A Preliminary Delineation of Shark Nursery Grounds in Two South Carolina Estuaries

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Introduction

Background

Atlantic shark populations are in a steep decline from historic numbers. Baum *et al.* (2003) have estimated that sixteen species of Atlantic sharks have decreased by at least 50% since 1992. These species include three species of hammerhead, the white, tiger, two species of thresher, the blue, oceanic whitetip porbeagle and six species of coastal sharks. Blue sharks, a common pelagic species often caught on high seas longlines as bycatch, have declined by as much as 80% since 1977 (Simpfendorfer *et al*. 2001). Although new legislation has reduced fishing pressure on shark stocks (1996 amendments to the Magnusson-Stevenson Act), commercial operations such as the gillnet fishery for small coastal sharks in the southeastern United States, still jeopardize the stocks of several species (Carlson and Cortes 2002). If current trends continue, the health of the entire oceanic ecosystem could be impacted and only low trophic level species will remain (Christensen *et al*. 2003).

Shark populations continue to decline from fishery pressure (both recreational and commercial) and habitat degradation (See appendix A for Contaminant Data). The earliest shark fishery has been dated to the sixteenth century (Stone et al 1998). These early fisheries were subsistence fisheries and the pressure on stocks was relatively low. In direct contrast are the contemporary commercial fisheries (i.e. fareast finning fleets). Finning of sharks, although outlawed in US waters, entails removal of fins and then returning the still alive animal to the ocean. Technology has also taken its toll on fish stocks. The rod and reel has been replaced by trawls and purse seines. Longlines and gill nets often stretch for miles, and if these tools are lost or abandoned they continue to catch sharks, a phenomenon known as “ghost fishing”.

Commercial landings of sharks steadily rose starting in the 1980s and peaked in 1988 with 7,851,522 kgs of sharks caught. The following years saw a dramatic decrease in kgs, and by 1995, the landings were only 3,922,822 kg. The numbers continued to decline and as of 2002 were only 830,527 kg. . (Personal communication National Marine Fisheries Service, Fisheries Statistics and Economics Division, Silver Spring, MD). This decrease in biomass is representative of two occurrences. Shark populations began to fall drastically, and the subsequent implementation of fishing quotas.

Although not as well documented, recreational fisheries also impact fish stocks. These numbers tend to be less than those for commercial fisheries, except for 1995. Recreational catches peaked in 2000 (Cortes 2002). Recreational catches do not take into account of post-catch mortalities while Manire and Gruber (1990) have estimated as high as 49%.

Commercial and recreational fisheries play a large role in reducing elasmobranch populations; however, they are only a fraction of the mortality due to bycatch (Cortes 2002), especially from swordfish, tuna, and shrimp fisheries (Stone et al. 1998; Hall et al 2000; Cortes 2002).

A final threat to sharks is habitat destruction. Stone et. al.(1998) concluded that shark populations would be adversely affected by habitat destruction or disruption. Habitats can be disrupted in several ways, including physical disruptions as dredging or installation of jetties, or it can be due to an increase in contaminants from point source or non-point source pollutants. Example of physical disruption exists in Bimini, Bahamas where construction of a casino is threatening the lagoon which acts as an important nursery for lemon sharks. Similar projects can been seen along the coasts of the United States as continued development of coastal lands puts increased pressure on the natural environment. Pollution from agriculture, industry, and suburban run-off are also degrading the quality of the habitat and limiting the ability of the wetlands to buffer against anthropogenic effects.

Essential Fish Habitat

The idea of protecting the areas of great importance to marine species spurred the essential fish habitat, EFH, additions to Magnuson-Stevens Act amendments in 1996. Congress defined EFH as “Those waters and substrate necessary for fish spawning, breeding, feeding, or growth to maturity” (NOAA 2003). Congress took an active and precautionary role to protect the environments that numerous commercially important species depend upon. One of the novel ideas behind this act is that it was designed to protect stocks from both fishery and non-fishery related activities. The act also determined who was responsible for designating EFH, enforcement and agencies responsible for following regulations. By taking an ecosystem approach, all species will be afforded the same protection.

Nursery Habitat

The coastal regions of North and South Carolina are characterized by a system of inlets and estuaries. These systems range in size and ecological importance; many act as nursery habitat for multiple fish species (Mallin et al. 2000; Gillanders 2002; Reichert, 2003; Holland et. al. 2003). Habitat utilization has been studied in only a few elasmobranchs, including scalloped hammerhead, *Sphyrna lewini*, (Bush, 2002), lemon shark, *Negaprion brevirostris,*(Gruber 1988) and sandbar shark, *Carcharhinus plumbeus,* (Grubbs 2000). Bush (2000) found that juvenile species of *S.* lewini inhabit the estuarine systems of Hawaii, and concluded that predator avoidance acts as a greater incentive for habitat selection than does prey availability. Gruber (1988) has concluded that female lemon sharks, *N.* brevirostris, move close to the mangrove forests of Bimini, Bahamas to drop their pups. Grubbs (2000) also concluded that the Chesapeake Bay system is a primary nursery ground for the sandbar shark.

Nursery habitats have a three-fold ecological function. First is to protect the smaller species from predators that would be encountered in less sheltered areas. Creeks and inlets are small and difficult for large predators to enter thus affording the smaller species a place of refuge. Additional shelter from vegetation protects a large number of vertebrates and invertebrates. Lazzari *et al.* (2001) showed a significant relationship between increased vegetation and higher survival rates for neonate and juvenile teleost species. *Spartina alterniflora* is the dominant species in most South Carolina estuaries and is essential in the functioning of the systems. *S. alterniflora* may not provide direct shelter for elasmobranches, but may indirectly by sheltering potential prey items from larger would-be predators (i.e. the smaller shark pups will be able to get into areas inaccessible by larger predators). Second, due to the large amounts of primary productivity, there is an ample source of food for the developing species resulting in accelerated growth rates and decreased time of vulnerability to large predators. A third function of estuaries is not ecological but instead economic. By weight, 75% of commercially important fish species are dependant on the estuaries during some portion of their life cycle (Lazzari 2001). If estuaries are damaged and disrupted, there is a higher probability for negative impacts against commercial fisheries.

Study objectives

The purpose of this study was twofold. First was to determine if a nursery habitat for elasmobranches did exist in the northeastern part of South Carolina. Two candidates were selected as possible locations, Murrells Inlet, which is located approximately 20 mi south of Myrtle Beach, and North Inlet which is located 20 mi south of Murrells Inlet. Both of these systems appear to provide an abundance of food, and shelter from predation. However, Murrells Inlet differed from North Inlet; it has a higher percentage of impervious surface around it, and a higher degree of urbanization. The second objective was to determine if there were population differences between the two locations with respect to elasmobrnach species.

I hypothesize that urbanization Murrells Inlet will affect the total number of elasmobranches present and the species diversity of elasmobranchs. I believe predation is the controlling factor for newborn sharks and young juveniles, and so I would expect to find those individuals in the areas least accessible to adult sharks. This idea is supported by Gilliam and Fraser (1987) who looked at foraging behavior in response to predation pressure. They found fishes will move to the habitats that afford them the greatest chances of survival. However, once animals grow in size, and the risk of being eaten becomes substantially less, they move to areas that are less environmentally stressful.

Materials and Methods

SAMPLING SITES

Two locations chosen for this study were Murrells Inlet and North Inlet, SC. These locations were chosen to determine if there are differences in elasmobranch diversity between an impacted habitat, Murrells Inlet, and a relatively pristine habitat, North Inlet. Both of these inlet systems are relatively small. However Vernberg et. al. (1992) has suggested that these smaller systems may be of greater importance than larger systems because their added areas account for more habitat. Numerous studies have been done on the differences between these two systems in relation to water chemistry and runoff effects (Vernberg et al. 1992; Fulton et al. 1993; Kawaguchi, et al. 1997; Wirth et al. 1998; Lewitus et al. 2003; White et al. 2003). However little has been done in relation to the associated fauna; the few studies I found dealt mostly invertebrates (Wirth et. al. 1998), and no studies have been performed to evaluate the differences in elasmobranch richness or diversity.

Murrells Inlet is located approximately 20 miles north of North Inlet and both systems are bar-built estuaries, although Murrells Inlet has been stabilized by jetties. They are both dominated by *Spartina alterniflora*. Both have high salinity due to the lack of river input to the system (White et. al. 2003). Freshwater received by these two systems comes from runoff and rain water. Both inlets are subject to semi-diurnal tides and have a mean flux of about 1.4 m (White et. al. 2003). The inlets do differ in size with Murrells Inlet being the smaller of the two. The open water area of Murrells Inlet measures 5.76×10^6 m^2 and the marsh area is 15.45×10^6 m^2 (Mathews et. al. 1980).

North Inlet has a open water area of 7.33×10^6 m^2, and a marsh area of 19.97×10^6 m^2 (Finley 1975).

The major differences between these two systems come from the amount of urban development to which they have been subjected. North Inlet has seen little development save for a small housing development on its northern side. This results in substantially less boat traffic, fewer impervious surfaces, and less anthropogenic disturbance. The rest of North Inlet is surrounded by forested lands. Murrells Inlet is a sharp contrast and has housing developments and hotels located on its northern, eastern, and western sides. This development leads to an increase in impervious surfaces and a subsequent increase in runoff to the system. Holland et al. (2003) established a classification system for estuaries based on the amounts of impervious surface. North Inlet was classified as a forested estuary because the urbanization was < 30% and the impervious surface did not exceed 10%. But Murrells Inlet was classified as a suburban estuary as the impervious surface was between 10% and 50% while the amount of urban development fell between 30% and 70% (Holland et. al. 2003). Murrells Inlet, located near Myrtle Beach, SC is also prone to an increase in population during the spring and summer months due to an increase in tourism. Mallin (2000) has listed tourism and its effects on increased urbanization as in increasingly detrimental effect to estuaries due to the increase in non-point source pollution. A higher number of golf courses located in the proximity of Murrells Inlet leads to increasing inputs of fertilizers, pesticides, and herbicides to the system.

FIELD

Sampling lines were set in three locations: Murrells Inlet, North Inlet, and along Garden City Beach. Garden City is located adjacent to Murrells Inlet, lines set outside of the jetties were designated to be in Garden City. Collection sites were chosen at random within the two estuaries, all accessible locations were sampled. Sampling in Garden City was limited by the weather because it is substantially less sheltered. Because the creeks were subject to tidal flux and could be almost dry at low tide, several areas were inaccessible during periods of low tide. Sampling was done during all cycles of the tide, and during all times, including night sets.

To catch the elasmobranchs, demersal long-lines were deployed within the creeks. The lines varied in both size and the amount of hooks contained dependant on the length and width of the creek. The decision for which line to set was made on the spot and not subject to preconceived plans. The lines were 300ft, 200ft, or 100ft and were composed of a tarred nylon. Loops were located at each end and another approximately twenty-five feet down from the ends to accommodate the buoy and anchor respectively. The lines were all set along the bottom and resulted in a general “U” shape (Figure 1). Each line contained 15, 10 and 5 hooks for the largest to smallest lines respectively. The hooks themselves were 13/0 circle hooks. Circle hooks were chosen because elasmobranches rarely swallow them.

