
18:0	Stearic acid	5.3 ± 0.32	4.9 ± 0.37	0.4775	*10.9 ± 0.25*	*8.5 ± 0.37*
18:1n7	Vaccenic acid	2.9 ± 0.19	2.8 ± 0.22	0.9436	*2.2 ± 0.69*	*3.8 ± 0.54*
18:1n9	Oleic acid	17.6 ± 1.17	18.9 ± 1.39	0.4832	*28.3 ± 1.24*	*23.6 ± 0.73*
18:2n6	Linoleic acid	2.9 ± 0.34	1.8 ± 0.40	**0.0532**	*4.9 ± 0.51*	*3.2 ± 0.14*
18:3n6	γ-Linoleic acid	0.2 ± 0.02	0.2 ± 0.02	0.3314	*0.1 ± 0.03*	*0.1 ± 0.01*
18:3n3	α-Linolenic acid	0.9 ± 0.10	0.4 ± 0.11	**0.0024**	*1.5 ± 0.47*	*0.6 ± 0.06*
18:4n3	Stearidonic acid	0.3 ± 0.03	0.2 ± 0.03	**0.0054**	*NR*	*NR*
20:0	Arachidic acid	0.2 ± 0.02	0.2 ± 0.02	0.7543	*0.1 ± 0.03*	*0.2 ± 0.03*
20:1n7	Paullinic acid	7.1 ± 0.46	4.0 ± 0.55	**0.0003**	*0.5 ± 0.11*	*0.6 ± 0.04*
20:1n9	Gondoic acid	ND	ND	**-**	*0.06 ± 0.02*	*0.1 ± 0.02*
20:2n6	Eicosadienoic acid	0.1 ± 0.01	0.2 ± 0.02	**0.0189**	*0.1 ± 0.04*	*0.4 ± 0.02*
20:3n3	Eicosatrienoic acid	0.02 ± 0.01	0.16 ± 0.02	**<0.0001**	*0.1 ± 0.02*	*0.1 ± 0.02*
20:3n9	Mead acid	0.01 ± 0.006	0.04 ± 0.007	**0.0020**	*0.5 ± 0.26*	*0.0 ± 0.02*
20:3n6	Dihomo-γ-Linoleic acid	0.13 ± 0.008	0.09 ± 0.010	**0.0055**	*0.6 ± 0.06*	*0.4 ± 0.02*
20:4n6	Arachidonic acid	1.3 ± 0.25	2.3 ± 0.29	**0.0171**	*12.8 ± 1.42*	*11.7 ± 0.50*
20:4n3	Eicosatetraenoic acid	0.3 ±0.03	0.2 ± 0.03	0.2237	*0.1 ± 0.03*	*0.1 ± 0.02*
20:5n3	Eicosapentaenoic acid	6.9 ± 0.44	5.1 ± 0.52	**0.0155**	*2.1 ± 0.14*	*4.0 ± 0.31*
22:0	Behenic acid	0.1 ± 0.02	0.1 ± 0.02	0.4783	*0.1 ± 0.01*	*0.2 ± 0.02*
22:1n9	Erucic acid	4.0 ± 0.40	1.8 ± 0.47	**0.0016**	*0.1 ± 0.02*	*0.0 ± 0.01*
22:4n6	Adrenic acid	0.1 ± 0.03	0.2 ± 0.03	0.0570	*2.1 ± 0.22*	*1.5 ± 0.07*
22:5n6	n6-Docosapentaenoic acid	0.15 ± 0.04	0.17 ± 0.05	0.6935	*0.8 ± 0.29*	*0.3 ± 0.02*
22:5n3	n3-Docosapentaenoic acid	0.9 ± 0.06	0.5 ± 0.07	**0.0005**	*1.9 ± 0.20*	*1.1 ± 0.07*
22:6n3	Docosahexanoic acid	4.7 ± 0.40	4.4 ± 0.47	0.6854	*1.7 ± 0.42*	*1.7 ± 0.11*
24:0	Lignoceric acid	0.04 ± 0.009	0.03 ± 0.010	0.5774	*0.0 ± 0.01*	*0.1 ± 0.01*
24:1	Nervonic acid	0.4 ± 0.05	0.3 ± 0.06	0.0813	*NR*	*NR*
Fatty Acid Groups						
Highly Unsaturated Fatty Acids (HUFA)		14.5 ± 0.71	13.2 ± 0.84	0.2847	*NR*	*NR*
Monoenes		48.2 ± 0.91	47.1 ± 1.08	**0**.4288	*NR*	*NR*
Total n 6 fatty acid		4.9 ± 0.46	4.9 ± 0.55	0.9801	*21.4 ± 1.75*	*17.6 ± 0.63*
Total n 3 fatty acids		13.9 ± 0.71	11.0 ± 0.85	**0.0147**	*7.4 ± 0.67*	*7.6 ± 0.53*
n-3: n-6 Fatty acid ratio		0.4 ± 0.08	0.6 ± 0.09	0.1619	*0.38*	*0.44*
Poly Unsaturated Fatty Acids (PUFA)		18.8 ± 0.73	15.9 ± 0.87	**0.0187**	*NR*	*NR*
Saturates		33.0 ± 0.84	37.0 ± 0.99	**0.0056**	*33.2 ± 0.64*	*31.5 ± 1.78*

