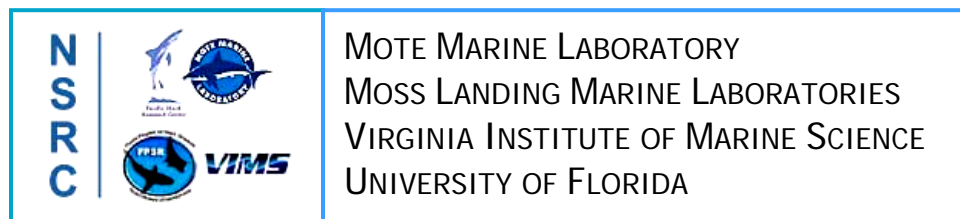


HIGHLY MIGRATORY SHARK FISHERIES RESEARCH
BY THE
NATIONAL SHARK RESEARCH CONSORTIUM
2002-2007



FIVE-YEAR TECHNICAL REPORT
TO NOAA/NMFS



MOTE MARINE LABORATORY TECHNICAL REPORT NO. 1241

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EXECUTIVE SUMMARY

The National Shark Research Consortium (NSRC) is a scientific collaboration comprising four leading shark research organizations in the U.S.: the Center for Shark Research (CSR) at Mote Marine Laboratory (MML), Sarasota, Florida; the Pacific Shark Research Center (PSCR) at Moss Landing Marine Laboratories (MLML), Moss Landing, California; the Shark Research Program (SRP) at the Virginia Institute of Marine Science (VIMS), Gloucester Point, Virginia; and the Florida Program for Shark Research (FPSR) at the University of Florida, Florida Museum of Natural History (UF/FMNH), Gainesville, Florida. Consortium projects involve NOAA/NMFS-related research and educational activities required for assessing the status of shark stocks, managing U.S. shark fisheries, and helping the U.S. take the leading role in worldwide conservation and management of shark populations. Fisheries-relevant multi-regional research is conducted on shark and ray populations in the Gulf of Mexico and Atlantic and Pacific Oceans, including collaborations with independent, academic and government institutions located along the U.S. Gulf, Atlantic and Pacific coasts and in many foreign nations. The primary objectives and service to the public of projects conducted by the NSRC are to: 1) conduct and advance basic and applied scientific research on sharks and their relatives, the skates and rays; 2) communicate scientific research and educational information about sharks, skates, and rays; 3) provide scientific information on sharks, skates, and rays to public policy makers; 4) expand scientific cooperation in national and international issues involving sharks, skates, and rays; and 5) increase public understanding of the biology and importance of sharks, skates, and rays. In its first five years of operation (2002-2007), the NSRC conducted more than 120 projects, produced more than 360 publications, theses and reports, organized or participated in more than 100 conferences and workshops, trained more than 100 graduate and undergraduate students, pioneered innovative research techniques and technology, and provided critical data for U.S. stock assessments and worldwide surveys of the status of shark, skate, and ray species.

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NARRATIVE REPORT

MOTE MARINE LABORATORY CENTER FOR SHARK RESEARCH

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1a. Relative Abundance.

1a1. Large Coastal Shark Surveys and Associated Data Analysis.

Objectives. Surveys for large coastal sharks inhabiting the Florida Gulf coast were initiated by the CSR in 2001, and continue to the present. The main objective is to collect fishery-independent catch data to estimate relative abundance and species composition of large coastal sharks. These surveys were designed in a standardized and random-stratified fashion such that CPUE results are comparable for the analysis of historical trends.

Methods. This work was largely accomplished with nearshore-to-offshore (out to approx. 25 miles) fisheries-independent surveys for large coastal sharks aboard Mote's 50-foot research vessel, the *R/V Eugenie Clark*, other Mote research vessels, and the Florida Institute of Oceanography vessel *R/V Suncoaster*. These surveys were conducted along the southwest Florida coast from Tampa Bay to Sanibel Island and utilized two different gear types (longline and single hook drumline) to catch large coastal sharks.