The hooks were attached to the bottom line by gangions. The gangions were composed of a tuna clip, two feet of 200 lb monofilament line, and the hook. Lines were set by hand from the bow of the boat. Times of the first hook in and last hook out were recorded and used to determine total sampling time for each line. The set time was designed to be thirty minutes; however, increased catches on the line occasionally pushed the set time longer. Set time was standardized when computing CPUEs (Catch-per-unit-effort). The CPUE for this project was the number of animals caught per twenty-five hook hours.

Precaudal length (PCL), fork length (FL), and the total length (TL) were measured for all sharks captured. Disk width and distance between spiracles was measured for skates and rays. The sex was determined and then they were visually inspected for any injury, or parasites. Injuries were noted and parasites were removed prior to their release. A roto tag (approximately 3 cm long) was then inserted into the dorsal fin for all animals above fifty centimeters. Animals smaller that fifty centimeters could be adversely affected by the size of the tags, so they were given a hole punch in their right pectoral fin to indicate capture. After insertion of the tag, the animals were released and rated on a scale of 1-5 (1=excellent health; 5=dead).

Physical conditions were recorded as well. They include: water temperature, air temperature, salinity, dissolved oxygen, current speed, time from high slack tide, cloud cover, wind speed and direction, and sea state. The salinity, water temp and DO were all measured using a YSI (yellow springs instrument) meter, and air temperature was attained with a mercury thermometer. Current speed was measured using a current meter (General Oceanics). Wind speed was estimated along with sea state and cloud cover. Wind speed was categorized by 5 knot increments, 0-4, 5-9, 10-14 etc. Sea state was also broadly categorized as flat, calm, light chop, moderate chop, or heavy chop. Cloud cover was distinguished between clear, mostly sunny, partly sunny, partly cloudy, cloudy, or rain.

STATISTICS

Two indices of species diversity were calculated. The indices were calculated for each estuary as well as Garden City. The first uses the Simpson's diversity index given by:

$$D = \sum (n / N)^2$$

Where n = the total number of organisms of a particular species, and

N = the total number of organisms of all species

and the second was the Shannon Weaver index given by:

$$H = -\sum_{i=1}^{S} p_i \ln p_i$$

Where S= the total number of species in a community, and

Pi= proportion of *S* made up of the *i*th species

These calculations take evenness and species richness into account and will provide for a general look at the species structure. The two methods were used to ensure accuracy and account for assumptions between methods.

Correlation between variables and CPUEs were calculated using two non-parametric tests: Spearman's rho and Kendall's tau-b. Both are measures of the relationship between variables. Both of these calculations were run in SPSS software. The statistical difference between the mean CPUE's of each location was computed using the non-parametric Wilcoxon test. The Wilcoxon test was used to compare North Inlet to Murrells inlet, Murrells Inlet to Garden City and North Inlet to Garden City. The Wilcoxon test was performed using SPSS software.

Results

Physical Data

The physical characteristics of the two estuaries were very similar. The mean values for each estuary and for Garden City are summarized in table 1**.** The mean salinity and dissolved oxygen are slightly higher for Murrells Inlet than for North Inlet, and seem to be much more in line with the values recorded for Garden City Beach. North Inlet has a higher mean water temperature than does Murrells inlet and again the values for Garden City closely mirror those for Murrells Inlet. The mean visibility was lower in Murrells Inlet and in North Inlet than what was observed in Garden City. The mean depth was also markedly lower for the two estuaries than it was for the beach area sampled.

Monthly variations also occurred. The warmest water temperatures were experienced during the summer months, with temperature highs being reached in June, July and August for Garden City, North Inlet, and Murrells Inlet respectively. Salinities also followed a seasonal trend with the higher salinities coinciding with the warmer temperature. Dissolved oxygen followed no monthly trend for any of the locations. No trend could be detected for changes in visibility on a monthly average, but there were observed differences during different tidal stages. The rising tide tended to have a higher visibility than did the falling tide.

Effort

The amount of effort in each area can be seen as a function of the amount of hooks and lines that were set as well as the amount of time the lines were in the water. These data are summarized in table 2**.** A total of 135 lines were set in Murrells Inlet which encompassed 1740 hooks and 4963 minutes of fishing time. This is more than three times the effort in North Inlet which had 41 lines 485 hooks, and 1640 minutes of fishing time. Due to the lack of a set sampling schedule for Garden City it received the least amount of effort with only 20 lines being set with 250 hooks over 779 minutes.

Catches

A total of 90 animals were captured and tagged during this study. Over the three locations, a total of nine species sampled were sampled**.** Figure 2 illustrates the species caught by number by gender. Six of the species were sharks: *Rhizoprionodon terrenovae, Carcharhinus plumbeus, Ginglymostoma cerratum, Sphyrna tiburo, Carchahinus limbatus,* and *Carcharhinus isodon.* Two species were rays: *Dasyatis americana* and *Gymnura micrura.* The ninth species caught was a skate, *Raja eglanteria*.

Murrells Inlet had the fewest catches at 24 total animals caught. The distribution of catches by percent can be seen in figure 3. Only two species of shark were caught: four *S. tiburo,* and one *G. cerratum*. Murrells inlet catches were dominated by rays, 75%, and almost half of the animals caught were *D. americana* 46%. The CPUE for the inlet was also the lowest at 0.474 for the entire sampling period. Out of the 24 animals that were captured in Murrells Inlet only one was male (*D. americana).*

Garden City only had a few more animals caught than did Murrells Inlet (27 vs. 24), but a lower effort lead to the highest total CPUE out of the three locations, 4.159. In contrast to Murrells Inlet, there was only one ray caught while all the other animals were sharks. The distribution of catches can be seen in figure 4. The catches were dominated by *R. terrenovae,* and constituted 89% of the catches. 42% of those were male.

North Inlet had the highest number of animals caught at 39, and the second highest CPUE, 3.253. The catches for North Inlet can be seen in figure 5. Both *S. tiburro*, and *R. terrenovae* were the most frequently caught at 48% and 31% respectively. Female *R. terrenovae* composed the majority of the catches (66%). All 19 of the *S. tiburo* caught were female. The remaining species sampled had only one male, *C. isodon,* between them.

Diversity

The results from two diversity indices used can be seen in table 3. The diversity for Garden City was the lowest at 0.79 and 0.42 for the Simpson's and Shannon-Weaver respectively. The other two locations had very similar results. Murrells Inlet had only 24 total animals caught, but they were between five different species. Murrells Inlet values were 0.32 and 1.28 (Simpson's and Shannon-Weaver respectively). North Inlet was dominated by two species, but there was a total of six species caught resulting in values of 0.34 and 1.28 (Simpson's and Shannon-Weaver respectively).

Variable Correlations

The CPUE for each location was compared physical factors in order to test for a significant relationship. The physical factors measured were water temperature (figure 6), dissolved oxygen (figure 7), visibility, (figure 8), salinity (figure 9), and tidal state (figure 10). To determine the correlation factor between each physical factor and CPUE, both a Speraman's Rho, and Kendall's tau-b were calculated. Due to the non-normal distribution of the data, these two methods were chosen over the Pearson method which assumes a linear relation between the variables.

Murrells Inlet only showed a significant correlation for one variable with CPUE. Water temperature was significantly correlated to CPUE by both the Spearman's Rho, and Kendall's tau-b methods (p=0.05). This is illustrated in figure 6 by a "hat" over the blocks of statistical significance. Despite the effort in the lower temperatures, the catches were the highest in the higher two blocks of water temperature. All other parameters for Murrells Inlet showed no significant correlation. Adjacent to Murrells Inlet, Garden City showed no significant relation between CPUE and any of the physical parameters.

North Inlet showed a correlation between one of the parameters and CPUE. There was a significant correlation between tidal state, and CPUE for both Speraman's Rho, and Kendall's tau-b methods (p=0.05). The statistically significant blocks are indicated by "hats" in figure 10. All other parameters however failed to have a significant correlation to CPUE.

Discussion

Shark usage of Estuarine Habitat

Elasmobranchs are dependent on estuaries as a nursery habitat (Bush 2002, Grubbs 2000, Gruber 1988). However, many coastal shark species in the Southeast United States are still poorly understood. The sharpnose shark, *Rhizoprionodon terrenovae* the finetooth shark, *Carcharhinus* isodon, and the bonnethead, *Sphyrna* tiburo are among the small coastal species that have been shown to reside in and around South Carolina estuaries (Castro 1993). It is not known if all the estuaries contribute equally to the populations, or a few of the most preferred habitats are selected over others (Gillanders 2002).

The most important estuarine function for sharks, is the separation of pups from parents (Branstetter, 1990). The most common predator on young sharks are adult sharks, both interspecies predation and intraspecies cannibalism are sources for juvenile mortality. Stomach samples from adult species yielded an occurrence of another elasmobranch species as often as 15% of the time (Branstetter, 1990). This idea of increased protection as being the main focus of an elasmobranch nursery ground was supported by Bush and Holland (2002). They concluded *S. tiburro* continued to reside in an area that was not providing the optimal amounts of food due to increased predation risks at other locations. Gilliam and Fraser (1987) also found that differences in foraging time are related to predation risk in teleost species. Increased chance of being eaten resulted in reduced foraging time. These studies indicate that predator avoidance is the primary factor when selecting for suitable habitat.

Physical Variables

The physical factors of each inlet seemed to be in line with the previous research that has been done in the area (Vernberg et al. 1992; Fulton et al. 1993; Kawaguchi, et al. 1997; Wirth et al. 1998; Lewitus et al. 2003; White et al. 2003) (See appendix A for contaminant results). Murrells Inlet and North Inlet have very similar values for salinity, DO, and temperature. This is due to the similar physical structures of the inlets. Murrells Inlet’s smaller size may however lead to more efficient flushing of the inlet explaining why the numbers are slightly closer to open water values. Garden City values closely mirror that of Murrells Inlet as well. This is also not surprising given Garden Cities close proximity to Murrells Inlet.

Effort

The amount of effort in Murrells Inlet is markedly higher than that of North Inlet and Garden City. There are several reasons for this: Garden City was not one of the primary sampling areas. However, the dynamics of Garden City prompted the desire to set lines in that area. But because it was not a primary sampling site, it did not receive as much attention as did Murrells Inlet. Weather also played a role in the sampling of Garden City. The placement of the jetties can cause for a great deal of bottlenecking in that area during certain times of the tidal state, and when the winds are coming from the East. This resulted in large swells that were difficult to pass. In the interest of boat and crew safety, the Garden City locations were forgone on several occasions. North Inlet required a pass to access the boating area. For a brief time the pass was inaccessible, and subsequently so was the boating slip.

Catches/Diversity

Table 3 illustrates that the similar diversity between Murrells Inlet and North Inlet when calculated mathematically. However, the systems are dominated by different species. Murrells Inlet is dominated in catches by *D. americanas*, with 46% of the catches coming from that species. The second highest catches were from *Gymnura micrura.* Almost 80% of the catches were of either ray or skate species. This indicates a low level of utilization by the shark species. There were only two species of shark caught in the inlet, *S. tiburro* and *G. cerratum*, the latter of the two only being captured once and very close to the mouth. North Inlet in contrast had a large percentage of sharks being caught, almost 85%, and relatively few rays. Whereas only five individuals of *S. tiburro* were captured in Murrells Inlet, 19 were caught in North Inlet. On paper the diversity of the two systems seems to be very similar; however, it is obvious that the species composition is in fact very different between the two locations. This has important management implications. Fisheries managers must realize that a complete species composition must be examined prior to any decision making.

