Liver Condition And Maternal Offloading In The Bonnethead Shark (Sphyrna Tiburo).

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ABSTRACT

Many marine organisms, including sharks, may be susceptible to accumulating high concentrations of toxins from exposure to their environment and as top predators through biomagnification. The bonnethead shark (Sphyrna tiburo) is a coastal elasmobranch belonging to the hammerhead family. Bonnethead reproduction involves a close connection between mother and embryo through a placental analogue. Maternal offloading refers to the transfer of various toxins from the system of a mother to her offspring and may be an important source of contaminant loading in species with an umbilical connection throughout development. The presence of maternal offloading in this species was tested by examining non-pregnant female bonnethead sharks, pregnant female bonnethead sharks, and the respective unborn offspring of the pregnant individuals captured from middle Tampa Bay, FL. The Hepatosomatic index and the Condition Factors were calculated for 15 non-pregnant, 4 pregnant, and 26 embryos as a means of analyzing the health of the organisms. Measurements in the offspring were then compared to measurements of the respective mothers and non-pregnant individuals. These results may have implications for shark populations residing in areas with high levels of pollution, specifically for sharks with placental viviparity, whereby mothers may pass significant levels of contaminants to their unborn offspring in much higher relative concentrations than found in each of the mothers.

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INTRODUCTION

Global contamination of waterways is an issue that has implications for the health of entire ecosystems. This is especially so for marine organisms that occupy higher trophic levels. Through the process of bioaccumulation, various persistent and often lipophilic substances, such as metals and organochlorines, may collect in the system of an organism (Epko, 2008). In turn, these toxins may frequently be found in the fatty tissues of the liver, as well as in other organs and muscles. Elasmobranchs, particularly those living in contaminated environments, may collect high concentrations of toxins from exposure to their environment and as top predators through the process of biomagnification. In addition to being extremely susceptible to the collection of contaminants from their surrounding environments and consumption of prey, organisms may be exposed to various toxins prior to birth. Maternal offloading refers to the transmission of substances from a mother's system to her young. In species with an umbilical connection throughout development, this can serve as an important source of toxin accumulation in the system of an offspring.

The bonnethead shark (Sphyrna tiburo) was selected for this study as it has been well characterized in its reproduction, diet, and development. This species is a coastal elasmobranch belonging to the hammerhead family. As one of the smaller members of the hammerhead genus, females may reach sizes of approximately 130cm with males reaching sizes of 110 cm. On average, sexual maturity is reached from ages two to three in females with a gestation period lasting between 4.5 to 5 months. This is one of the shortest known gestation periods among sharks (Manire, 1995). The relatively early age of maturity and short gestation period may contribute to their abundance and, in turn, their listing as a species of least concern by the International Union for Conservation of Nature. Although they are currently considered populous, Bonnetheads are fished both commercially and recreationally in the United States, Mexico, Ecuador, as well as in Trinidad and Tobago (Cortés, 2016).

Bonnethead reproduction is placental viviparous, referring to live birth involving a placental analogue between mother the embryo (FIG 1). During embryonic development, bonnetheads obtain nutrients from a vitellogenic yolk during the first half of development prior to receiving nutrition from the placental transfer for the second half (Manire, 2004). Due to this connection, both nutrients and potential toxins have the means of entering the systems of the embryos. After being passed into the system of an individual, lipophilic substances tend to accumulate in the livers of sharks. The liver is a key organ in elasmobranchs as it is responsible for storing energy through synthesizing and storing lipids. The accumulation of contaminants in this organ may have implications such as negatively affecting the liver health of the embryos prior to birth. Unhealthy livers in elasmobranchs have been linked to adverse effects particularly affecting major processes such as growth, reproduction, foraging, and the ability to migrate

(Pethybridge, 2014).

Previous studies have examined the circumstance of maternal offloading in species, such as the scalloped hammerhead (Sphyrna lewini), white sharks (Carcharodon carcharias), the common thresher shark (Alopias vulpinus), and bull sharks (Carcharhinus leucas) (Lyons, 2015) (Mull, 2013) (Lyons, 2013) (Olin, 2014). These studies looked to quantify amounts of contaminants including organochlorines, heavy metals such as mercury, and Polychlorinated biphenyls (PCBs) in mothers and their respective young. Lyons, 2014 analyzed the concentrations of total mercury levels, PCBs, DDT and other pesticides in white sharks, thresher sharks, salmon sharks (*Lamna ditropis)*, and shortfin mako sharks (*Isurus oxyrinchus)*. This study in particular supported that the levels of pollutants in newborns of these four lamniform shark species tended to reflect the contaminant burdens within their mothers (Lyons, 2013).

