ANALYSIS OF FATTY ACID PROFILES IN EASTERN BOX (*TERRAPENE CAROLINA CAROLINA) AND COMMON SNAPPING (CHELYDRA SERPENTINE) TURTLES FOR WILD AND IN-HUMAN CARE ENVIRONMENTS*

Khushboo, Dass[1*]; Kimberly Ange-van Heugten[1]; Liz Koutsos[2], Larry J. Minter[3]

[1]Department of Animal Science, North Carolina State University (NC State), Raleigh, NC, 27606, USA.
[2]Koutsos Consulting LLC & Adjunct Professor, Department of Animal Science, NC State, Raleigh, NC, 27606, USA.
[3]North Carolina Zoo, Asheboro, NC & Adjunct Asst. Professor Department of Clinical Sciences, NC State School of Veterinary Medicine, Raleigh, NC, 27606, USA.

Abstract
Diets of wild animals are often more diverse and offer higher concentrations of nutrients than those of animals' in-human care (zoos, rehabilitation facilities, etc.). Managing wild animals within human care facilities is often necessary, and we hypothesized that chelonian dietary differences within circulating fatty acid profiles would be reflected in wild vs human care data. The current study examined the effect of species and environment on fatty acids concentrations in two omnivorous species of chelonians native to North Carolina within two environments: Eastern box turtles, *Terrapene carolina carolina,* and common snapping turtles, *Chelydra serpentine,* located in the wild and in-human care. Whole blood was collected and placed on spot cards for later analysis of all 26 fatty acids in a total lipid fatty acid profile. This novel research indicated that snapping turtles have significantly *(P<0.05)* higher values of Linolenic acid, Dihomo- γ -Linolenic acid (DGLA), Tetradonic Acid, Docasatetraenoic acid (DTA), Docosapentaenoic acid (DPA), Eicosadienoic acid, Erucic acid, and overall saturated fatty acids. Among all the wild animals, there tended *(P<0.06)* to be higher values for α-Linolenic acid, DGLA, Arachidonic acid, Eicosadienoic acid, Eicosatrienoic acid, DPA, and DTA. Docasonic acid, DTA, DPA, Eicosadienoic acid, and Nervonic acid showed significant *(P<0.01)* differences via species x environment interactions. Interestingly, both wild species showed higher concentrations of dihomo-γ- Linolenic acid (20:3n6), known to be directly affected by diet and display anti-inflammatory effects. This research may allow us to better formulate diets for chelonian kept in-human care. Additionally, fatty acids are used for many important body functions including proper immune system usage and therefore our research provides new biologically important data for the reptile diagnostic field.

Introduction
The diets of wild animals are often more diverse and offer higher levels of nutrients than those of animals' in-human care (zoos, rehabilitation facilities, etc.). Managing wild animals within human care facilities is often necessary and thus careful consideration should be given when formulating diets for these animals. The fatty acid levels in these diets are of particular importance because they are needed to maintain proper immune, anti- inflammatory and reproductive functions (Fritsche 2006; Saker *et al.* 1998). Another potential benefit from two particular fatty acids, Docosahexaonic acid (22:6n3) and Eicosapentaenoic acid (20:5n3) are the significant cardio-protective effects attained by

fighting arrhythmias and thrombosis while working to lower the heart rate and effects of harmful triaclyglerols (Mozaffarian et al. 2007). Most published data are from human and feline-model based studies although there is potential translation into more animal models, including reptiles.

Historically, utilizing tissue samples from the liver, muscle, or adipocytes provide better insight of the long-term fatty acid status than using a serum sample although they can be difficult to obtain. Whole blood samples provide a solution to this problem by relaying information about long-term status of fatty acids with greater accessibility (Baylin *et al.* 2005). The novel technology used within this study requires only a single drop of whole blood on a spot card and will therefore allow studies to evaluate small exotic animals previously only used in terminal work and large wildlife in the field where previous samples were less reliable as they were unable to be kept frozen for analyses.