Total catches between locations also varied greatly. Murrells Inlet had the lowest CPUE and was followed by North Inlet and Garden City. The Wilcoxon test indicated that both Garden City and North Inlet were statistically higher than Murrells Inlet. All results indicate that Murrells Inlet most likely does not act as a nursery ground.

These results indicate that a difference between Murrells Inlet and North Inlet does exist in regards to elasmobranch community structure. I propose that the differences in community structure stem from the amount of anthropogenic effects along Murrells Inlet, more specifically the large amount of boat traffic present. Two recreational boat slips along with a multitude of private docks allows access for a very large number of commercial and recreational boaters. I believed that this large amount of boat traffic is causing increased stress on the sharks that enter the inlet, and forces them to retreat to areas of lower disturbances. The inlets at low tide are shallow, and several of them become dry at low tide. A demersal species such as a shark would be very close to a boat prop as it cruised overhead. Large sharks commonly prey on smaller sharks, a passing boat could be perceived as a threat, or add stress to the animal. Animals will instinctively move to areas that afford them the greatest chance for survival (Gilliam and Fraser 1987). The areas of less stress in this case are the areas of deeper water. This causes them to move out of the inlet and around the jetties. This hypothesis is further supported by the high levels of catches around the jetties in Garden City. Jetties still act as a nursery ground in some sense such that the rocks will provide more shelter from predation than open water, and there is still an ample amount food source.

The animals in North Inlet were not subject to the increased boat traffic, and may explain why they remained in the preferred habitat. The biology of skates and rays makes them less susceptible to stress from the surface. Because rays are benthic, and substantially less affected by the heavy boat traffic, they are more likely to remain inside the inlet where food availability is higher. Defense strategies differ between families. Skates and rays will bury in order to hide, while coastal shark species flee.

Variable correlations

Despite all the variables recorded, CPUE was only correlated to one variable in each of the inlets. The catches in North Inlet were closely associated with the tidal state. Mid-tide in North Inlet can yield very high water velocities. During times of non-slack tide more energy is needed to swim against the current, or find suitable places to avoid high current speeds. During times of low tide there are more animals in a smaller area thus making it easier for predators to feed, and low water velocities require less energy for locomotion. Both Murrells Inlet and North Inlet have approximately the same tidal flux of about 1.4 m (White et. al. 2003), and because both inlets have the same time between slack tides (approximately 6 hrs), North Inlet's size will result in more water moving out per hour than will Murrells Inlet. This may explain why this same correlation is not evident in Murrells Inlet; the water velocity is slower at mid-tide. The feeding biology of rays is also differs from sharks, and may affect feeding times.

Murrells Inlet CPUE was correlated to water temperature. There were no animals caught below 21° C and as indicated in figure 6 the majority of the catches were in warmer waters (> 26°C). This trend was not however seen in North Inlet. North Inlet was not sampled until June, when the waters were beginning to warm.

Garden City conditions were much more constant and trends were difficult to determine. The water temperature never dropped below 21° C, and tidal effect was lessened because there was no bottleneck effect. Because only 20 lines were set, many of the blocks for each parameter did not have sufficient data points to be statistically significant. Garden City did not have the large range in variables that are characteristic of estuary systems, and thus graphic representations of all three locations can be misleading.

Implications

As has been previously discussed, the coastal Atlantic shark populations are in danger of becoming threatened, endangered, or if trends do not stop even locally extinct. The problems of over fishing, habitat degradation, and bycatch are magnified by the low reproductive potential of all elasmobranch species. Shark species are K-selected in that they grow slowly, have a late age of sexual maturity, and have relatively few offspring (Cortes 2000), thus more susceptible to fishing pressure than are teleost species. Because sharks are a K-selected species, a healthy nursery system is of greater importance than to teleost species capable of spawning every year to compensate for bad recruitment years. Once a pup is dropped, survival depends on finding suitable habitat. This is a reasonable hypothesis underlying selective breeding areas. Hoff and Musick (1990) has labeled this time of the young sharks life as being the most critical time for characteristics such as recruitment, survival and mortality.

Nursery areas are included under EFH regulations for all commercial fish stocks. The bonnethead, sharpnose, and finetooth are all subject to a gillnet fishery in the southeast United States (Carlson et al. 2003), and are thus eligible for EFH protection. The idea of EFH is designed around the principle of habitat protection. Under EFH, both fishing and non-fishing related activities are examined. These activities include: using stationary and mobile fishing gear, dredging and filling, agricultural runoff, direct vessel discharge, and the introduction of exotic species. This is a wide list of possible activities that could have adverse effects on the environment; however, there is no authority to regulate non-fishing activities or activities in state waters.

The idea of EFH, while hailed as a step in the right direction, has often been criticized for its inability force a change in behavior. The idea of EFH comes down to the difference between authority and responsibility, NOAA has the responsibility to regulate, but no authority to evoke change. Since EFH was first created it was accused of not having enough muscle behind it (Fletcher 2000). It is designed to act on all fishing and non-fishing activities; however, the regional fishery counsels only have the authority to act over the fishing activities.

A final obstacle that is not related to NOAA is the culture of the area. Murrells Inlet is a favorite location for recreational fisherman. Many of these sea goers are retired men who spend the vast majority of their time on the water. To tell these men that they are no longer allowed to fish in the area would be devastating to them. Through discussions at the docks and boat slips I have found that although they think this research is important, they are not willing to sacrifice their boat time, for increased shark stocks in the inlet.

There is the potential for shark nursery grounds to thrive in the northeastern inlets of South Carolina, but there is no federal legislation capable of protecting these areas. It has been shown that Murrells Inlet is very similar to North Inlet in physical characteristics. However, differences exist between elasmobranch diversity and community structure.

As shark populations continue to decline, all steps need to be taken to prevent their local extinction. These species control the food webs below them, and their loss could result in a top-down collapse of the system. This poses an interesting problem for management in that it would be virtually impossible to remove all the recreational fishermen from the area for prolonged periods. Economic use of the inlet is also very important for many seasonal businesses that rely on Jet Ski rentals or deep sea fishing ventures. Although seasonal closures, limited boating days or restricted use of the inlet should be examined, it is suggested that already protected areas such as North Inlet should receive increased attention to ensure their pristine nature.

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Figures

Figure 1:

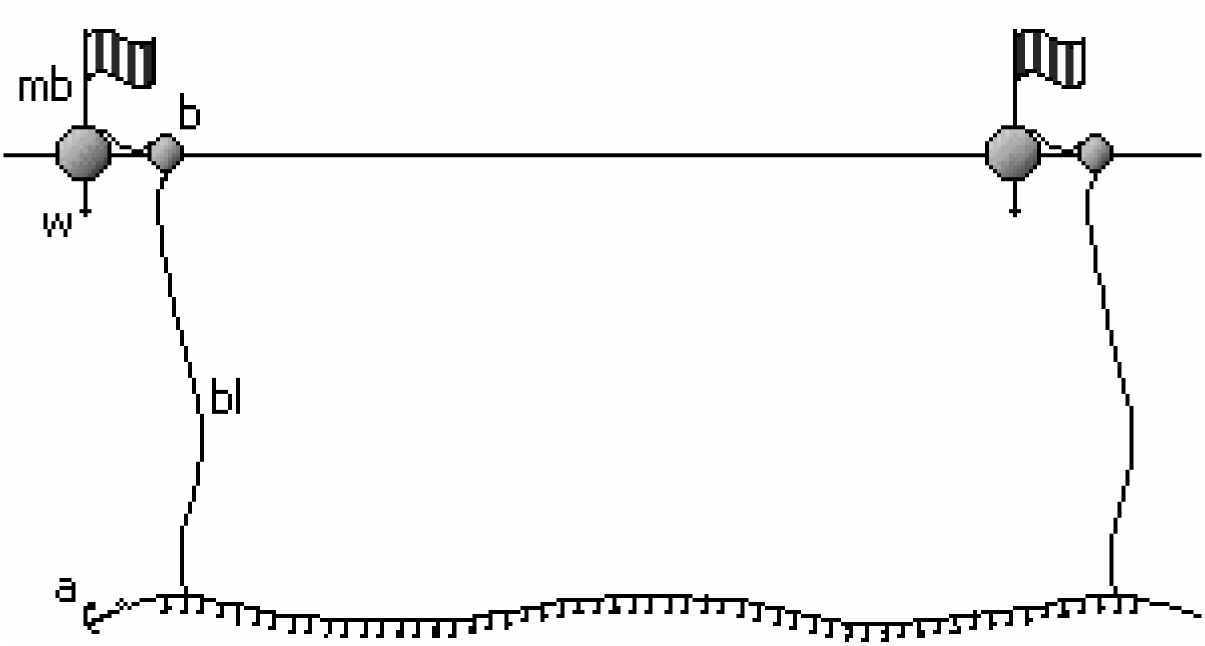
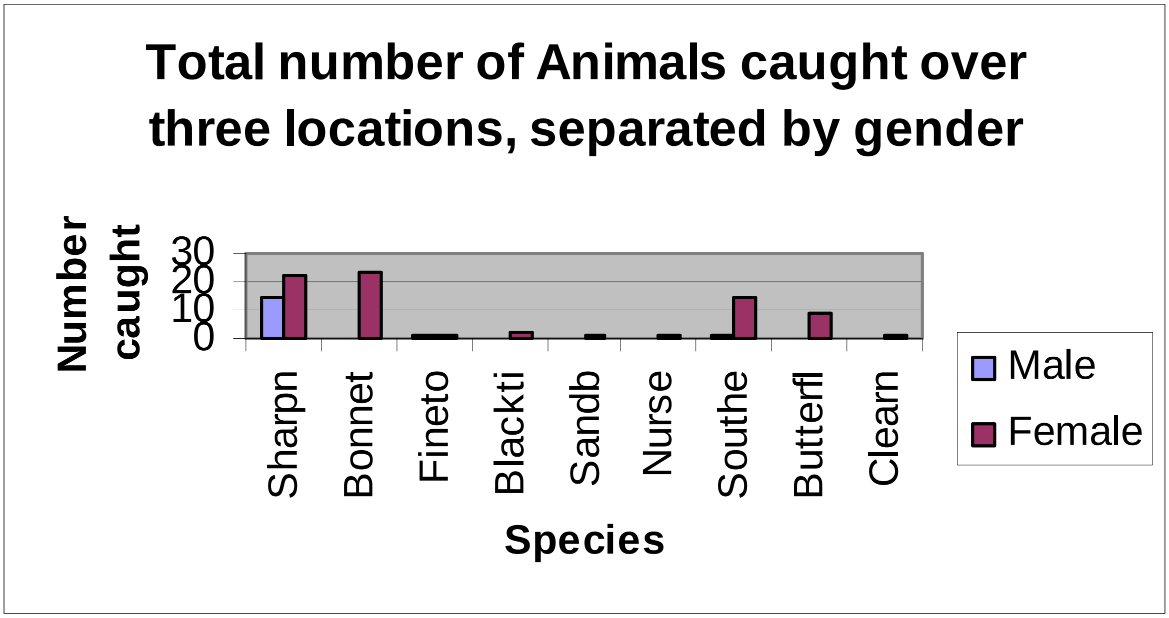


Figure 2:



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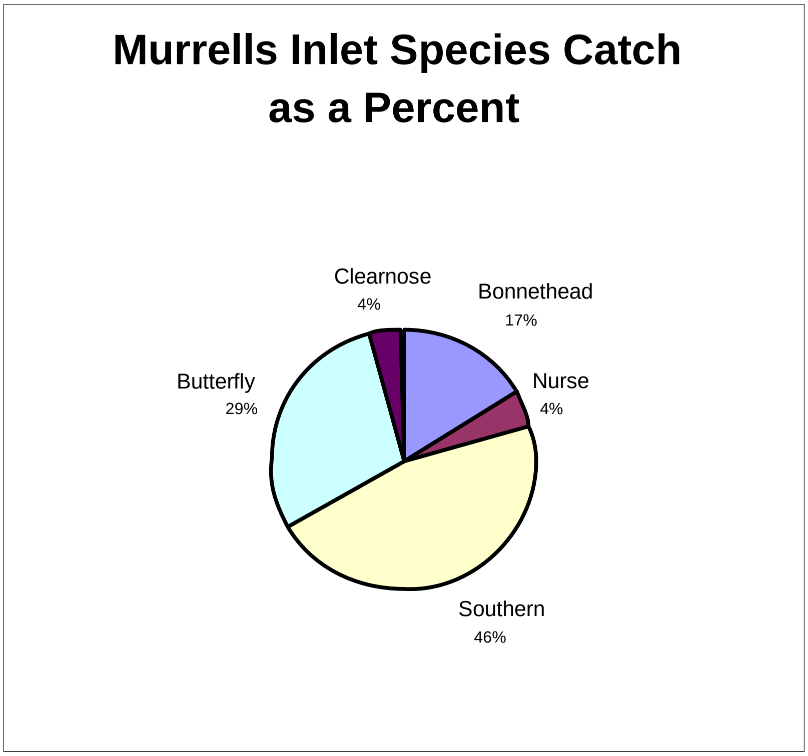

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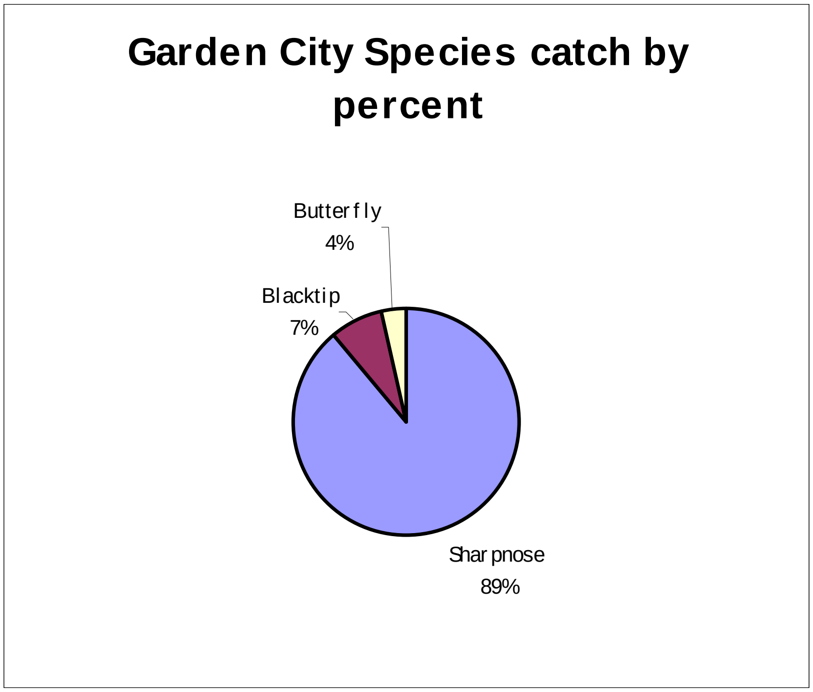

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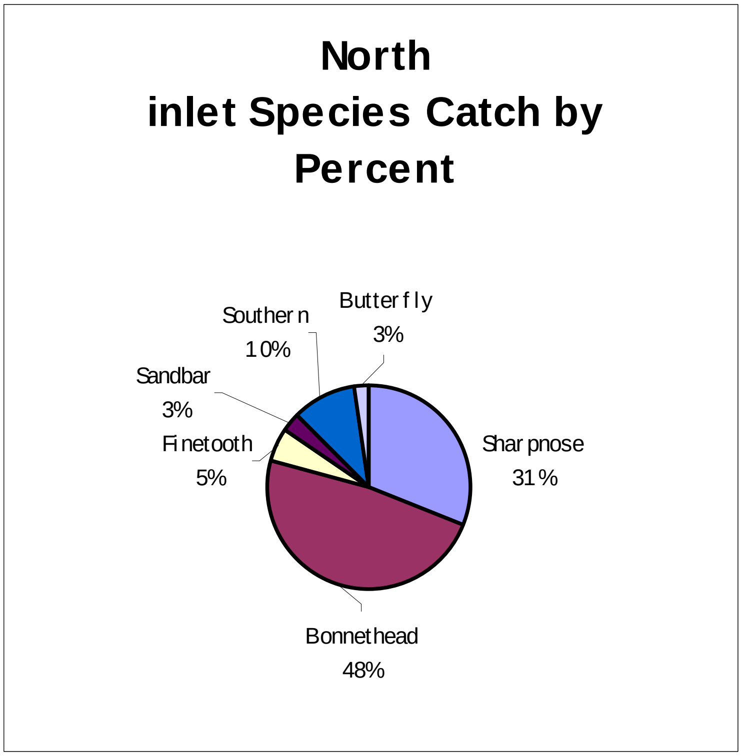

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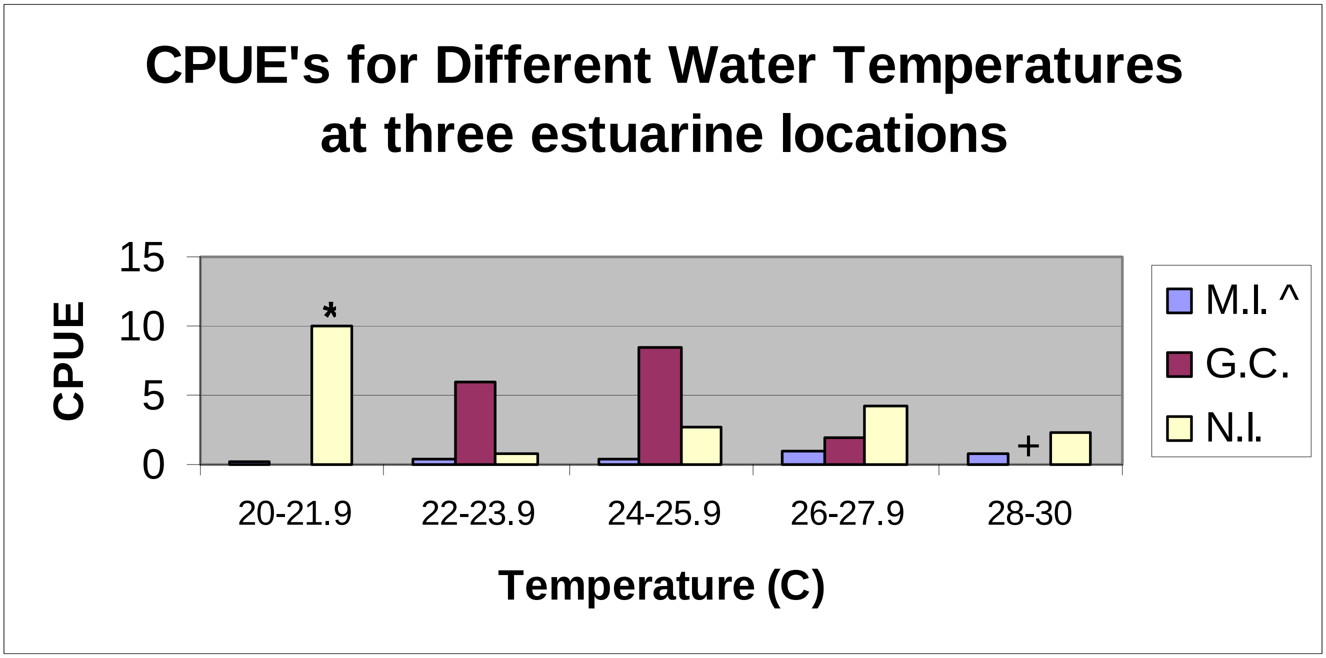

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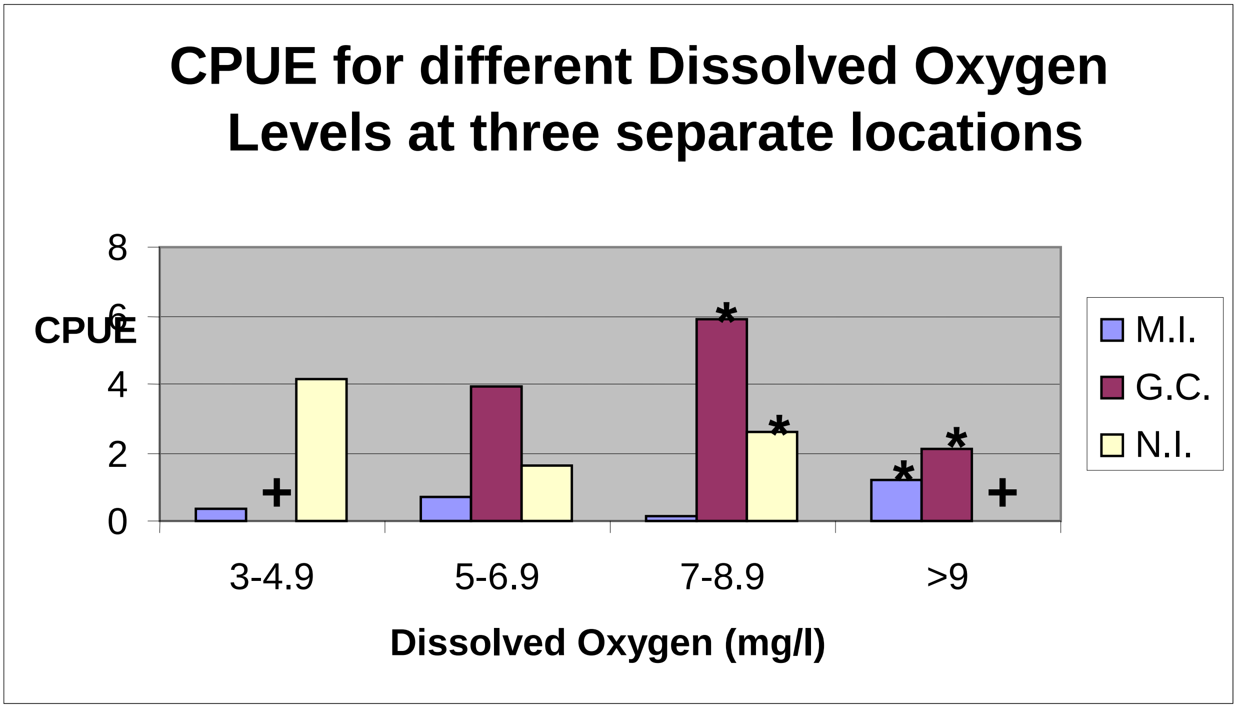