Studies examining the maternal offloading of toxins in the bonnethead have not yet been published, although Walker, 2014 supported that bonnetheads show evidence of bioaccumulation of mercury to amounts that could potentially cause negative health effects to individual sharks as well as to those consuming their meat (Walker, 2014). Additionally, Gelsleichter examines the presence of organochlorines in bonnetheads; noting that these animals are extremely vulnerable to the effects that organochlorine contaminants may have during the embryonic development of these sharks (Gelsleichter 2005, 2008). In a study conducted by Manire, 2004, the transfer of steroid hormones through embryonic and ovarian yolks were also analyzed in the bonnethead.

The present study examined the general health condition as well as the health of the livers of the pregnant females, non-pregnant females, and male and female embryos. By examining

liver conditions, the aim is to identify whether exposure to contaminants prior to birth could potentially impact the liver health and general condition of the embryos. This is achieved by calculating the hepatosomatic index and the condition factor of the sharks within the three different life stages. The Hepatosomatic Index serves as an indication of liver health which then can be used to estimate the amount energy reserved in an organism. It has been reported to be lower in species exposed to Cadmium and Zinc as well as lower in organisms living in poor environmental conditions. Another index that is frequently used among various species of fish to compare various conditions for growth is the Condition Factor. This is calculated by comparing length and weight of an individual. Higher condition factors indicate better environmental quality while lower condition factors indicate poorer environmental quality. Lower condition factors indicate poor growth, which has been linked to largely impacting the reproductive health of an organism.

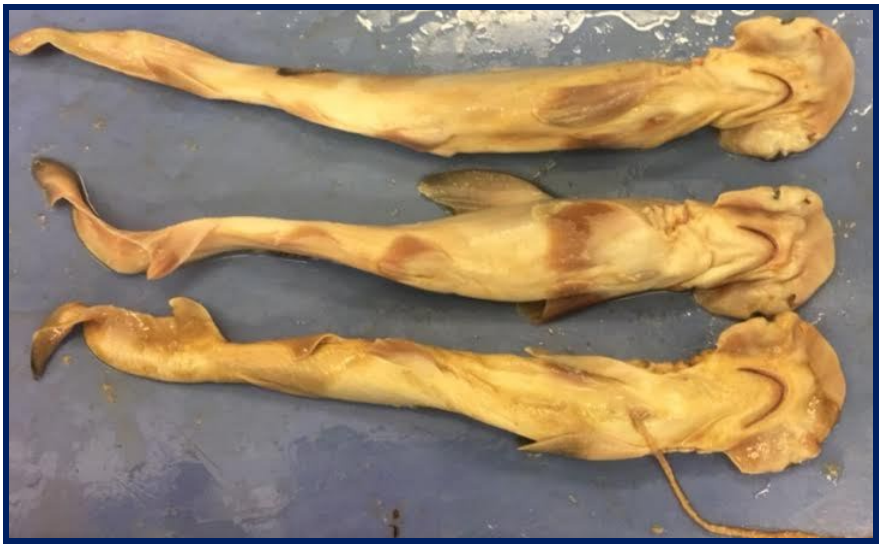
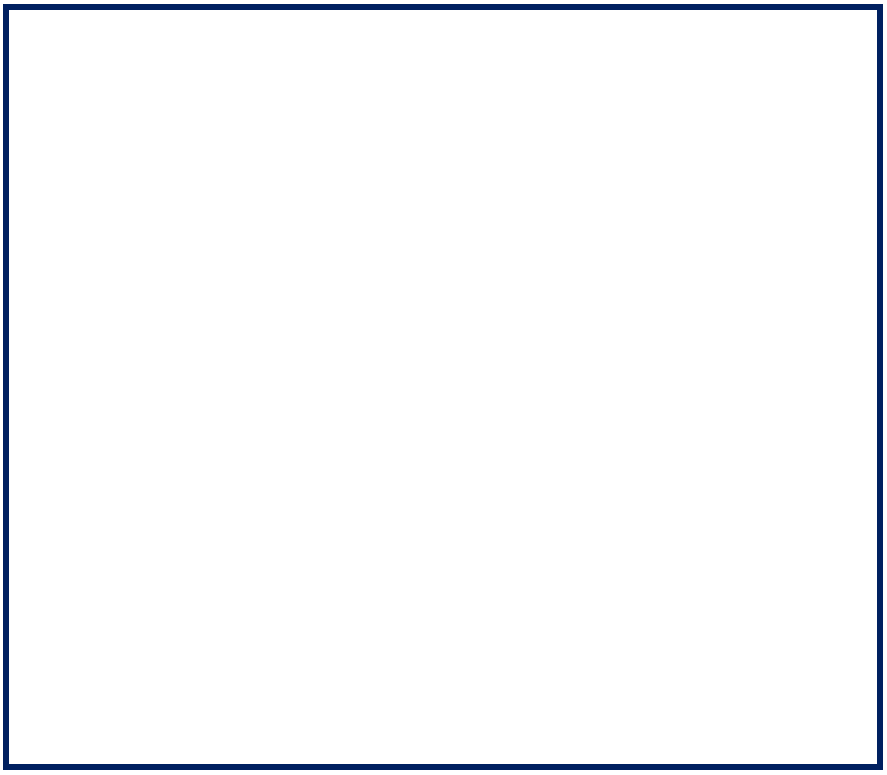