There have not been previous studies examining circulating fatty acid profiles in the eastern box (*Terrapene Carolina Carolina)* or common snapping turtle (*Chelydra serpentine)* as the previous studies have examined liver, muscle, and fat depot samples. Samples fat depots, liver, and muscle from wild and cultured Common Snapping Turtles found that the fatty acid profiles of cultured turtles reflected the dietary levels of fatty acid, which shows that serum values can likely be influenced by dietary modifications (Carroll 1965; Takeuchi and Watanabe 1977, Farkas *et al.* 1980, Sheridan 1988.) Interestingly, Docosapentaenoic acid (22:5 n3) was one of the only fatty acids present in the tissue samples that was absent from the diet. This indicates that Common Snapping Turtles, and perhaps chelonians overall, may be able to convert the fatty acids present in the diet, such as 20:5 n3 into the required fatty acids, like 22:5 n3 (Maxwell *et. al* 1998).

Despite having a commercially formulated diet for the common snapping turtles, these animals will sometimes be fed a commercial alligator diet while in human care. Therefore, we should compare and contrast the essential fatty acid requirements and profile of the American alligators to the common snapping turtle (Staton 1990). For example American alligators are able to convert Linolenic acid (18:2n-6) to Arachidonic Acid 20:4n-6 and thus the former is of primary concern when formulating a commercial diet. If common snapping turtles possess this same ability then the fatty acids that are used as precursors to synthesis others will be of greater concern in the turtle's diet.

This current study examined the effect of species and environment on fatty acids concentrations in two omnivorous species of native North Carolinan chelonians within two environments: Eastern box turtles, *Terrapene Carolina Carolina,* and common snapping turtles, *Chelydra serpentine,* located in the wild and kept in-human care for up to weeks. We hypothesized that there would be inherent differences in the levels of fatty acids between the two species of turtles. We also expected to see that both species of the wild groups would have elevated levels of certain fatty acids as a reflection of their natural diets.

Materials and Methods

A total of 19 turtles, from the two species and two environments were sampled. The four treatment groups were as follows: wild Eastern box turtles from the wildlife rehabilitation center at NC Zoo *(n=4),* Eastern box turtles kept in human care (as a part of the long term collection; *n=5)*, wild common snapping turtles caught and immediately released from a large pond at NC Zoo *(n=5)*, and common snapping turtles kept in human care at NC State's turtle rehabilitation team *(n=5).* Only animals kept in rehabilitation centers for less than 6 weeks were considered "wild".

The Eastern box turtles in human care were fed a diet including kale (17 -30 g), grated carrots (20 g), chopped green beans (30 g), sweet potato, grated squash (20 g), peas (20 g), chopped apples (30-48 g), peeled bananas (62 g), peeled oranges (65 g), tomatoes, blueberries (30 g) on Mondays, Wednesdays and Friday. They were supplemented with 3 crickets once a week, 1 night crawler once a week, and 1/8 teaspoon Repti-Vite twice a week.

The captive common snapping turtles at the NC State Rehab facility were fed a diet of frozen and then thawed fish although data records for species and amounts were inconsistent.

None of the animals presented abnormally and were assumed to be healthy for this study. The whole blood samples were collected from the subcarapacial sinus and coccygeal vein in Eastern box and common snapping turtles, respectively. A drop of whole blood was placed on a blood spot card. Lipid Technologies LLC (1600 19th Avenue SW, Austin MN 55912) used the blood spot cards to run total lipid fatty acid profile and analyze all 26 fatty acids.

Fatty acid differences between species and environmental location were determined using a one-way ANOVA. P-values of <0.01 were used to determine significance and <0.10 were used to determine tendencies. For significant species*environment interactions, an All Pairwise Multiple Comparison was run. For data that was did not have normal distribution or variance, a log transformation was used to normalize the data.