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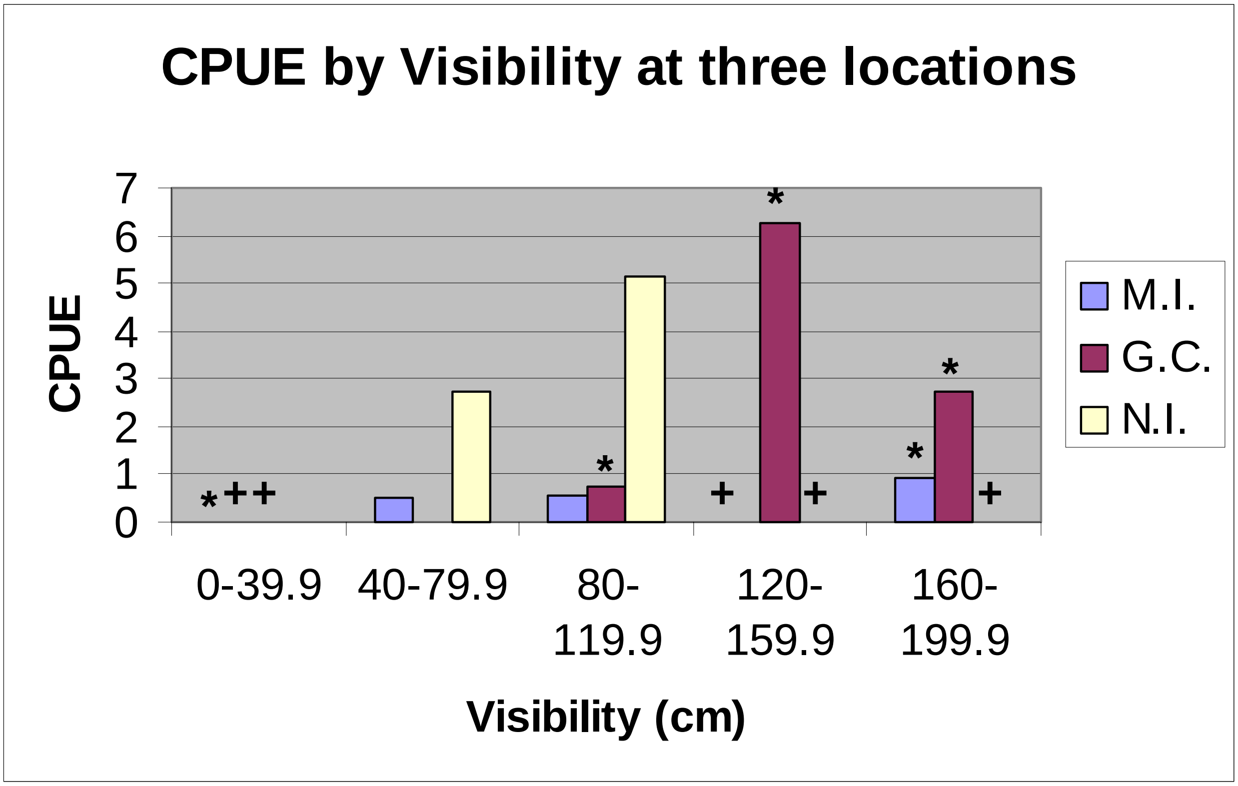

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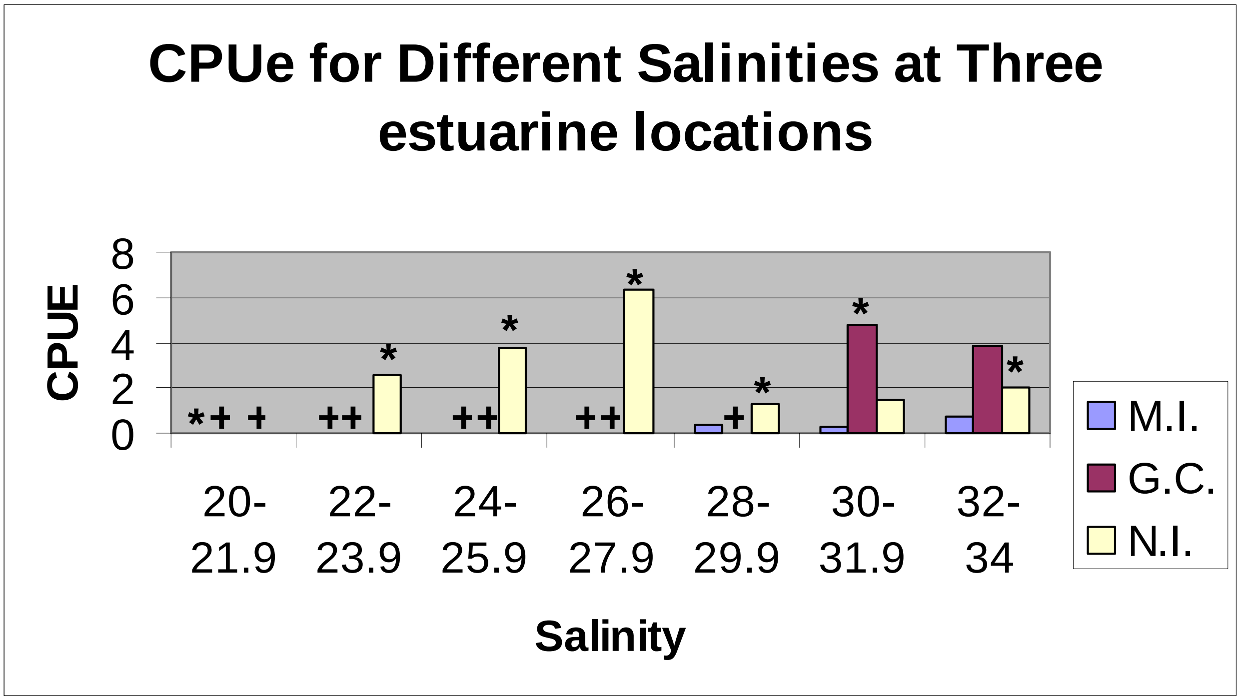

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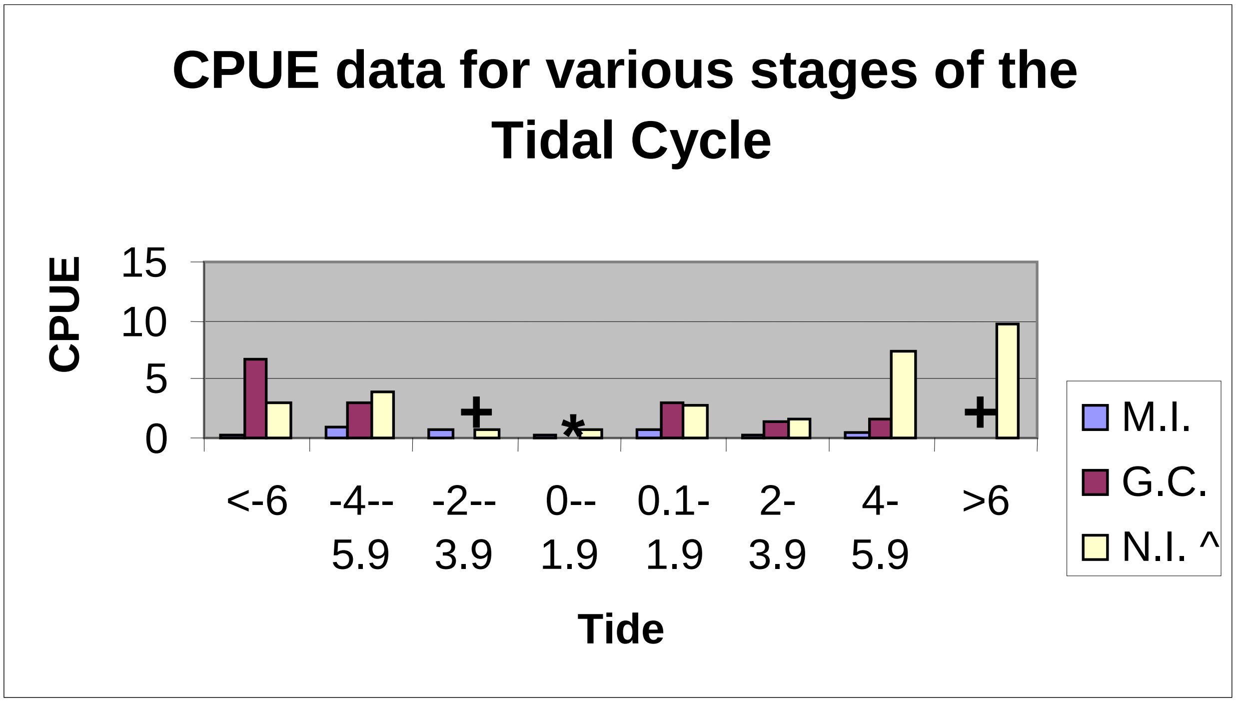

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Figure Captions

Figure 1: Illustration of a typical longline. Courtest of **www.savethealbatross.org.za/ longline.htm)**

Figure 2: total catches for all three locations sampled, separated by species and gender.

Figure 3: Catch composition of Murrells Inlet by percent species.

Figure 4: Catch composition of Garden City by percent species.

Figure 5: Catch composition of North Inlet by percent species.

Figure 6: CPUE for each location as it relates to water temperature (C°). "*****" indicates less than ten lines for that bin in a given location. "**+**" indicates no effort for that bin in a given location. "**^**" indicates a significant relationship between the variable and the CPUE for that location (Significance determined by Kendall's tau-b and spearman's Rho calculations).

Figure 7: CPUE for each location as it relates to dissolved oxygen (mg/l). "*****" indicates less than ten lines for that bin in a given location. "**+**" indicates no effort for that bin in a given location. "**^**" indicates a significant relationship between the variable and the CPUE for that location (Significance determined by Kendall's tau-b and spearman's Rho calculations).

Figure 8: CPUE for each location as it relates to secci disk depth (cm). "*****" indicates less than ten lines for that bin in a given location. "**+**" indicates no effort for that bin in a given location. "**^**" indicates a significant relationship between the variable and the CPUE for that location (Significance determined by Kendall's tau-b and spearman's Rho calculations).

Figure 9: CPUE for each location as it relates to salinity (ppt). "*****" indicates less than ten lines for that bin in a given location. "**+**" indicates no effort for that bin in a given location. "**^**" indicates a significant relationship between the variable and the CPUE for that location (Significance determined by Kendall's tau-b and spearman's Rho calculations).

Figure 10: CPUE for each location as it relates to tidal state. The x-axis is hours from high tide. Positive values indicate hours after high slack tide. Negative numbers indicate hours before high slack tide. "*****" indicates less than ten lines for that bin in a given location. "**+**" indicates no effort for that bin in a given location. "**^**" indicates a significant relationship between the variable and the CPUE for that location (Significance determined by Kendall's tau-b and spearman's Rho calculations).