Single hook drumlines consisted of a cement block anchor attached to 20-40 m of line (depending on water depth) running to a surface float, and a 30 m heavy monofilament gangion (800 lb test) secured to the bottom anchor by a swivel and terminating with a baited circle hook (18/0). Individual drumlines (10-20) were set approximately 1 km apart and allowed to soak for 2 to 4 hours before being checked for sharks and/or rebaited. Drumlines were ideal for catching relatively large sharks, by minimizing bycatch and providing higher survivorship as they permitted the hooked shark to swim in circles around the anchor. Longlines consisted of 80-120 hooks (9/0 J or 18/0 circle), 3 m gangions with a 1 m leader (stainless steel or monofilament) and a 1.6 km mainline. Sets made during 2002 and 2003 used stainless steel leader material in order to compare results with those of the Virginia Institute of Marine Science shark research program, but a change was made to monofilament gangions in early 2004, to improve catch rates. Longline

gear was typically soaked for about 4 hours. All live sharks were tagged with CSR dart tags and/or fin tags (such as rototags) before release; some sharks were double-tagged with NMFS-style tags as appropriate for the size of shark.

For a preliminary analysis of the catch rate per year, the number of sharks caught in each set for every year was converted to CPUE. CPUE for the longline sets was calculated by dividing the number of animals caught by the hook-hours (number of hooks used multiplied by the total soak time), and CPUE for the drumline sets was calculated by dividing the number of animals caught by the soak time of the drumline. CPUE data were standardized using the natural logarithm of the CPUE + 1 before being analyzed. Regression analyses of the mean annual catch rates for both gear types were used to assess if there has been an increase or decrease in the annual catch rates.

Outcomes and Significance. Between June 2001 and June 2007 (i.e. beginning one year before the start of this project and then continuing through the five years of the project), 72 longline sets (6,729 hooks) and 1,920 drumline sets (1,920 hooks) captured a total of 696 sharks comprising 11 species. The blacktip shark (*Carcharhinus limbatus*) was the most commonly encountered species comprising nearly 22% of the catch, followed by the nurse shark (*Ginglymostoma cirratum*), spinner shark (*Carcharhinus brevipinna*), blacknose shark (*Carcharhinus acronotus*) and sandbar shark (*Carcharhinus plumbeus*) (Table. 1). Approximately 78% of all sharks captured were tagged and released.

Species	No. of Sharks	Percent
<i>Carcharhinus limbatus</i>	151	21.70
<i>Ginglymostoma cirratum</i>	134	19.25
<i>Carcharhinus brevipinna</i>	169	24.28
<i>Carcharhinus acronotus</i>	80	11.49
<i>Carcharhinus plumbeus</i>	83	11.93
<i>Carcharhinus leucas</i>	48	6.90
<i>Negaprion brevirostris</i>	14	2.01
<i>Sphyrna mokarran</i>	11	1.58
<i>Rhizoprionodon terraenovae</i>	2	0.29
<i>Galeocerdo cuvier</i>	2	0.29
<i>Sphyrna lewini</i>	2	0.29

Table 1. Catch composition of shark species captured during large coastal shark surveys using longline and drumline sets from 2001 to 2007.

For the large coastal shark complex, the catch rates for both longline and drumline gear did not demonstrate a clear increasing or decreasing trend (Figs. 1 and 2). However, a more rigorous analysis will be performed using a general linear model (GLM) for both gear types to determine if there are differences in catch rates between locations, months (seasons), years and species. Nominal and standardized catch rates and other data from these fishery-independent surveys were used by NMFS in the stock assessment for the Large Coastal Shark Complex (2006) and for the Small Coastal Shark Complex (2007) (see section 1a4).