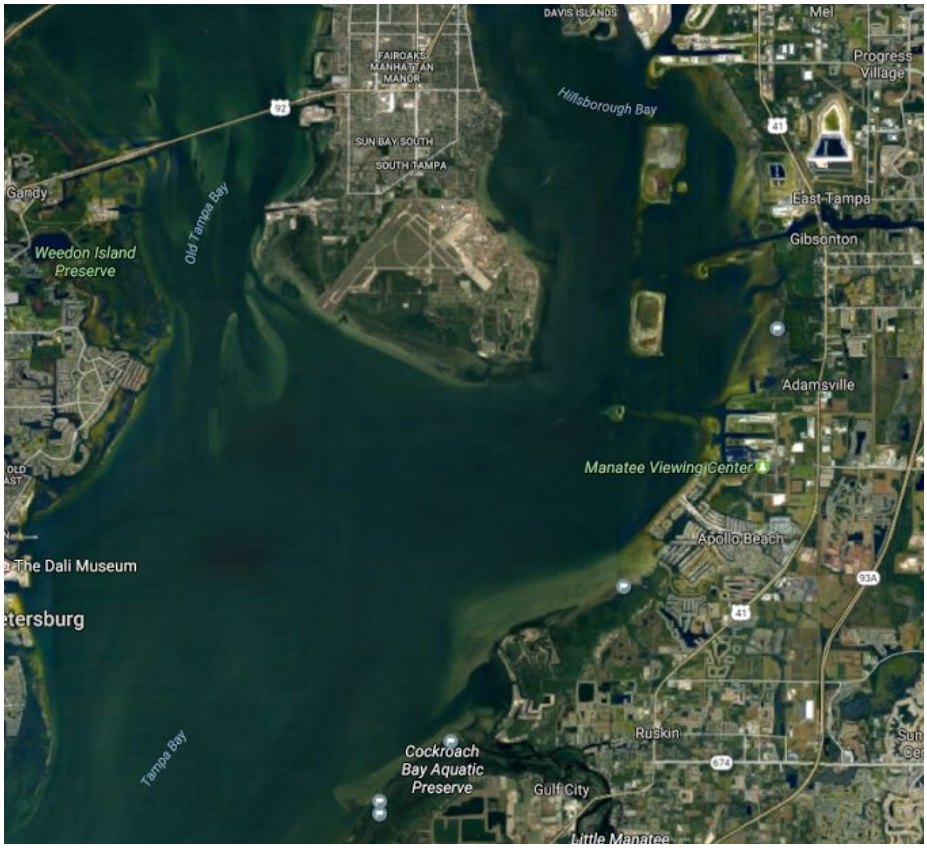
Figure 1: Placental analogue visible in embryos from 16BH13.

MATERIALS AND METHODS

Collection of Samples

The collection of organisms took place from the months of May through August during the summers of 2015 and 2016. Free swimming bonnetheads were collected through use of 100 millimeter gillnets in water less than 1.25 meters in depth in the Middle Tampa Bay region. Water temperatures ranged between 26.3 C and 31.1 C with the salinity ranging between 24.0 and 27.8 ppt. Gill nets were soaked for a maximum of three hours during which the nets were continuously monitored and sharks quickly removed from the netting. In order to euthanize the samples, Tricaine Methanesulfonate (MS-222) was mixed into saltwater, and samples were immediately placed on ice and returned to the laboratory. Total numbers of specimens used in this project include fifteen non-pregnant females, four pregnant females and twenty six embryos, thirteen of which were male and thirteen of which were female.





Identification

For identification, individuals are indicated by the year they were caught, with either a 15 or 16 referring to 2015 or 2016 for year they were caught, BH to note their species, and pups indicated by a P followed by a M or F for their sex. For example, a female caught during the summer of 2016 would be identified as 16BH1, with her first male pup being indicated by 16BH1MP1. Each individual used in the study has been identified by this method in Tables 1, 2 and 3.

Dissection

In the laboratory, each of the samples were thawed prior to the total weights of each

individual being taken to the nearest gram, along with measurements of their total lengths, fork lengths, and precaudal lengths. A metal ruler was used to measure these lengths in centimeters with the caudal fin resting neutrally. The measurement of length used in this study was the Precaudal Length (PCL), defined as the distance from the tip of the snout to the precaudal pit.