[1] [5]. * Fatty acids: Lauric acid (12:0), Pentadecenoic acid (15:1), Myristoleic acid (16:1n5), 13-octadecenoic acid (18:1n5) were analyzed but did not have detectable values. [#] Rehabilitation sea turtles with significant differences by species have *p*-values in bold highlight ($p = 0.05$). ND = not detected; NR = not reported.

Table 3. Total whole-blood individual and fatty acid grouping profiles (%, mean ± SEM) for juvenile green (GST; *Chelonia mydas)* and Kemp's ridley (KR; *Lepidochelys kempii*) turtles in rehabilitation housing as analyzed by time, both immediately after sampling (New) and after four years frozen at −80 °C (Old).

		Green Sea Turtle (GST) DATA		Kemp's Ridley (KR) DATA		
Individual Fatty Acids *						
Fatty Acid	**Common Name**	**GST New (*n* = 7)**	**GST Old (*n* = 7)**	**KR New (*n* = 5)**	**KR Old (*n* = 5)**	**Time Main Effect *p*-Values [#]**
14:0	Myristic acid	8.7 ± 0.82	8.0 ± 0.82	10.4 ± 0.97	9.7 ± 0.97	0.4480
14:1	Myristoleic acid	0.39 ± 0.202	0.93 ± 0.202	1.15 ± 0.239	0.82 ± 0.239	0.6174
15:0	Pentadecylic acid	0.41 ± 0.046	0.64 ± 0.046	0.51 ± 0.054	0.64 ± 0.054	**0.0020**
16:0	Palmitic acid	18.7 ± 0.98	18.4 ± 0.98	22.2 ± 1.15	20.1 ± 1.15	0.3011
16:1n7	Palmitoleic acid	14.5 ± 0.93	16.5 ± 0.93	18.9 ± 1.10	17.7 ± 1.10	0.6986
18:0	Stearic acid	5.1 ± 0.45	5.4 ± 0.45	4.8 ± 0.53	5.0 ± 0.53	0.6695
18:1n7	Vaccenic acid	2.3 ± 0.26	3.4 ± 0.26	2.1 ± 0.31	3.6 ± 0.31	**0.0002**
18:1n9	Oleic acid	19.1 ± 1.66	16.2 ± 1.66	18.2 ± 1.97	19.7 ± 1.97	0.7041
18:2n6	Linoleic acid	2.8 ± 0.47	3.0 ± 0.47	1.4 ± 0.56	2.2 ± 0.56	0.3762
18:3n6	γ-Linoleic acid	0.18 ± 0.025	0.20 ± 0.025	0.16 ± 0.030	0.16 ± 0.030	0.6966
18:3n3	α-Linolenic acid	0.91 ± 0.136	0.81 ± 0.136	0.41 ± 0.161	0.28 ± 0.161	0.4577
18:4n3	Stearidonic acid	0.33 ± 0.037	0.29 ± 0.037	0.13 ± 0.044	0.23 ± 0.044	0.4914
20:0	Arachidic acid	0.19 ± 0.021	0.22 ± 0.021	0.19 ± 0.025	0.22 ± 0.025	0.2568
20:1n7	Paullinic acid	5.2 ± 0.65	9.1 ± 0.65	2.7 ± 0.77	5.3 ± 0.77	**0.0002**
20:2n6	Eicosadienoic acid	0.11 ± 0.018 [a]	0.10 ± 0.018	0.21 ± 0.021	0.11 ± 0.021	**0.0130**
20:3n3	Eicosatrienoic acid	0.03 ± 0.020	0.01 ± 0.020	0.29 ± 0.024	0.03 ± 0.024	**<0.0001**