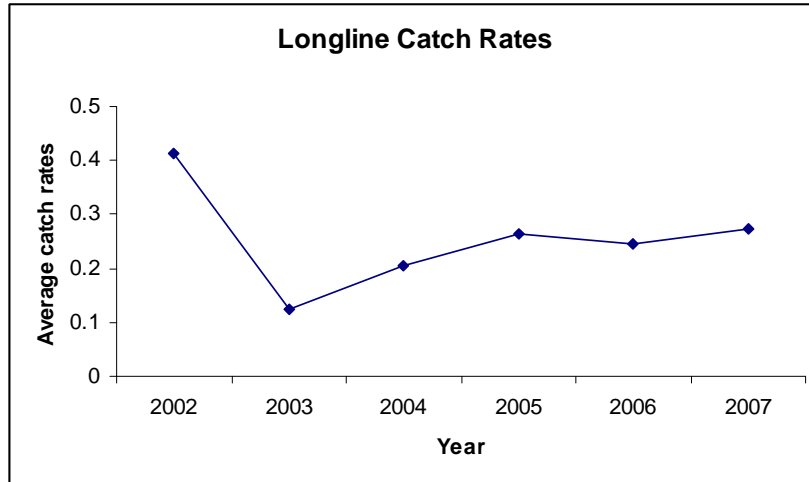


Figure 1. Average catch rates of combined large coastal shark species from longline sets.

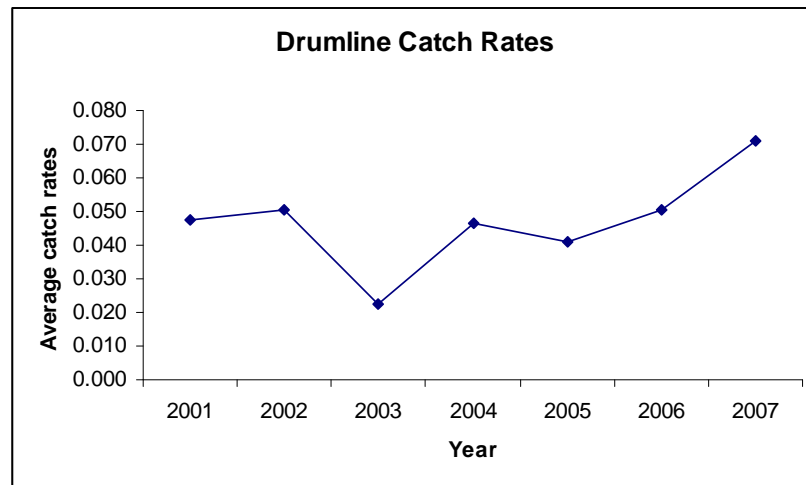


Figure 2. Average catch rates of combined large coastal shark species from drumline sets.

1a2. Blacktip Shark Relative Abundance Surveys.

Objectives. Blacktip sharks are one of the two most important species in the U.S. east coast shark fishery, the other being the sandbar shark. Given the paucity of fishery-independent sources of shark abundance data, the CSR built upon its shark nursery studies originating in 1991 by conducting standardized gill-net surveys in blacktip nursery areas providing relative abundance data for juvenile blacktip sharks as well as other species such as the bonnethead (*Sphyrna tiburo*). The primary objective of these repeated yearly surveys was to determine if the relative abundance of blacktip pups has changed since the initial CSR abundance surveys were conducted (1995-1997), in order to gauge the status of the blacktip shark stock and evaluate the results of changes to regulations in the shark fishery. The tagging of live sharks during these surveys fulfilled the secondary objective of gaining further valuable data on the movement and migration patterns as well as life history information on these sharks.

Methods. Field trips focused on two nursery areas in the Florida Gulf (Charlotte Harbor/Pine Island Sound and Yankeetown) in efforts to provide an estimate of relative abundance of blacktip shark neonates, young-of-the-year (YOY), and other juveniles in these nurseries. In each of these areas, CSR field biologists conducted one quantitative survey once per month during the periods of July-September 2002, May-August 2003, and May-August 2004. These standardized, random-stratified fishery-independent surveys were conducted in a fashion identical to previous CSR studies of the same areas since 1995. Each survey comprised ten gill-net sets in specific sites randomly selected from the established grid areas of each nursery. These quantitative sets were made for one hour (first mesh in to last mesh out) and utilized a 400 yd x 10 ft x 4⁵/₈" SM weighted net composed of #208 (0.52 mm diameter) monofilament. All sharks captured were identified, sexed, measured and weighed and live sharks were tagged with CSR dart tags and released.