By performing a ventral dissection, livers were individually removed and weighed (FIG 2). For future studies concerning measurements of toxins within each individual, tissue samples were removed from the kidneys, livers, hearts, and muscle tissues from the left dorsal flank. In the pregnant females, the gonads were removed, weighed, and then further dissected to remove individual embryos from excess gonadal fluid and tissues. The dissection process above was then repeated for each of the twenty-six well-developed embryos.

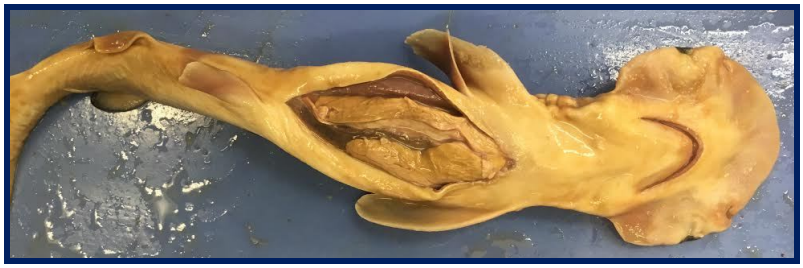


Figure 2: Ventral Dissection of embryo with liver largely visible prior to removal.

Calculations: Determining HSI

Hepatosomatic Indices were calculated by looking at the ratio of weight of the liver to the total weight of the individual. The calculation was made by following:

$$HSI = [M_{TL}\ (Kg)/\ M_{TB}\ (Kg)] \times 100$$

Where M_{TL} represents the total body mass of both lobes of the liver and M_{TB} represents the total body mass of each individual.

Calculations: Determining Cf

Condition Factor serves as more general health indicator as it solely takes into account the total body weight versus the total body length of the individual. In order to calculate the Condition Factor, the formula below was used:

$$C_F = [M_{TB}\ (Kg)/\ PCL\ (cm)^3] \times 10^5$$

Where M_{TB} represents the total body mass of each individual and PCL represents the Precaudal Length (FIG)

Data Analysis

A Shapiro Wilk test was run on both the data for the condition factors and the hepatosomatic indices to assure normally distributed data before further analyzation. According to the Shapiro Wilk test, the data for the hepatosomatic index was not normally distributed, therefore a square root transformation was performed. When the Shapiro Wilk Test was run on the transformed data for the hepatosomatic index, a normal distribution was indicated, which allowed for parametric analysis to be run. The data for the calculated Hepatosomatic Indices and

the Condition Factors was then grouped based on litter size, litters from each mother, and by life stage (in reference to the pregnant, non-pregnant females, and embryos). These three groupings were then analyzed using one-way analysis of variance (ANOVA). If the ANOVA yielded a significant p-value, a Tukey HSD Test was run to further identify within the group the significance between any two of the sampled means within the group.

Table 1. Non Pregnant Adult Females: Precaudal lengths, body and liver weights, hepatosomatic indices and condition factors of non-pregnant adult females.

ID	PCL	Whole Body Weight (g)	Liver Weight	Hepatosomatic Index (%)	Condition Factor
15BH2	60.5	2300	88.5	3.847826	1.038632031
15BH3	59.1	1500	77.6	5.173333	0.7266559206
15BH5	58.1	1980	56.4	2.848485	1.009570828
15BH6	56.8	1820	53.6	2.945055	0.9931763762
15BH7	57.4	1747	41.3	2.364053	0.9237559054
15BH8	58.1	2015	113.7	5.64268	1.027416777

15BH9	61	2250	91.2	4.053333	0.9912723972
15BH10	56.2	1900	104	5.473684	1.070396436
15BH11	80	4600	135.3	2.941304	0.8984375
15BH12	67.3	2900	113.8	3.924138	0.9513773446
15BH13*	55.2	1300	27.8	2.138462	0.7729050041
16BH8	74	5000	*	*	1.233885456
16BH9	59.6	2700	*	*	1.275337074
16BH10	60	2000	120	6	0.9259259259
16BH11	75.8	6000	*	*	1.377665026

*Liver weights not obtained for 16BH8, 16BH9, and 16BH11.

Table 2. Pregnant Adult Females: Precaudal lengths, body and liver weights, hepatosomatic indices and condition factors of the pregnant females.

ID	PCL	Whole Body Weight	Liver Weight	Hepatosomatic Index (%)	Condition Factor
15BH4	70	4000	155.5	3.8875	1.166180758
16BH5	70.8	4800	300.6	6.2625	1.35251305
16BH12	65	3500	162	4.628571	1.27446518
16BH13	66.8	3200	176.9	5.528125	1.073545834

Table 3. Embryos: Precaudal lengths, body and liver weights, hepatosomatic indices, and condition factors of the embryos.