Results

For each of the four-treatment groups, the least square mean and standard error of the mean was calculated. *Table 1.* For Eicosadienoic acid (20:2n6), Eastern box turtles in human care were significantly higher than the other treatments. For Arachidonic acid (20:4n6), both the Eastern Box and Common Snapping Turtles were different from each other as well as both wild treatments. For Docosapentanoic acid, Common Snapping Turtles was higher than the other treatments groups. *Chart 1.*

Fatty acids that had an affect by species included Tetradonic acid(14:0), which was higher for Common Snapping Turtles and Linoleic (18:2n6) and h- Y-linolenic acid (20:3n6), which were higher for Eastern Box Turtles. *Table 2.*

Fatty acids that had an affect by environment included α-Linolenic acid (18:3n3), Arachidic acid (20:0) and Eicosatrienoic acid (20:3n3), and Docosanoic acid (22:0) were higher for wild chelonians than those kept in human care. *Table 3.*

Average percent of ω -3 was higher for wild animals. Ratio of ω 6/ω 3 was higher for captive than wild.

Discussion

Of the various fatty acids that showed significant differences by species, environment or both, we must consider how they affect the biological process of chelonians.

For example, the proper ratio of ω -6 to ω -3 in humans has generated a lot of discussion. Our ancestors 10,000 years ago likely had 1-2:1 ratio of ω 6: ω-3 due to limited intake of saturated and trans-fats that contribute to ω-6 (Candela *et al.* 2011) Fast forward to present day where the western diet has a 20-30:1 ratio of ω-6: ω-3 due to the increased consumption of saturated and trans-fats from grains and vegetable oil often used in highly processed foods. This unbalanced ratio may lead to chronic diseases such as obesity, arthritis, mental illness, autoimmune disorders, cancer and cardiovascular disease (Candela *et al.* 2011).

In the current study, we see that the percent of ω-3 was higher for both species of wild chelonians but the ratio of ω -6 to ω -3 was higher for both species kept in human care. This abundance of ω -6 and deficiency of ω -3 could lead to health issues for animals kept in human care for extended periods of time. This finding is consistent with a previous study on essential fatty acids in the crocodiles that found ω -3's to be higher in wild animals than those in captivity. This study concluded that these differences were a result of dietary differences and thus could be adjusted so that the captive animal's fatty acids more closely mimicked their wild counterparts (Morpurgo *et al.* 1993). Another study that also examined fatty acid composition in liver and fat deposits in Indian Ocean Loggerhead Turtles, *Caretta Caretta,* also found similar results that there was an increased percentage of ω -3's in the adipose over the liver (Davidson *et al.* 2014). This variation may reflect the function of these fatty acids in the various organs depending on their short versus long term functionality.

One study that showed cultured common snapping turtles fed a 40% protein commercial alligator diet had significantly lower levels of α-linolenic acid (18:3n3) in the muscle and fat depot samples. The results of the present study showed both species of wild turtles had higher levels of α-linolenic acid than those kept in human care. The current results and the findings from the study of snapping turtles fed the commercial alligator diet support our hypothesis that wild species will have elevated levels of fatty acids due to insufficiencies in commercial and rehabilitation diets (Maxwell *et al.* 1998). The advantage of this study is that the exact diet was known for the cultured turtles and could thus be broken down. For the current study, a formulated diet was not used for the turtles in-human care, which may have led to variation to the amounts consumed and nutrients obtained. Additionally, the diets for the wild turtles were not known and thus could not be controlled amongst the various animals.

Another study that examined fatty acid profiles of wild and captive black seabream (*Spondyliosoma cantharus*) showed multiple examples of deficiencies and surpluses between the two environments. Specifically, 20:4n−6, 20:5n−6 and 22:6n−3 were considerably higher in the wild fish, whereas 18:1, 20:1, and 22:1n−9 as well as 18:2n−6 and 20:5n−3 were more higher for the captive group (Rodriguez *et al.* 2004). Further studies may be more indicative of whether these fatty acid insufficiencies across species are diet based. While we cannot equate a commercial alligator diet or rehabilitation facility diet for turtles to a zoo diet for rhinos, this finding does help to highlight a particular fatty acid that we may be overlooking across multiple species managed in human care.