Outcomes and Significance. A total of 220 relative abundance gill-net sets were conducted resulting in the capture of 2,987 sharks of five species including 1,393 blacktip sharks. Of the blacktip sharks captured, 863 were tagged and released. The data from these efforts were analyzed and compared with previous data collected from these same locations to identify any population changes. Overall, 970 quantitative gill-net sets were made between 1995 and 2004 resulting in the capture of 8,257 sharks of thirteen species, of which 3,842 were juvenile blacktip sharks. A general linear model was used to standardize the log-transformed CPUE data. Significant differences in the juvenile blacktip catch were found between years, nursery areas, and between grids within a nursery area. When looking at the blacktip catch rates over the entire 10-year period (Fig. 3), there were no apparent trends that would be indicative of population level changes despite stock assessments indicating an increasing abundance of blacktip sharks in the U.S. Atlantic and Gulf of Mexico since the mid 1990s. Bonnethead abundance, although appearing to increase somewhat over the course of the study, did not significantly change (Fig. 4). The ability of these surveys to detect changes in recruitment in the nursery areas may have been influenced by environmental perturbations such as red tide blooms and localized salinity drops from severe storm events. Data from these surveys have been utilized in stock assessments for both large coastal (SEDAR 11) and small coastal sharks (SEDAR 13) (see section 1a4, below).

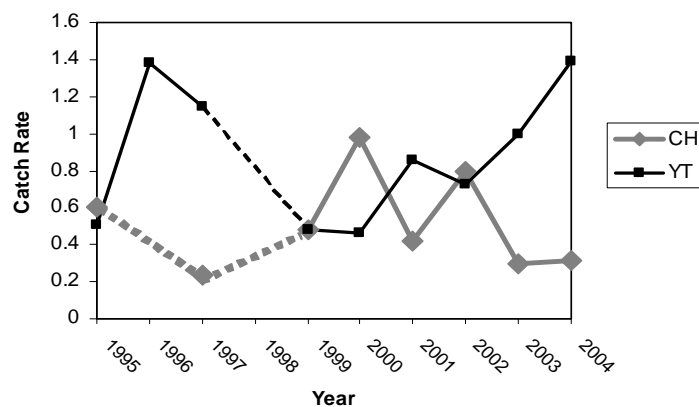


Figure 3. Blacktip shark (*C. limbatus*) relative abundance in the Charlotte Harbor (CH) and Yankeetown (YT) nursery areas, 1995-2004.

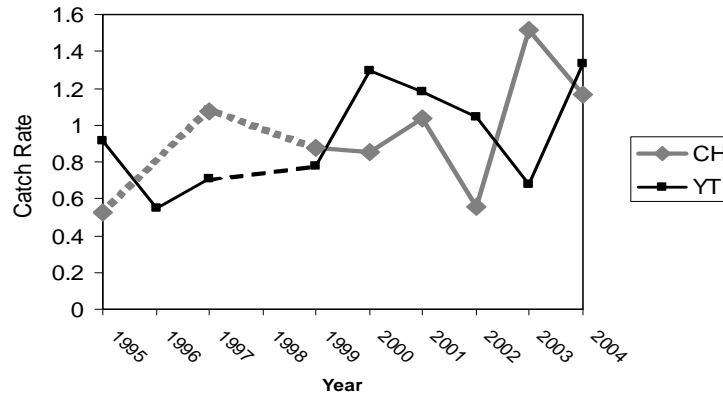


Figure 4. Bonnethead (*S. tiburo*) relative abundance in the Charlotte Harbor (CH) and Yankeetown (YT) nursery areas, 1995-2004.

1a3. Relative Abundance of Shark Populations in the Florida Keys.