ID	PCL	Whole Body Weight	Liver Weight	Hepatosomatic Index	Condition Factor
15BH4FP1	11.2	14.2	0.8	5.633802817	1.010727952
15BH4FP2	12.1	19.5	0.6	3.076923077	1.100724164

15BH4FP3	12.3	16	0.7	4.375	0.8598142694
15BH4MP1	12.5	17.6	0.9	5.113636364	0.90112
15BH4MP2	12.6	19.5	1	5.128205128	0.9748167345
15BH4MP3	11.9	14.7	0.6	4.081632653	0.8723212468
15BH4MP4	12.7	16.9	1	5.917159763	0.8250410202
15BH4MP5	12.4	13.9	0.3	2.158273381	0.7290372931
16BH5MP1	10.7	13.6	0.4	2.941176471	1.110165113
16BH5MP2	10.3	12.8	0.5	3.90625	1.171381324
16BH5MP3	10.1	11.1	0.4	3.603603604	1.077355064
16BH5MP4	11.1	14.3	0.4	2.797202797	1.045603675
16BH5FP1	11	14	0.3	2.142857143	1.051840721
16BH5FP2	10.6	11.7	0.4	3.418803419	0.9823545611
16BH5FP3	10.2	12.8	0.3	2.34375	1.206172588
16BH5FP4	10.3	10.2	0.4	3.921568627	0.9334444925
16BH5FP5	10.7	12.6	0.5	3.968253968	1.028535325
16BH5FP6	10.9	13.1	0.7	5.34351145	1.011560359

16BH12FP1	15.2	39.8	0.8	2.010050251	1.133319361
16BH12MP1	16.5	45.3	1.4	3.090507726	1.008431422
16BH12MP2	16.5	48.4	1.6	3.305785124	1.077441077
16BH12FP2	16.7	53.2	1.8	3.383458647	1.142252767
16BH12MP3	16.8	53.7	2	3.724394786	1.132521461
16BH13FP1	14.7	28	2.1	7.5	0.8814669373
16BH13FP2	16	36.4	2.9	7.967032967	0.888671875
16BH13MP1	15.3	34.9	3.2	9.169054441	0.9744310956

RESULTS

Condition Factor and Hepatosomatic Index across life stages:

The Condition Factor and the Hepatosomatic Index were compared amongst the non-pregnant females, pregnant females, and embryos. The P-value for the mean of the Condition Factors was significant with a value of .024. The Tukey HSD Test was then used to further analyze the significant differences within the group of three life stages. As noted in the Figure 3, the pregnant females (A) had mean condition factors that were significantly different from the means of the condition factors than the embryos and the non-pregnant females (B). The Tukey HSD test yielded P- values of $P< .05$ when comparing the pregnant to nonpregnant females, and $P<.01$ when comparing the pregnant sharks to the embryos. The P-value obtained

for the means of Hepatosomatic Indices of each life stage was not significant.