Another interesting result is the elevated levels of 22:5 n3 in common snapping turtles among the other treatments. Maxwell *et al.*'s study showed that common snapping turtles had 22:5 n3 present in all tissue samples despite its absence from the diet, which would mean they were able to convert other fatty acids such as 20:5 n3 into the essential fatty acids needed. If we were able to obtain and analyze the fatty acid content of the diets for the Common Snapping Turtles used in this study, we may be able to support this claim with our findings of elevated levels of 22:5 n3 above all other groups.

For the captive environment, there were two subgroups that the turtles were collected from: the NC Zoo and NC State CVM. These two subgroups were not examined individually due to the small sample size and therefore any variation that may have resulted from these two separate environments has not been accounted for. To confirm and expand the results found from this study, a larger population would be required.

Table 1. Percentage of Total Whole Blood Fatty Acid Profiles for Common Snapping Turtle (CST), *Chelydra serpentine*, and Eastern Box Turtle (EBT), *Terrapene Carolina Carolina*, in Wild and In-Human-Care (IHC) Environments

	CST Wild (%)	**CST IHC (%)**	**EBT Wild (%)**	**EBT IHC (%)**
Fatty Acids				
14:00	2.1 ± 0.27	1.9 ± 0.34	1.4 ± 0.32	0.9 ± 0.20
16:00	16.8 ± 0.7	19.6 ± 0.47	16.6 ± 0.58	16.5 ± 1.40
16:1n7	7.5 ± 2.7	7.1 ± 1.1	5.2 ± 1.8	3.9 ± 0.90
18:00	8.2 ± 0.65	7.1 ± 0.73	6.6 ± 0.77	8.6 ± 0.90
18:1n9	24.5 ± 0.98	28.2 ± 1.3	28.3 ± 2.1	28.5 ± 3.6
18:2n6	16.3 ± 2.4	14.3 ± 0.9	24.8 ± 3.8	16.9 ± 1.8
18:3n6	0.5 ± 0.15	0.1 ± 0.13	0.1 ± 0.03	0.3 ± 0.25
18:3n3	2.6 ± 0.46	1.0 ± 0.13	1.1 ± 0.18	2.1 ± 0.74
20:00	0.3 ± 0.03	0.3 ± 0.04	0.2 ± 0.03	0.4 ± 0.07
20:1n7	0.6 ± 0.07	0.8 ± 0.09	0.9 ± 0.23	0.9 ± 0.13
20:1n9	0.1 ± 0.01	0.2 ± 0.05	0.2 ± 0.06	0.5 ± 0.23
20:2n6	0.40 ± 0.04 *b*	0.33 ± 0.03 *b*	0.32 ± 0.06 *a*	0.94 ± 0.24 *b*
20:3n3	0.2 ± 0.02	0.1 ± 0.01	0.1 ± 0.02	0.2 ± 0.08
20:3n9	0.2 ± 0.02	0.17 ± 0.01	0.5 ± 0.09	0.60 ± 0.06
20:3n6	1.0 ± 0.08	0.8 ± 0.11	0.4 ± 0.07	0.7 ± 0.18
20:4n6	12.0 ± 0.98	11.6 ± 1.5	7.9 ± 0.61	10.7 ± 2.3
20:4n3	0.15 ± 0.04	0.10 ± 0.03	0.16 ± 0.06	0.11 ± 0.06
20:5n3	1.7 ± 0.15	0.9 ± 0.06	1.5 ± 0.34	1.5 ± 0.69
22:00	0.24 ± 0.03 *a*	0.41 ± 0.05 *b*	0.27 ± 0.05 *b*	0.42 ± 0.07 *b*
22:1n9	0.17 ± 0.09	0.10 ± 0.05	0.04 ± 0.02	0.02 ± 0.02
22:4n6	1.08 ± 0.09 *b*	1.40 ± 0.13 *ab*	1.06 ± 0.10 *a*	2.10 ± 0.35 *b*
22:5n6	0.07 ± 0.01 a	0.34 ± 0.08 *b*	0.06 ± 0.02 *a*	0.08 ± 0.02 *a*
22:5n3	1.22 ± 0.09	0.86 ± 0.04	1.38 ± 0.15	1.54 ± 0.50
24:0	0.06 ± 0.01	0.12 ± 0.06	0.04 ± 0.01	0.08 ± 0.02
22:6n3	0.35 ± 0.67	1.0 ± 0.26	0.67 ± 0.45	0.25 ± 0.04
24:1	0.35 ± 0.03	0.56 ± 0.08	0.29 ± 0.06	0.49 ± 0.09