Tables

Table 1:

Total Mean Values	Mean Depth	Mean Temp	Mean DO	Mean Salinity	Mean Visibility
Murrells Inlet	6.09	24.32	6.38	31.83	93.88
Garden City	11.5	24.9	6.64	32.3	148.46
North Inlet	6.8	26.14	5.12	27.78	74.28
Monthly Mean Values					
Murrells Inlet					
March	6.67	15.67	6.54	30.67	92
April	7.11	18.61	8.67	30.1	109.2
May	8.71	23.26	6.95	30.39	98.53
June	8.38	25.95	5.81	32.14	139.32
July	5.17	26.33	5.27	33.5	97.73
August	6.22	26.58	6.61	33.37	82.92
September	4.86	25.34	6.87	33.1	88
October	7.03	22.09	5.63	32.29	81.83
Garden City					
May	13.38	22	7.68	31.2	115.5
June	9.3	26.45	5.85	32.39	N/A
July	13.3	25.5	6.83	33.6	176.44
August	7.27	25.97	5.86	33.7	90
September	12.5	24.9	9.05	32.5	N/A
October	14.7	22.1	5.94	32.4	97
North Inlet					
June	9.23	24.4	5.02	27.21	86.4
July	7.28	26.92	4.84	27.28	62.16
August	3.93	26.17	5.84	29.48	75.19

Table 2:

Total Values	Lines	Hooks	Set Time
Murrells Inlet	135	1740	4963
Garden City	20	250	779
North Inlet	41	485	1640
Effort by Month			
Murrells Inlet			
March	7	60	201
April	11	125	401
May	35	520	1618
June	28	275	990
July	28	340	900
August	21	250	680
September	5	60	140
October	7	90	204
Garden City			
May	3	50	185
June	10	135	130
July	2	25	99
August	3	30	112
September	1	15	47
October	1	15	35
North Inlet			
June	10	115	409
July	20	250	772
August	11	120	459

Table 3:

	M.I.	*G.C.*	*N.I.*
Simpson's	0.326389	0.796982	0.346483
Shannon-Weaver	1.28	0.419	1.287

Appendix A

Polycyclic Aromatic Hydrocarbon (PAH) Levels present in *Rhizoprionodon terrenovae* gall bladders sampled from two South Carolina Estuaries

Introduction

Impervious surfaces from urbanization increase run-off and supplies the waters with increased nutrients. Holland et. al. (2003) showed that the ability of a creek to function as a nursery ground is severely degraded once the amount of impervious surface exceeds 20%. These anthropogenic effects can often be harmful to the system by resulting in toxic algal blooms, shellfish closures, fish kills and a decrease in water quality. Areas that are not completely flushed, these large blooms may result in anoxic conditions once the phytoplankton dies off and bacterial decay begins (Mallin 2000). This pollution and degradation of the environment is putting a large strain on the system and surrounding areas. Potentially harmful substances (i.e. heavy metals, polycyclic aromatic hydrocarbons, pesticides, herbicides) are exported from the estuaries to surrounding areas (Gillanders et. al. 2003). Habitat degradation and contaminant transfer greatly jeopardize estuarine functionality.

Increased urbanization of Murrells Inlet, SC has been linked an increase in polycyclic aromatic hydrocarbons (PAH's) (Vernberg et. al. 1992; Fulton et. al. 1993 and Wirth et. al. 1998), low iron availability (Kawaguchi et. al. 1997 and Lewitus et. al. 2003), bioaccumulation of toxins in invertebrates (Fulton et. al 1993; Wirth et. al. 1998 and Vernberg et. al. 1992) and a decrease in macropelagic fauna (Vernberg 1992).

PAH's are byproducts of both anthropogenic and environmental decomposition (Wirth et. al. 1998). They enter estuarine systems by storm water runoff and have serious effects on the system. Levels were found to be significantly higher in Murrells inlet than in North Inlet (28.05 ng/L to 0.83ng/L respectively Fulton et. al. 1992). Wirth et. al. (1998) showed a reduction in grass shrimp of 90% in areas with high PAH levels. Wirth *et al.* (1998) also showed that increased PAH levels affect the reproductive cycle of copepods. Mallin et. al. (2000) concluded that copepods are essential for the distribution of energy through the food web. A decrease in fecundity of the copepod, in conjunction with a decrease in grass shrimp populations (another potentially important prey species) predatory species will be affected.

PAH level and type is characteristic of specific activities. The heavier substances will settle out and bind to the sediment (Baumard et al. 1998). Escartin and Porte (1999) found differences in both concentration and type of contaminants in the tissues of two species of fish, the red mullet, *Mullus barbatus*, and the sea comber, *Serranus cabrilla*. *S. cabrilla* feeds more along rocky substrates on small fish and thus had PAHs of a lower molecular weight than did *M. barbatus*, whch tends to feed along sandy or muddy substrate

The purpose of this project was to examine the PAH levels in *R. terrenovae* from Murrells Inlet, SC (urbanized) and North Inlet, SC (pristine). Based on previous research (Vernberg et. al. 1992; Fulton et. al. 1993 and Wirth et. al. 1998), the levels from specimens in the Murrells Inlet area are expected to be greater than those from North Inlet.

Materials and Methods

A total of nine sharpnose sharks, *R. terrenovae*, were dissected to remove the gall bladders and test bile metabolites for PAH substances. Five animals were removed from

North Inlet, and four from Garden City Beach, adjacent to Murrells Inlet. Due to the lack of sharpnose catches within Murrells Inlet, no samples were taken from that location. Because the contaminant reaching the pups removed from Garden City originated from Murrells Inlet, these pups are referred to as having come from the Murrells Inlet area. To prevent damage to the gall bladder during dissection, surrounding liver tissue was also removed. Samples were immediately placed on ice and transferred to -80° C conditions.

Prior to homogenization of the samples all glassware was triple rinsed in methylene chloride ($MeCl_2$) or acetone. Tissue samples weighing 4-5 grams were placed in centrifuge tubes along with 15 ml $MeCl_2$, 5g sodium sulfate and 25 µl of a 50 µg/µl polystyrene /$MeCl_2$ solution. The samples were then homogenized manually and using a electronic tissue homogenizer for approximately two minutes. The samples were centrifuged at 2500 rpm for five minutes and then decanted. An additional 15 ml of $MeCl_2$ was added to the original sample an the procedure repeated. The samples were then condensed to 5ml by gentel heating. 0.75 g of Attagel 40 was added, and vortexed, to remove lipids from solution. The solution was centrifuged for five minutes at 3000 rpm, and then decanted. The remaining solution was then condensed to 1 ml via gentle heating. This final solution was used for high performance liquid chromatography (HPLC) to determine the presence of PAHs.

A size exclusion chromatography column, consistent with Hufnagle *et al.* (1998), was used to separate out the sample components. The HPLC system included a 2µm prefilter, a 50 x 7.8 mm Phenomenex Phenogel guard column, one 300 x 7.8 mm Phenomenex Phenogel 5, 100 Å size exclusion column and two Perkin-Elmer LS 40 Fluorescence detectors. Three sets of excitation/emission wavelengths were used to capture the full array of potential PAH substances: 260/380 nm wavelengths were designed to capture the presence of phenanthrene and alkylphenanthrenes. 290/335 nm wavelengths were capable of indicating the presence of naphthalene, alkyl-napthalenes, dibenzothiophene and alkyl-dibenzothiophenes. 380/430 mm were used to detect pyrene, alkyl-pyrene, fluoranthene and alkyl-fluoranthenes. Samples of 200 µl were run at a speed of 2ml/min and allowed to run for fifteen minutes. Absorption versus time results were plotted in Excel©. Areas under the curves were determined to establish the amount of toxin present in each sample. Mean values for each of the sample locations was calculated and used to make a qualitative assessment about the amount of elasmobranch exposure in each location.

Results

Species caught in Murrells Inlet showed a larger concentration of PAH’s at all three wavelengths than did those sampled from North inlet. Figure 1 illustrates these differences for the excitation/emission levels of 260/80 and 290/335. Figure 2 shows the differences for the wavelengths of 380/430. The two lighter weight PAH categories, NPH and HPN, were the most common found. BaP substances were very limited in both systems.

There were also variations in the types of compounds present. The different PAH’s could be determined as exclusive or shared based on the retention time of the sample. Due to the size separation of the column, each individual PAH was recorded at a specific time. The times could then be compared between sites to check for similarities. In several of the samples there were PAH compounds that were present in Murrells Inlet extracts that were not present in any from North Inlet. Likewise, North Inlet had four contaminants that were exclusive to that location.

For the wavelengths of 260/380, a total of six PAH's were found (Figure 3 and 4); each location had samples that contained at least one, and as many as four of the contaminants, and two were found in both locations. Samples run under 290/335 showed a total of five contaminants. Each sampled showed at least one contaminant present, and one sample ran contained four. Two of these were exclusive to Murrells inlet, while one was found only in North Inlet. The remaining two were found in both locations. Differences are illustrated in figure 5 and 6. The final wavelengths of 380/430 only yielded three different contaminants (figure 7 and 8); each sampled yielded the presence of two PAH's. One of the three was shared between the two sites, and each site also had a unique contaminant.

Discussion

The results from the toxicology analysis clearly indicate that there is a higher concentration of PAH's in Murrells Inlet than are in North Inlet. This finding is supported by previous research (Marshall et al 1996; Wirth et al. 1998; Fulton et al. 1993; Vernberg et al. 1992). The higher concentration levels in the tissues are most likely a direct result of the urbanization of Murrells Inlet. Highway 17, a major highway of eastern South Carolina runs parallel to the coast and is located less than one mile west of the inlet. The entire Northeastern beach is covered with hotels, condominiums, and multiple public boat docks allow for a variety of boats to come and go as they please. These roads and building increase the amount of impervious surface and allow for greater runoff into the system. The low levels of PAH's in North Inlet however is a direct result of the lower amount of anthropogenic disturbance. Because PAH's are a naturally occurring substance that can be the direct result of organic decay and microbial processes (Ruddock et al. 2000; Escartin et al. 1999; Baumard et al. 1998; Wirth et. al. 1998; Fundamentals of Aquatic Toxicology), some levels were to be expected. But as hypothesized the levels were substantially lower than those of an urbanized site.

The presence of the lighter compounds in the bile extracts is was expected. The heavier compounds will settle out and become associated with the sediment where they are not easily incorporated into the food web. *R. terrenovae*, being a top predator, does not spend any time in the sediment, and only limited time foraging there. Stomach samples taken from the species sampled indicated a 100% composition of teleost species in filled stomachs. This indicates that the sharks are feeding higher on the trophic level, and are subjected to a higher contaminant load via bioaccumulation. Previous studies have also suggested that the lower level food sources have a greater chance of accumulating toxins and thus passing them up the food web (Bauamrd et al 1998; Marshall et al. 1996). Studies have also indicated that the amount of a contaminant present in the tissue is not only a function of age, but also on feeding behavior, trophic level, and habitat (Escartin et al. 1999; Baumard et al.1998). It is unknown if the adults will have a higher concentration in their tissues, but several studies have shown that fish have a higher metabolism for PAH's and could thus excrete more than they would accumulate (Orbea et al. 2002; Escartin et al. 1999; Baumard et al. 1998; Bryan 1979) depending on the fat content of the tissues. A higher metabolic rate doe not however indicate that there is any less danger or potential threat posed to the animals.

The effects of these toxins in the tissues of the animals are not yet known. PAH's are lipid soluble and thus would tend to accumulate in areas of high fat content (Bryan 1979). The high oil content of shark livers would make them an ideal location for the accumulation of PAH's. Muscle tissue of several crustacean species has also been shown to hold PAH's and would thus be a plausible location to test (Hufnagle et al. 1998). Further studies should include these areas as well as include specimens from all across the life cycle.