20:3n9	Mead acid	0.01 ± 0.009	0.00 ± 0.009	0.07 ± 0.011	0.01± 0.011	**0.0010**
20:3n6	Dihomo-γ-Linoleic acid	0.12 ± 0.012	0.13 ± 0.012	0.07 ± 0.014	0.10 ± 0.014	0.1781
20:4n6	Arachidonic acid	1.3 ± 0.35	1.3 ± 0.35	2.0 ± 0.41	2.6 ± 0.41	0.4704
20:4n3	Eicosatetraenoic acid	0.27 ± 0.038	0.25 ± 0.038	0.22 ± 0.045	0.20 ± 0.045	0.6490
20:5n3	Eicosapentaenoic acid	9.0 ± 0.62	4.8 ± 0.62	5.8 ± 0.73	4.5 ± 0.73	**0.0005**
22:0	Behenic acid	0.05 ± 0.024	0.16 ± 0.024	0.13 ± 0.029	0.12± 0.029	0.0918
22:1n9	Erucic acid	2.3 ± 0.57	5.7 ± 0.57	0.87 ± 0.67	2.7 ± 0.67	**0.0004**
22:4n6	Adrenic acid	0.11 ± 0.037	0.11 ± 0.037	0.209 ± 0.044	0.18 ± 0.044	0.7440
22:5n6	n6-Docosapentaenoic acid	0.14 ± 0.055	0.15 ± 0.055	0.21 ± 0.066	0.13 ± 0.066	0.5806
22:5n3	n3-Docosapentaenoic acid	1.11 ± 0.079	0.61 ± 0.079	0.60 ± 0.093	0.41 ± 0.093	**0.0008**
22:6n3	Docosahexanoic acid	6.4 ± 0.56	3.0 ± 0.56	6.0 ± 0.67	2.9 ± 0.67	**<0.0001**
24:0	Lignoceric acid	0.01 ± 0.012	0.06 ± 0.012	0.03 ± 0.014	0.03 ± 0.014	0.0783
24:1	Nervonic acid	0.30 ± 0.067	0.51 ± 0.067	0.12 ± 0.079	0.43 ± 0.079	**0.0020**
Fatty Acid Groups						
Highly Unsaturated Fatty Acids (HUFA)		18.5 ± 1.01	10.4 ± 1.01	15.4 ± 1.19	11.1 ± 1,19	**<0.0001**
Monoenes		44.0 ± 1.28	52.4 ± 1.28	44.0 ± 1.52	50.1 ± 1.52	**<0.0001**
Total n 6 fatty acid		4.8 ± 0.66	5.0 ± 0.66	4.3 ± 0.78	5.5 ± 0.78	0.3555
Total n 3 fatty acids		18.1 ± 1.01	9.8 ± 1.01	13.4 ± 1.20	8.6 ± 1.20	**<0.0001**
n-3:n-6 Fatty acid ratio		0.27 ± 0.109	0.52 ± 0.109	0.33 ± 0.129	0.81 ± 0.129	**0.0067**
Poly Unsaturated Fatty Acids (PUFA)		22.9 ± 1.04	14.7 ± 1.04	17.8 ± 1.22	14.0 ± 1.22	**<0.0001**
Saturates		33.1 ± 1.19	32.9 ± 1.19	38.2 ± 1.40	35.8 ± 1.40	0.3156

* Fatty acids: Lauric acid (12:0), Pentadecenoic acid (15:1), Myristoleic acid (16:1n5), Margaric acid (17:0), Heptadecenoic acid (17:1n7), 13-octadecenoic acid (18:1n5), Vaccenic acid 18:1n7, and Gondoic acid 20:1n9 were analyzed but did not have detectable values. [#] *p*-values in bold highlight the New vs. Old (2021 vs. 2017) overall comparisons that are significant ($p = 0.05$). Species [a] Time interactions not shown.