[a,b] Superscripts that differ indicate statistical differences (P=0.05)

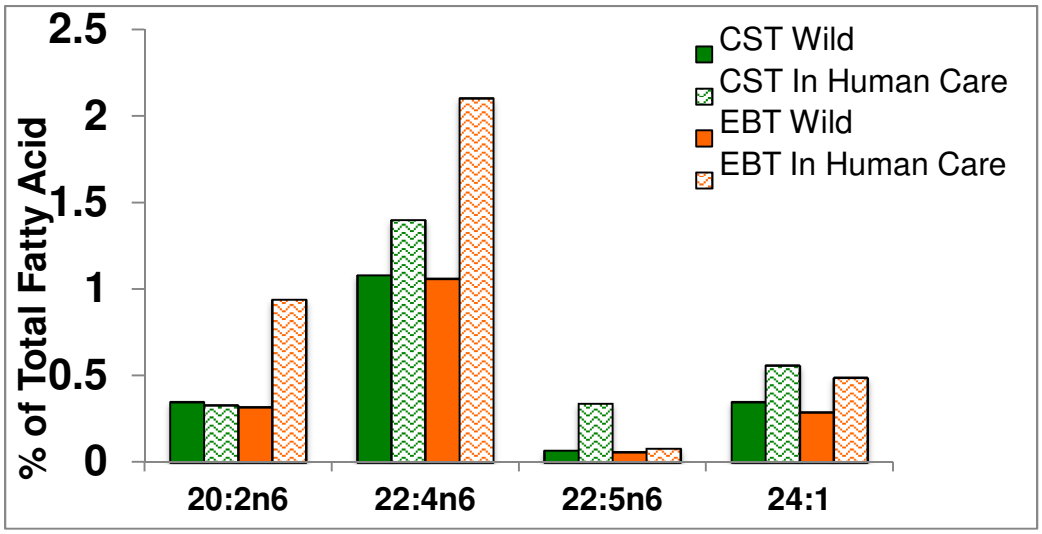

Figure 1. Interactions of Significant Fatty Acids for *Common Snapping Turtle (CST), Chelydra serpentine*, and Eastern Box Turtle (EBT*), Terrapene Carolina Carolina,* in Wild and In-Human-Care (IHC) Environments

Fatty Acid	CST	EBT	P value
14:0	2.02 ± 0.21	1.15 ± 0.22	0.011
18:2n6	15.3 ± 1.6	20.8 ± 1.9	0.047
22:4n6	1.24 ± 0.08	1.52 ± 0.24	0.02
20:3n9	0.18 ± 0.01	0.54 ± 0.06	<0.001
20:3n6	0.92 ± 0.08	0.525 ± 0.09	0.004
22:5n6	0.2 ± 0.05	0.07 ± 0.14	0.001

Figure 2. Percentage of Species Effect on Fatty Acids in *Common Snapping Turtle (CST), Chelydra serpentine, and Eastern Box Turtle (EBT), Terrapene Carolina Carolina*

Fatty Acids	Wild	In Human Care	P-values
18:3n3	2.4 ± 0.30	1.08 ± 0.3	0.007
20:0	0.35 ± 0.03	0.22 ± 0.03	0.009
20:3n3	0.20 ±0.027	0.11 ± 0.03	0.03

Figure 3. Environment effect on Fatty Acids for Common Snapping Turtle (CST), Chelydra serpentine, and Eastern Box Turtle (EBT), Terrapene Carolina Carolina, in Wild and In-Human-Care (IHC) Environments

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