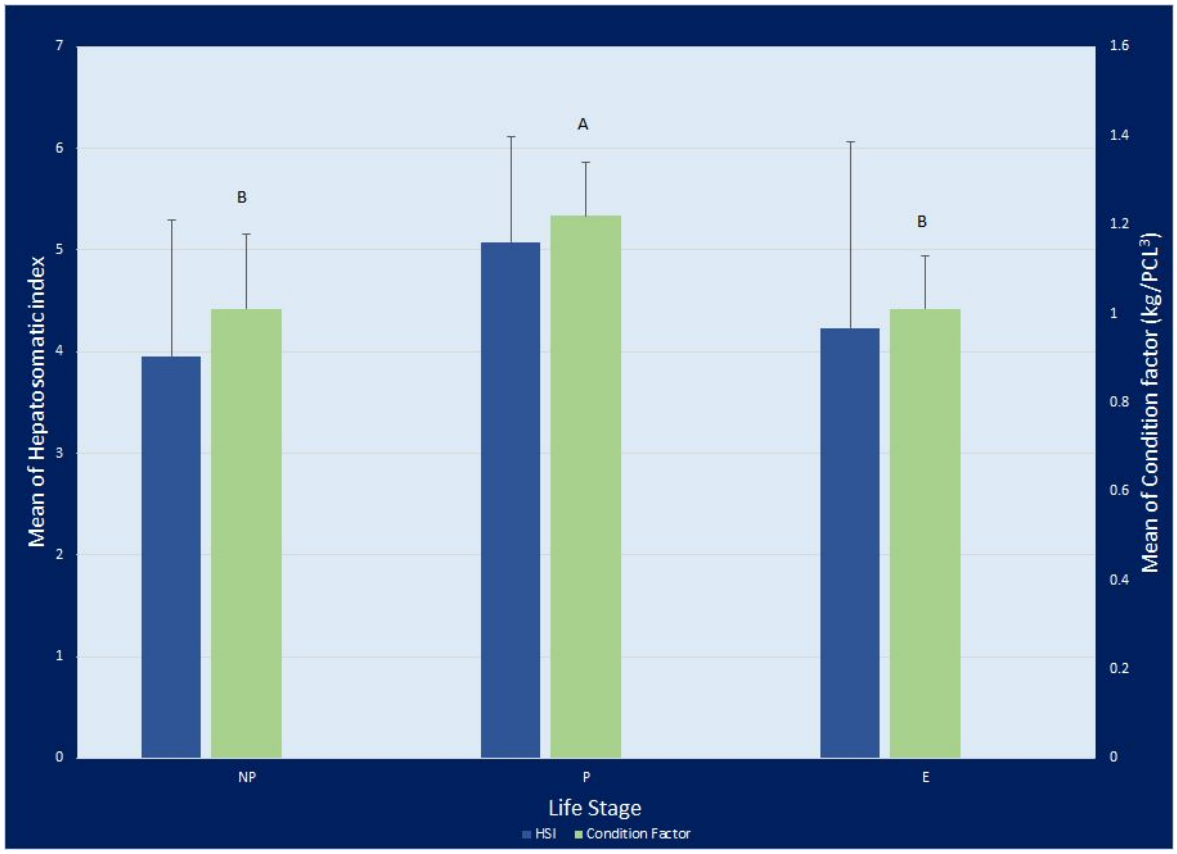


Figure 3. Mean of Hepatosomatic Indices and mean of Condition Factor amongst life stages.

Hepatosomatic Index and Condition Factor for Size of the Female

The Hepatosomatic Index and the Condition Factor were then compared amongst the litters of each of the four females, as distinguished by the Female Weights in Figure 4. The P-values obtained when comparing the means of Hepatosomatic Indices amongst litters and when comparing the means of the condition factors amongst litters both were significant. The P-value obtained for the mean of the Hepatosomatic Index was significant with a value of .0001. The Tukey HSD Test was then used to further analyze the significant differences within the four

litters. The Tukey HSD test yielded P-values of P< .01 when comparing mean condition factor of the litter from female 16BH13 weighing 3.2Kg (A) against the other females (B) including 16BH12 weighing 3.5Kg, 15BH4 weighing 4.0Kg, and 16BH5 weighing 4.8Kg.

The P-value obtained for the mean of the Condition Factors was significant with a value of .0014. The Tukey HSD Test was then used to further analyze the significant differences within the litters. The Tukey HSD test yielded P- values of P< .05 when comparing the means of the condition factor of the litter from 16BH13 (D) to the litter of 16BH12 (C). A P-value of P<.05 was also obtained when comparing the condition factor of the litter from 15BH4 (1) and the litter of 16BH5.