3. Diet

All the rehabilitation center diet items were representative of natural diets [5] and were consistent in 2017 and 2021. The rehabilitation center green sea turtle diets consisted of an average offering of 250 g of fish per day primarily consisting of capelin (*Mallotus villosus)*, although depending on seasonal availability, small quantities of Spanish and Northern mackerel (*Scomberomorus maculatus* and *Scomber scombrus*) and blue runner (*Caranx crysos*) were included. Shrimp (*Penaeus* sp.) was also occasionally added to the diet. Two green sea turtles were fed only squid (*Loligo loligo*) (average 182.5 g/day) while two others were fed a vegetable and fish mixture (average 119 g/day). Vegetables included romaine lettuce, cucumbers, and green peppers. The Kemp's ridleys were fed primarily a squid diet (average 513 g/day), although one animal was fed a choice diet of squid or mackerel. All green turtles and Kemp's ridleys were given a multivitamin (Member's Mark (Bentonville, AR 72712, USA) Adults 50+ Multivitamin, ASIN: B01N1ZJ41C) and calcium supplement (Member's

Mark Calcium 600mg with Vitamin D-3, ASIN: B01AVJ7XA4) on Mondays, Wednesdays, and Fridays.

4. Statistical Analysis

Data were analyzed for differences by Species (green turtles vs. Kemp's ridleys) and Time (2017 frozen samples vs. 2021 immediate analysis samples), with statistical significance set at $p < 0.05$, using Proc GLM procedures of SAS 9.4 (Cary, NC, USA). LS Means and standard error of the means (SEM) were calculated in the model statement. Qualitative comparisons were made with previously published values for free-ranging green turtles and Kemp's ridleys (Table 2) [5]. Overall comparisons of whole blood fatty acids (all 2017 values compared to all 2021 values) were conducted although total means by each year are not shown in the tables (just the p values (Table 3)).

5. Results

Median and range of weights, straight carapace lengths are shown in Table 1 by species and sample collection year.

Eight of the 36 measured individual fatty acids were below limits of detection (lauric acid (12:0), pentadecanoic acid (15:1), myristoleic acid (16:1n5), margaric acid (17:0), heptadecenoic acid (17:1), 13-octadecenoic acid (18:1n5), vaccenic acid (18:1n7), and gondoic acid (20:1n9)). All seven fatty acid groupings were represented in the data (Tables 2 and 3).

Overall comparisons of whole blood fatty acid profiles between green and Kemp's ridley sea turtles in rehabilitation are presented in Table 2. Fourteen individual fatty acids and three fatty acid groups differed by species ($p < 0.05$). Palmitic acid (16:0), palmitoleic acid (16:1n7), eicosadienoic acid (20:2n6), eicosatrienoic acid (20:3n3), mead acid (20:3n9), arachidonic acid (20:4n6), and saturates were significantly lower in green turtles. Linoleic acid (18:2n6), α-linolenic acid (18:3n3), stearidonic acid (18:4n3), paullinic acid (20:1n7), dihomo-γ-linoleic acid (20:3n6), eicosapentaenoic acid (20:5n3), euric acid (22:1n9), n3-docosapentaenoic acid (22:5n3), total n 3 fatty acids, and poly unsaturated fatty acids (PUFA) were higher in green turtles.