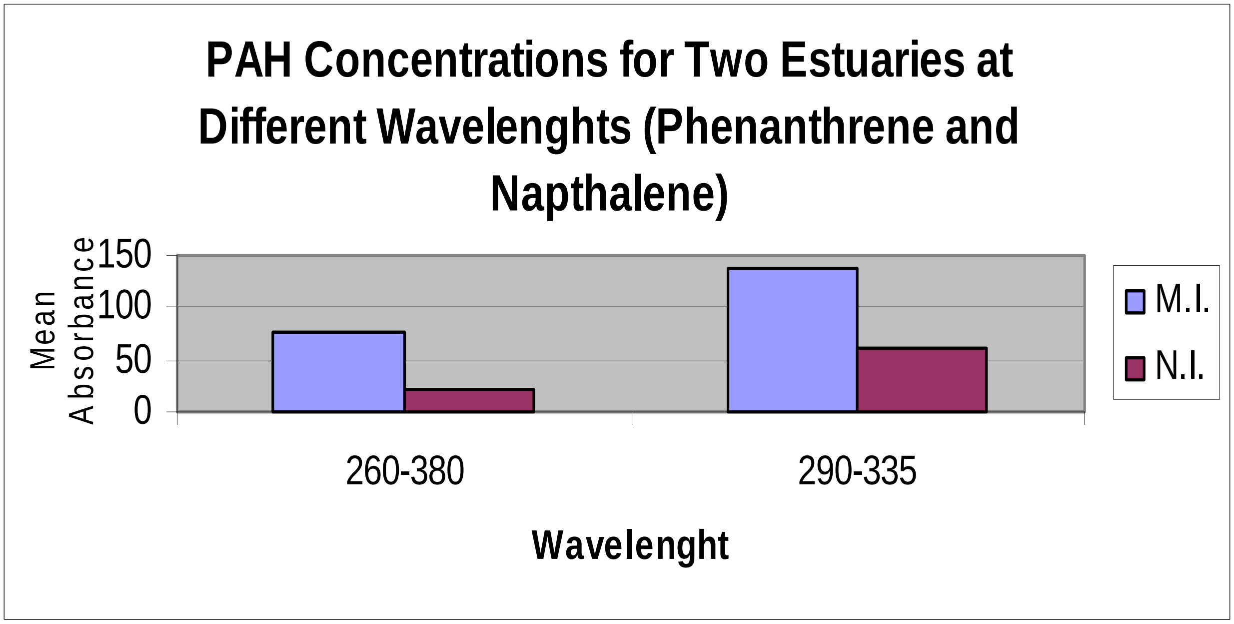
The specimens captured were young of year as was indicated by an open umbilical scar. Therefore any contamination load could have been delivered by the mother through the placental interaction. This however in unlikely since the females spend a large portion of their time offshore, and would thus not be subject to high levels of PAH's that are present in estuaries. Contaminant uptake most likely occurs after birth via uptake of food sources.

The conclusion from this analysis indicate that shark pups of the species *R. terrenovae* do uptake PAH's at a very early stage of their life. This is most likely due to the uptake of food sources and not from maternal interactions as indicated by the different contaminant levels between sites. The location of the PAH's in the gall bladder indicate that they are being metabolized and excreted through the feces (Orbea et al. 2002; Escartin et al. 1999; Baumard et al. 1998; Bryan 1979) . This suggests that there is not a high amount of bioaccumulation in the tissues, but future studies should test this hypothesis on older sharks. The effects of increased PAH levels has not been evaluated in sharks. Marshal *et al.* (1996) showed a preference for teleosts to feed on less contaminated prey items. PAH levels could act as a deterrent for species to enter

Murrells Inlet. Future studies should be directed at the effects of PAH levels on elasmobranch biology and behavior.

Figures

Figure 1:



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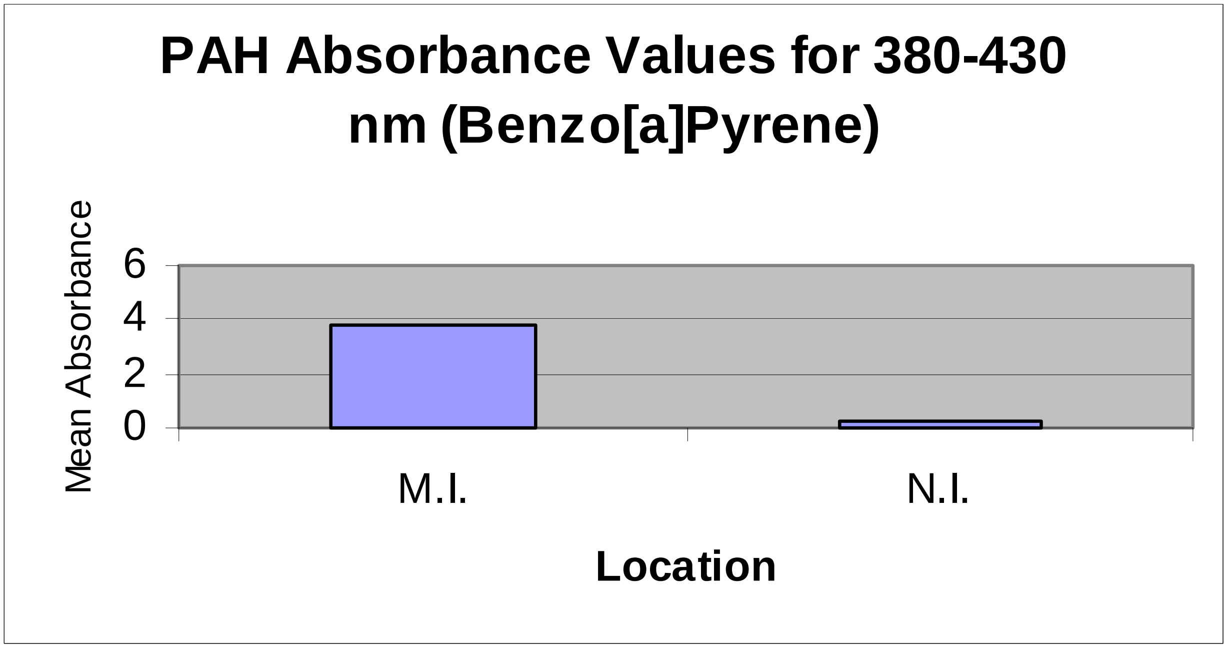

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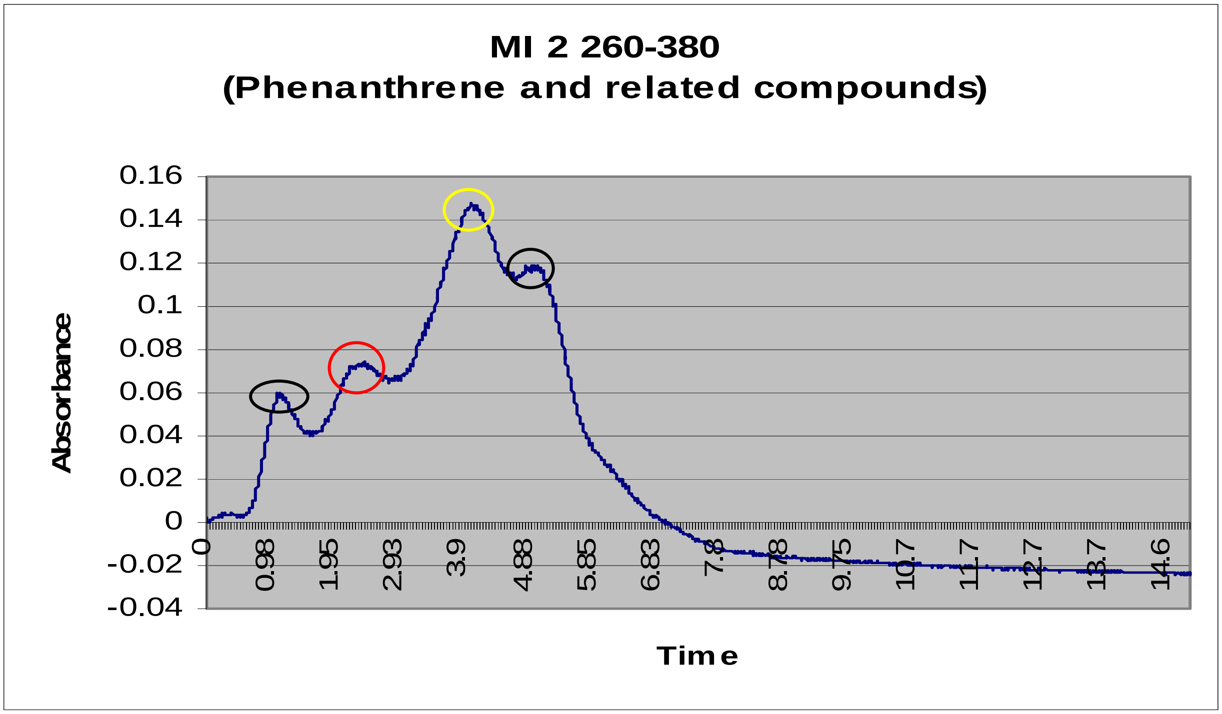

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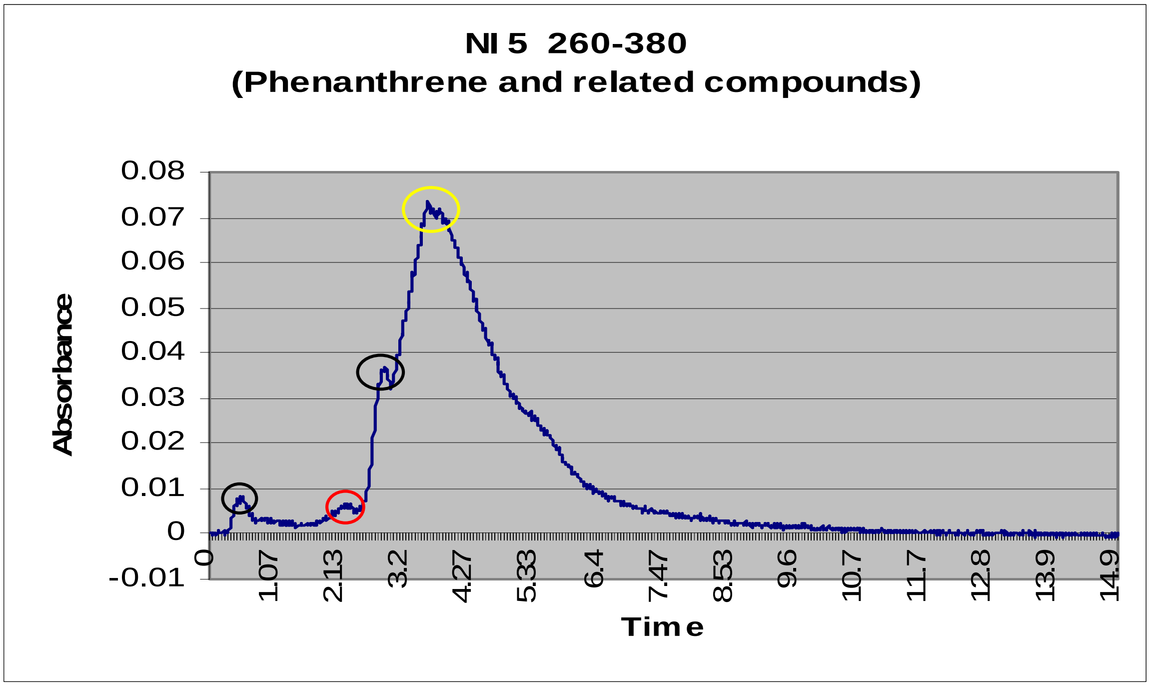