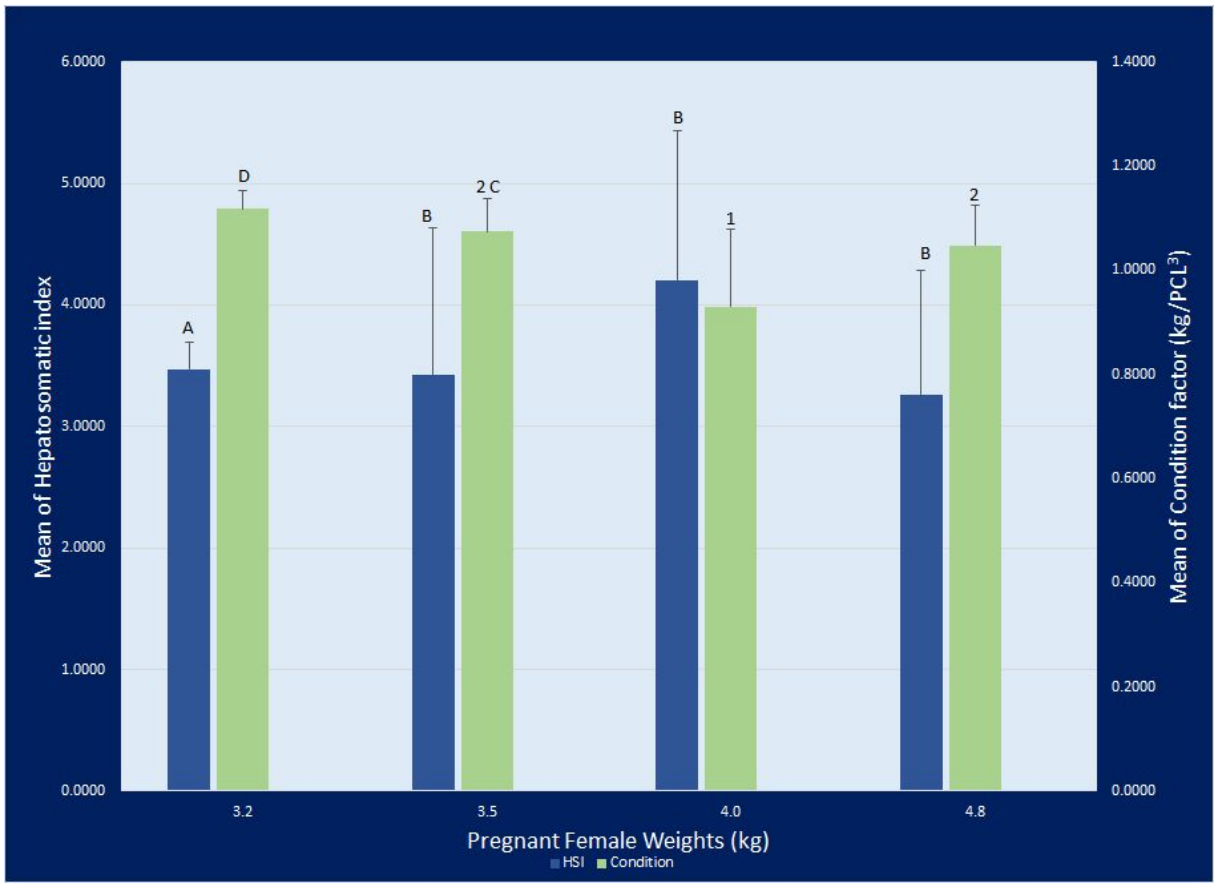


Figure 4. Mean of Hepatosomatic Indices and mean of Condition Factor amongst litters.

Hepatosomatic Index and Condition Factor amongst Litter Sizes:

The Hepatosomatic Index and the Condition Factor were also compared amongst the embryos based on the sizes of the litters (FIG. 5). The P-value obtained for the means of Hepatosomatic Indices of the embryos within each litter as categorized by litter size was significant with a value of .01 and the downward trendline is visible in Figure 5. The ANOVA treatment between groups for the Condition Factor was not significant.