Eleven individual fatty acids and five fatty acid groups differed by time (frozen 2017 versus immediate 2021 analysis). Pentadecylic acid (15:0), eicosadienoic acid (20:2n6), eicosatrienoic acid (20:3n9), mead acid (20:3n9), eicosapentaenoic acid (20:5n3), n3-docosapentaenoic acid (22:5n3), docosahexanoic acid (22:6n3), highly unsaturated fatty acids (HUFA), poly unsaturated fatty acids (PUFA) and total n 3 fatty acids were higher in freshly analyzed (2021) samples. Vaccenic acid (18:1n7), paullinic acid (20:1n7), euric acid (22:1n9), nervonic acid (24:1), monoenes, n-3: n-6 fatty acid ratio were higher in 2017 (old) samples. All the fatty acids that were higher in the 2017 (old) samples were present in very small quantities which likely affected comparisons.

To better understand the effects of the managed diet on both species we compared the immediately analyzed DBS cards from the current study to our prior juvenile wild data from the same species (Table 2) [5]. The profile percentages of both linoleic and linolenic were approximately half the amount for both species as was recorded in the wild data [5]. The profile percentages of arachidonic acid (20:4n6) were found at approximately 10% the level of the wild turtles and was noted to be lower in greens than Kemp's ridleys. Total n 6 fatty acids were four times higher in wild turtles for both species while wild turtle total n 3 fatty acids were approximately half the managed care percentages.

6. Discussion

Fatty acids are critical components of many biological processes, including energy metabolism, building blocks of cell membranes, involvement in cell signaling, influencing inflammatory cascades, cardiovascular protection, etc. [19]. Concentrations of fatty acids in the blood are indicative of biological processes as well as dietary intake [20–22]. Other factors that can influence levels of fatty acids include age, physiological status, and stress [23–26]. Therefore, it should be noted from Table 1 that the 2017 average turtles

from both green and Kemp's turtles were smaller and therefore likely younger than the 2021 animals. This was more obvious within the Kemp's ridley turtles which also had a much longer average rehabilitation stay prior to blood sampling (943 days for 2017 and 339 for 2021). As all animals were considered juveniles and fed a human managed diet for several months, it is not believed that these differences played a major role in the fatty acid profile percentage differences noted. Koutsos et al. (2021) reported that margaric acid (17:0) declined with increasing size in green turtles and that Kemp ridley's had increases in linoleic (18:2w6) and gondoic acid (20:1w9) with increasing size. Although margaric and gondoic acid were both not detected in this study and linoleic acid did not differ by time, it is still possible that some of the species and storage differences noted could be due to the different population ages, sizes, and total rehabilitation time. Future research with larger populations of rehabilitated animals could address these concerns although this is unlikely due to the endangered nature of the research species.

As the sea turtles in this study were all free-ranging animals in temporary rehabilitation, the goal was to provide the animals with enough nutrients to gain an adequate amount of weight, muscle, and fat stores in order to be released back into the wild. Linoleic (18:2n6) and linolenic (18:3n3) acids are essential fatty acids in most vertebrate species as they cannot be synthesized, resulting from the lack of the Δ12 and Δ15 desaturase enzymes needed to insert a cis double bond at the n-3 or n-6 position [22]. While the fatty acid requirements of sea turtles are unknown, given what is known about the requirements of linoleic (18:2n6), linolenic (18:3n3), and long chain omega-3 fatty acids in other marine organisms and humans, it is possible that they are also essential for sea turtles [5,19–21]. However, it is thought that all omega-3 and omega-6 fatty acids can be conditionally essential throughout their metabolic pathways due to the dietary, enzyme, and genetic limitations [27].