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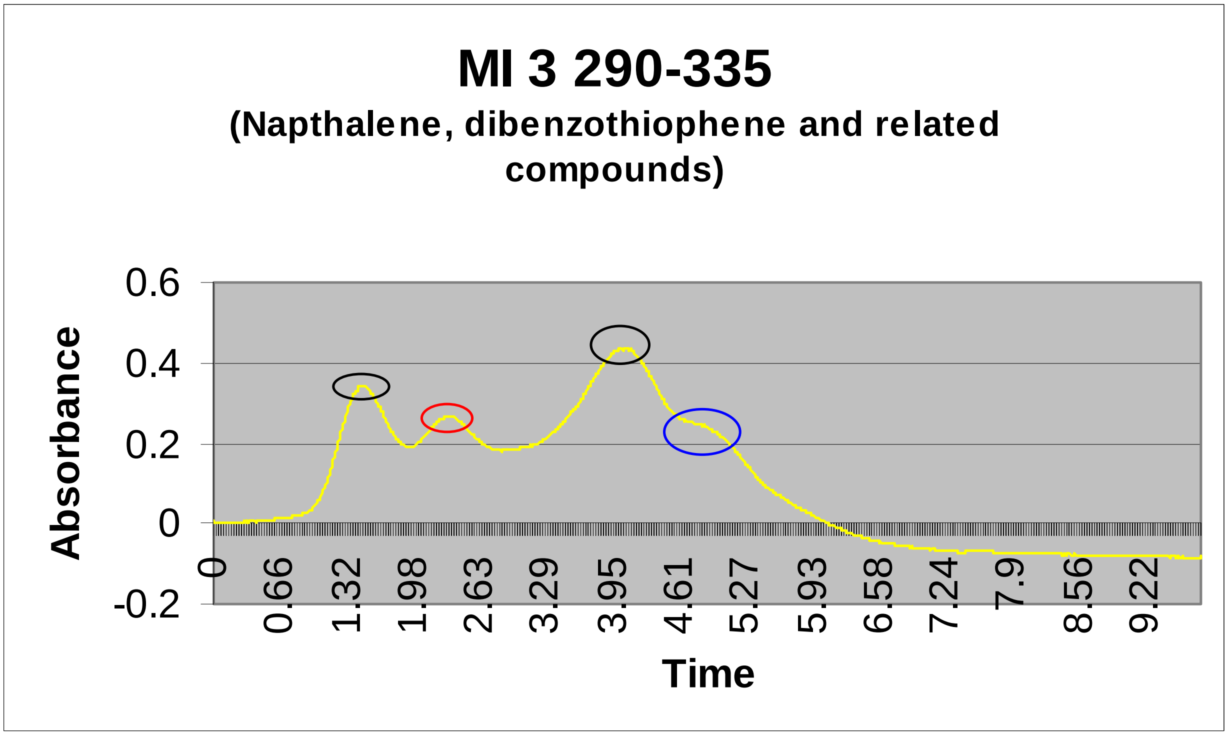

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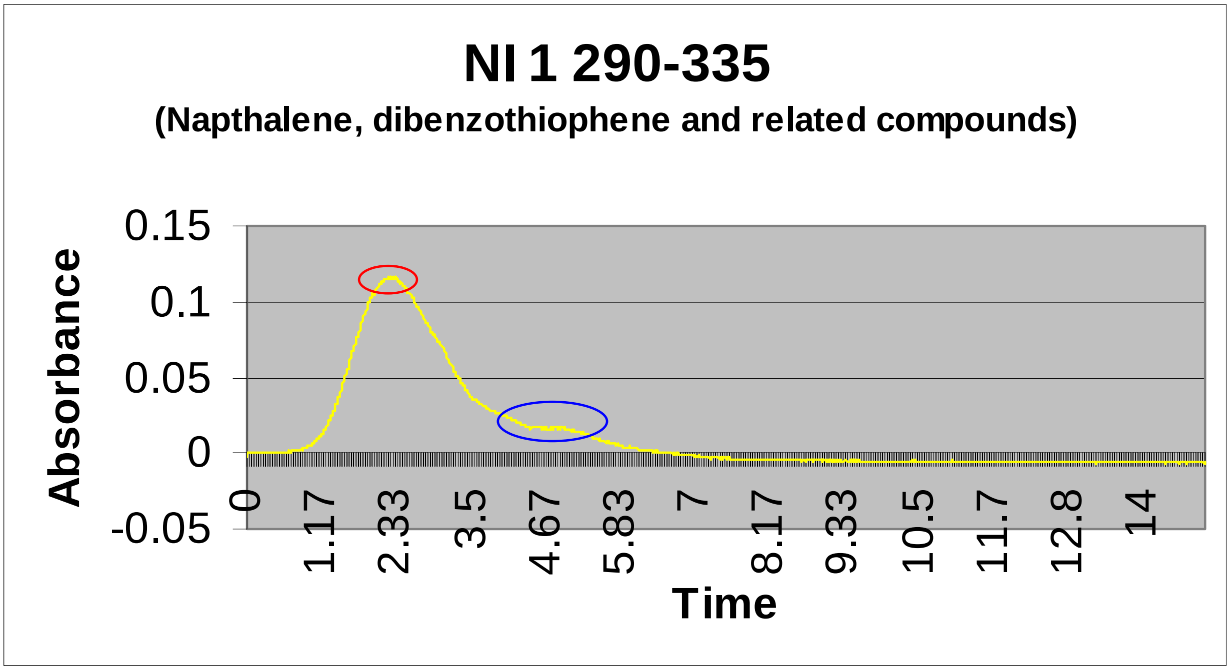


Figure 7:

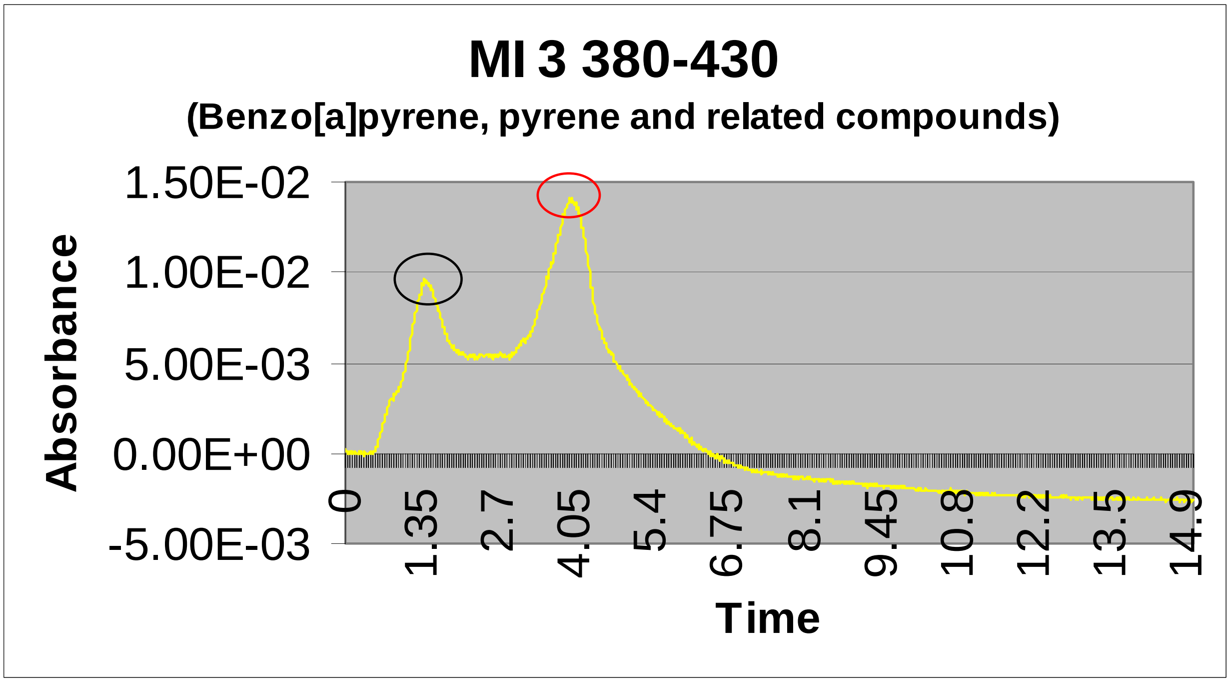
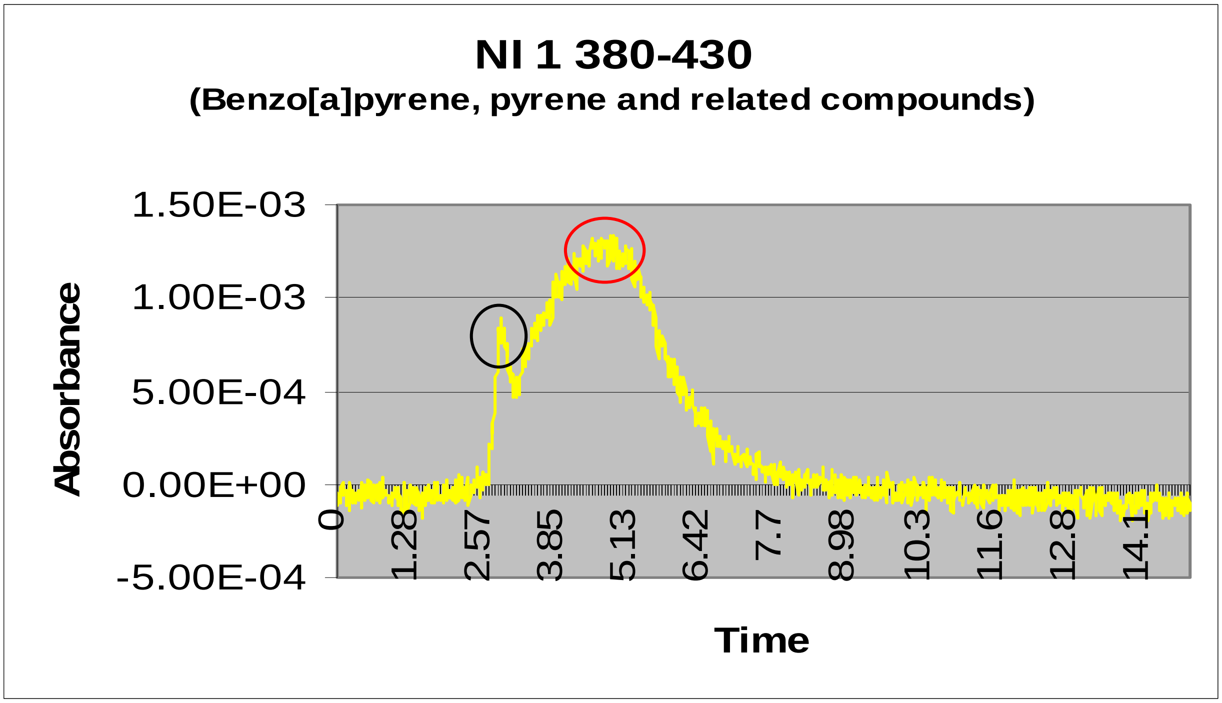


Figure 8:



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