Objectives. The Florida Keys contain a diverse array of coastal marine habitats for resident and migratory sharks of all life history stages. The importance of this region as a winter feeding and secondary nursery area for sharks has been underscored by CSR tag-recapture results. Using drumline and longline surveys, the spatial variation in shark communities of the Florida Keys and the influences of habitat, water temperature, and bait type on catch rates of sharks were investigated.

Methods. The study was conducted in the lower Florida Keys offshore of Summerland and Big Pine Keys. Habitats for drumline and longline sampling comprised Atlantic flats, backcountry channels, backcountry flats, barrier islands, channels, passes, Hawk Channel, Marquesas deep, Marquesas shallow, and reefs. Single hook drumlines consisted of a cement block anchor with a 30 m heavy monofilament gangion (800 lb test) secured to the bottom anchor by a swivel and terminating with a baited Mustad circle hook (16/0) with offset point. Up to 20 individual drumlines were set within one or two habitats concurrently and allowed to soak for approximately 2 hours before retrieval. Longlines were approximately 300 m in length and consisted of 50 hooks (Mustad 14/0 circle hook with offset point), gangions of 6 mm nylon rope attached to wire leaders, and a ~300 m mainline made of 6.4 mm braided nylon rope. Longline gear was typically soaked for about 30-60 minutes. All live sharks were tagged with CSR dart tags and/or fin tags (such as rototags) before release; some sharks were double-tagged with NMFS-style tags as appropriate for the size of shark.

Logistic regression was used to determine the influences of habitat, water temperature, and all two-way interactions on the probability that a longline set captured at least one shark, and the influences of habitat, water temperature, bait type, and relevant interactions on the probability of capturing a shark on a drumline. Final models were determined using a backward stepping procedure. Community similarity was investigated using several indices, such as Jacard's index, proportional index of community similarity, and Bray-Curtis index, which placed differing weights on the presence of rare species and species relative abundances.

Outcomes and Significance. A total of 1,375 drumline sets and 198 longline sets were made during the surveys, resulting in the capture of 553 sharks of 10 species (nurse, great hammerhead, blacktip, bull, blacknose, lemon, spinner, sandbar, tiger and bonnethead), plus 3 smalltooth sawfish, *Pristis pectinata*. Community composition varied among habitats. Catch rates of

smaller sharks were highest in protected shallow waters while large sharks were more abundant in deep channels. Overall probabilities of catching large sharks on drumlines did not vary with water temperature while catches of small sharks on longlines increased with increasing water temperature. Individual species differed in their responsiveness to variation in water temperatures and habitat. Bait type affected catch rates of some species, suggesting that fishing methods should be considered explicitly in studies describing shark communities or temporal trends in abundance. Catch rates of large-bodied sharks were higher in a remote, and protected, location compared to similar habitats near inhabited Keys. Furthermore, historical accounts of a shark fishery in the study area during the 1920's suggest substantial declines in large shark abundance and shifts in community composition. By implication, ecosystem impacts of changes in the large shark community may be dramatic and likely occurred before adequate baselines were established. This study was recently published in a peer-reviewed journal (Heithaus, M.R., D. Burkholder, R.E. Hueter, L.I. Heithaus, H.L. Pratt, Jr. and J.C. Carrier. 2007. Spatial and temporal variation in shark communities of the lower Florida Keys and evidence for historical population declines. *Can. J. Fish. Aquat. Sci.* 64:1302-1313).

1a4. Analysis of Large Coastal Shark Survey Data for SEDAR Workshops.

Objectives. The CSR contributed to the South East Data, Assessment, and Review (SEDAR), which is a cooperative Fishery Management Council process initiated in 2002. SEDAR is managed by the Caribbean, Gulf of Mexico, and South Atlantic Regional Fishery Management Councils in coordination with NMFS and the Atlantic and Gulf States Marine Fisheries Commissions. The goal is to improve the quality and reliability of fishery stock assessments in the South Atlantic, Gulf of Mexico, and U.S. Caribbean. SEDAR is organized around three workshops. The first is a data workshop where datasets are documented, analyzed and reviewed, and data for conducting assessment analyses are compiled. The second is an assessment workshop where quantitative population analyses are developed and refined and population parameters are estimated. The third and final is a review workshop where a panel of independent experts reviews the data and assessment and recommends the most appropriate values of critical population variables for fisheries management.