To the authors knowledge, only one prior paper has investigated fatty acid profiles in sea turtles [5]. This prior paper was in conjunction with the current authors and investigated healthy, wild, juvenile green turtles and Kemp's ridleys. To better understand the effects of the managed diet on these species we compared the overall average DBS cards (Table 2) from the current study to the wild data (Table 2). While in different percentages, the two main fatty acids were the highest in both studies (oleic acid and palmitic acid). Additionally, while the rehabilitation green turtles and Kemp's ridleys had several differences in fatty acid profile percentages for individual fatty acids and groupings they were overall similar to the wild publication. Of particular interest, the potentially essential fatty acid profiles of linoleic (18:2n6) and linolenic acid (18:3n3) were previously found to be higher in green turtles as compared to Kemp's ridleys in the wild [5] and the current data showed this to be the same within the rehabilitated turtles. However, it is important to note that the profile percentages of both linoleic and linolenic were approximately half the amount for both species as was recorded in the wild data. In previous wild sea turtle data, when levels of arachidonic acid (20:4n6) in green turtles and Kemp's ridleys were compared, no significant difference was found, suggesting the potential ability of both species to synthesize arachidonic acid from w6 precursors such as linoleic acid [5]. However, in this current study, profile percentages of arachidonic acid (20:4n6) were found at approximately 10% the level of the wild animals and was noted to be lower in green turtles than Kemp's ridleys. Arachidonic acid is considered a third essential fatty acid in mammalian carnivores [28]. As Kemp's ridleys are generalist carnivores in the wild but with prior demonstrated ability to synthesize arachidonic acid, this suggests that while in a managed situation both species may need to have supplemented arachidonic and/or linoleic acid. In the wild, it seems that diets consumed are sufficient to synthesize arachidonic acid de novo from linoleic acid [5]. The research presented here indicates arachidonic acid also may be essential, or conditionally essential during rehabilitation conditions, since most other species can convert linoleic acid into arachidonic acid, and despite our managed care diet having linoleic acid, the arachidonic acid levels remain low.

In the current rehabilitation study, green turtles had a higher overall blood concentration of omega-3 fatty acids than Kemp's ridleys that was not noted in prior wild turtles [5]. The rehabilitation diets of the green turtles consisted of primarily fish, while the Kemp's ridley diets consisted mainly of squid. The higher green turtle's percentages of omega-3s may be attributed to the particular species of fish in their diet. While squid are considered a good source for both omega 3 and omega 6 fatty acids, the fish fed in the current study may have been higher [29]. Squid (and fish) are also noted for being high in eicosapentaenoic acid (EPA, 22:5n3) and docosahexaenoic acid (DHA, 22:6n3) and therefore the high levels of these two fatty acids in the managed animals compared to the wild ones is also not surprising. However, since the conditionally essential fatty acids compete for the same elongase and desaturase enzymes in the metabolism reactions it should be noted that excess fatty acids in any category (omega 3, omega 6 or just one particular fatty acid) could have downstream negative reactions [17].

Wild green turtles are primarily carnivorous from the time of hatching to their juvenile period while they are in a surface-pelagic phase and feed mainly on zooplankton, mollusks, and crustaceans [19,30–32]. As juveniles, they move inshore and the diet subsequently shifts to primarily herbivorous over varying durations, leading them to rely more heavily on microbial fermentation to break down plant cell walls in order to produce VFAs, an important energy source [19,33–35]. Over time, microbial populations in the digestive system can adapt, but in rehabilitation situations this can result in decreased digestibility [19]. However, near-shore (littoral) juveniles are often in varying dietary stages as they can go back and forth between littoral and offshore environments and shift their diets and the post-hatchling dietary strategy is a pelagic phase [19,36–38]. Similarly, juvenile Kemp's ridleys transition from a pelagic to a littoral phase, but remain carnivorous throughout, shifting from pelagic to benthic organisms over variable durations. Therefore, the lower levels of total omega 3 and the higher levels of omega 6 fatty acids in the prior wild animals could reflect differences in age related feedings areas or that more vegetation and food choices should be fed in managed care. The low levels of total omega 6 fatty acids likely contribute to the lower arachidonic levels noted in managed animals as well.

Among the fatty acid groupings (HUFA, PUFA, n-3, n-6, n-6/n-3, monoenes and saturates); HUFA, monoenes, total n-6, and n-6: n-3 ratios did not differ between the two species (Table 2). Total n-3, and PUFA were higher in green turtles. As PUFA can only be synthesized by plants and phytoplankton, and vegetables are known to contain more n-3 fatty acids, these findings correspond to the higher levels of vegetables found in the diets of the GST [10,23]. Marine species often need higher levels of PUFA, longer chain n3 polyunsaturated fatty acids, as they play an important role in membrane fluidity and lipid characteristics, these are especially important in cold water environments as they have implications in survivability of cold-stunning events [5]. Eicosapentaenoic acid (EPA; 20:5n3) was found at higher levels in wild Kemp's ridleys compared to green turtles, likely attributed to the incidental levels of seagrass they intake [5]. In contrast, this study found that both species contained higher levels of EPA in the wild and green turtles had higher levels than Kemp's ridleys. This corresponds to the much higher levels of total n-3 grouping and much lower n 6 grouping that we found in the current study compared to the wild Koutsos et al., 2021 data. Additionally, the high levels of EPA require excess delta-6 desaturase for conversion which is an enzyme also needed for arachidonic acid conversion from linoleic acid and therefore the n6 excesses could be negative for the n 3 fatty acids.