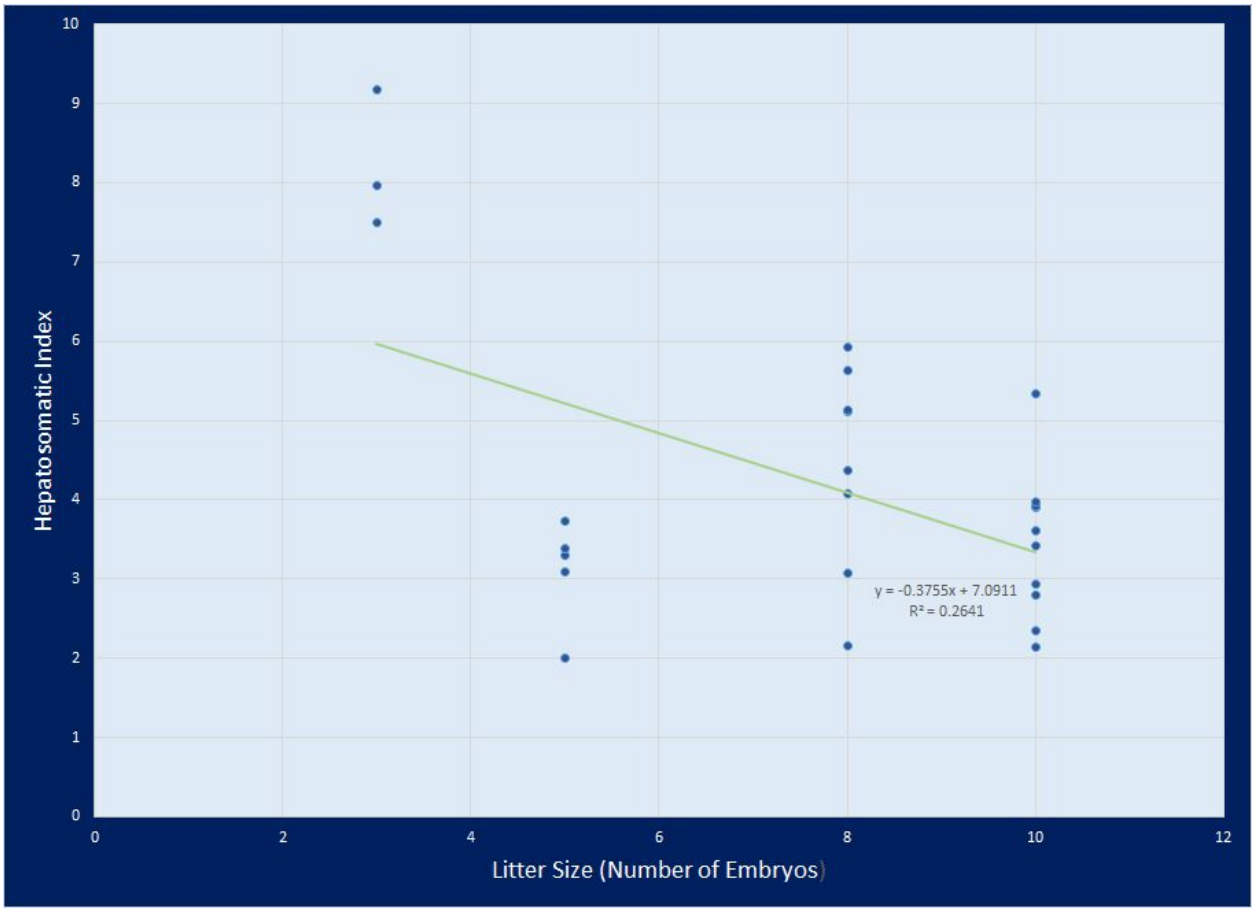


Figure 5. Hepatosomatic Indices within each litter as categorized by litter size.

DISCUSSION

As indicated in Figure 3, there was variation in the health of the organisms at different life stages, with the pregnant females having significantly higher Condition Factors. This can likely be attributed to Condition Factor being calculated by the weight corresponding to the length of an organism. Provided that pregnant females will weigh more for their given length then a non-pregnant female, the condition factor would in the likely be higher for those carrying offspring. To further analyze this factor, comparing the Condition Factors of the females with the gonadal weights removed may provide a more accurate means of comparison. However, it is possible that the pregnant females would be in overall better health condition than non-pregnant females of a similar length. This could be due to higher energy being reserved in an organism prior to giving birth, as well as the ability to reproduce can often be reflective of organismal health.

When examining Figure 4, there was evident variation in the mean Hepatosomatic Indices of the litter from female 16BH13 when compared to the mean Hepatosomatic Indices of the other litters. This could potentially be contributed to 16BH13 being the lightest in weight, however the Condition Factors were also found to be significant when comparing the means of amongst the litters from 16BH13 (D) to the litter of 16BH12 (C) and when comparing the litter from 15BH4 (1) and the litter of 16BH5. Although there was significance within the differences of these litters, the sample size of pregnant females is not large enough to draw significant conclusions when comparing the health based on the weight of the mother.

However, when analyzing Figure 5, there is a distinct downward trendline visible when viewing the hepatosomatic indices of the embryos when categorized by the size of the litter. This is indicated by the embryos from the smallest litter size n=3 from 16BH13 having the highest Hepatosomatic Indices, and the largest litter size n=10 from 16BH5 having the lowest Hepatosomatic Indices, with the other two litters also following this trendline. This can likely be attributed to females being able to disperse more energy into individuals within a litter when their are less embryos. However, it would be interesting in future studies to also examine whether there are significant differences within the levels of toxins amongst embryos when categorized by litter size.

Future studies examining the effect of the presence of toxins within the systems of embryos, as well as analyzing whether there is a correlation between embryonic health and these organisms could be key to supporting the concept of maternal offloading in this species. Gelsleichter 2008 notes the importance of studies examining the effects of contaminants as bonnetheads may be exposed to pollutant-impacted areas during periods of their life such as embryonic development and sexual maturation when these toxins may affect organismal health. With the samples used in this study, total mercury levels will be measured in the liver, heart, kidney, and muscle tissues. The concentration of total mercury levels measured in the offspring will then compared to the total mercury levels found in the liver and muscle tissues of the respective mothers and non-pregnant individuals. Mercury is commonly found in the tissues of marine organisms particularly those living in contaminated environments. Various studies have examined the effect of the growing industrial areas surrounding Tampa Bay on the increase in pollutants in the Middle Tampa Bay watershed, which is the site from which our samples were

collected. Additionally, numerous consumption warnings are in place in Tampa Bay for various fish species due to mercury levels.

CONCLUSIONS

These findings support the concept that the health of placental viviparous bonnethead shark mothers impacts the health of offspring, which could be a result of maternal offloading. This is consistent with the finding of other studies that support that mothers may pass significant levels of contaminants to their unborn offspring in much higher relative concentrations than found in the respective mothers. This study also notes that larger litter sizes may have adverse health impacts on individuals within that litter as less energy may be allocated to each offspring. Further studies on these factors when correlated to quantified toxin concentrations would lead to a to better understanding of the impact of pollution on embryonic health of elasmobranchs, particularly in areas of high contamination.

ACKNOWLEDGEMENTS

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