Saturated fatty acids (saturates) was the only fatty acid group that was higher in the Kemp's ridleys than green turtles in this study. Additionally, the managed Kemp's had higher saturates than the wild ones [5]. Saturates are considered non-essential as they are synthesized from glucose and acetate, meaning that these levels are indicative of precursor availability rather than dietary intake [5,39]. Similarly, the non-essential palmitoleic acid (16:1w7) and paullinic acid (20:1n7) were high within the rehabilitation turtles and much lower in wild ones. Paullinic acid, in particular, was dramatically higher than found in wild samples,

and is found in nature from plant sources, and therefore the high level found in rehab animals is very interesting. Oleic acid (18:1n9) was much lower in the rehabilitating turtles as compared to the wild ones [5]. The relevance, if any, of these differences are not known. Similarly, heptadecenoic acid (17:1n7) was not found in the current data for either species but was found in both wild species (with Kemp's ridleys having somewhat high-profile percentages). As this fatty acid is noted with higher bacteria populations this could be due to lack of natural gastro-intestinal microflora from wild diets [40].

With respect to individual fatty acid analyses by storage time, 31% of the individual fatty acids differed between the immediate 2021 analyses and the samples analyzed after being frozen for four years (Table 3). However, for the wild to managed care comparisons previously discussed we combined the 2017 and 2021 since most differences seemed to be for non-essential fatty acids or those with small profile percentages. Additionally, most individual fatty acid differences by time appears more numerical than substantive as the profile percentages were still closer together within the species than they were across species. However, five of the seven fatty acid groupings differed by analysis storage time. Total omega 3 fatty acids, highly unsaturated fatty acids and poly unsaturated fatty acids were the three groups with the largest decreases when immediate analyses were compared to frozen ones. Since fatty acid storage differences have been noted previously in white rhinoceros (*Ceratotherium simum simum*) as well [12], the authors suggest freezing fatty acid blood spot cards and analyzing as quickly as financially feasible for the researcher. However, it should be noted that our research population is small, animal sizes differed by analytical year and rehabilitation purchased diet items provided to the turtle species also differed by year. Individual turtle daily diets were also not available and fatty acids available in the rehabilitation supplement are unknown. Therefore, while the differences noted warrant some concern over the validity of long term ultra-cold freezer storage, larger studies are needed especially since a prior study has indicated long term storage at −80 °C for periods up to seven months can be appropriate [41].

7. Conclusions

This study provided data that reflected the significance of storage time on the whole blood analysis of fatty acids. Long term storage of fatty acid blood cards, even in ultra-low freezers, may not be optimal when trying to compare research studies or publish data on novel species. While many differences noted by storage time were small, some were large enough to shift potential diet preparations or confuse veterinary practitioners if trying to ascertain normal medical values. Thus, we recommend freezing collected DBS storage cards as soon as possible, using ultra-low freezers preferentially. Additionally, we recommend that these cards be protected from sunlight and humidity and sent for analysis as quickly as financially feasible using the same laboratory and as few analytical runs as possible to conduct comparisons. Future studies with larger groups of similar sized animals, and therefore less confounding variables, are needed before concrete conclusions can be made.

In general, the fatty acid profiles reflected in the sea turtles managed in the rehabilitation center differed considerably from free-ranging animals. While the significance of most of these differences are unknown, these data could be used in attempts to help plan rehabilitation diets that better reflect wild diets. It should be noted that both the current data and the previously published wild paper have small sample sizes. Thus, datasets with larger sample sizes are needed to better understand the differences noted although increasing the sample size is difficult due to the endangered status of these species.

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