Methods. The CSR was invited to participate in two SEDAR workshops, the large coastal shark assessment (SEDAR 11) and the small coastal shark assessment (SEDAR 13) workshops. Analyses of CSR gill net, longline and drumline catches of several species of sharks were conducted by CSR scientists as contributions for both SEDAR workshops.

Outcomes and Significance. Along with participation of our staff in the SEDAR 11 data and assessment workshops and the SEDAR 13 data workshop, the CSR provided a total of nine working papers, nominal and standardized catch rates, and other types of data. This information was used by NMFS in the stock assessment for both large coastal sharks (SEDAR 11) and small coastal sharks (SEDAR 13).

1b. Life History, Migration and Stock Structure.

1b1. Conventional Tagging Studies of Shark Movement and Migration Patterns.

Objectives. Using conventional tagging methods, we investigated the movements and migration patterns of small and large coastal sharks along Florida's Gulf coast.

Methods. This work was largely conducted using nearshore-to-offshore (out to approx. 25 miles) fisheries-independent surveys for large coastal sharks aboard Mote’s 50-foot research vessel, the *R/V Eugenie Clark*, other Mote research vessels, and the Florida Institute of Oceanography vessel *R/V Suncoaster*. These surveys were conducted along the southwest Florida coast from Tampa Bay to Sanibel Island and utilized longline and drumline techniques to catch large sharks. In addition, coastal surveys using smaller MML research vessels utilized gill nets and hook-and-line to target juvenile large coastal and small coastal sharks in selected Florida nursery areas, primarily Yankeetown and Charlotte Harbor. All live sharks were tagged with CSR dart tags and/or fin tags (such as rototags) before release; some sharks were double-tagged with NMFS-style dart tags as appropriate for the size of shark.

Outcomes and Significance. During the period of this report, more than 4,700 sharks were tagged with conventional tags. Data from 282 recaptures comprising 10 species were reported during this same period (Table 2). These data have helped demonstrate a pattern of philopatric behavior whereby juveniles of several large coastal shark species, including blacktip and bull sharks, return to their natal nursery areas in successive years. Many of these recaptures have also provided useful data for studying the age and growth of these species, some of which have been utilized in recent stock assessments (SEDAR 11 and SEDAR 13).

Species	No. Recaptures	Males	Females	Max. time at large (yrs)	Max. distance traveled (km)
<i>Carcharhinus acronotus</i>	3	0	3	2.02	83.3
<i>Carcharhinus brevipinna</i>	2	1	1	1.90	92.6
<i>Carcharhinus leucas</i>	68	35	33	3.01	714.8
<i>Carcharhinus limbatus</i>	87	27	55	8.65	1,064.9
<i>Carcharhinus plumbeus</i>	4	2	2	3.20	1,472.3
<i>Ginglymostoma cirratum</i>	25	16	9	6.75	535.2
<i>Negaprion brevirostris</i>	32	9	22	4.44	161.1
<i>Rhizoprionodon terraenovae</i>	21	20	1	10.75	292.6
<i>Sphyrna mokarran</i>	1	0	1	2.09	172.2
<i>Sphyrna tiburo</i>	39	16	23	6.16	124.1
Totals	282	126	150		

Table 2. Summary of CSR tag recaptures reported during the period of this report.

1b2. Satellite Telemetry Studies of Migration of Large Coastal Sharks.

Objectives. Satellite tracking methodology was utilized to study seasonal migrations of blacktip (*C. limbatus*), bull (*C. leucas*) and other large coastal sharks inhabiting the eastern Gulf of Mexico and Florida Keys. This work further aimed to investigate the habitat preferences of these species and evaluate how these preferences change with age, size of shark, season and other factors.

Methods. Pop-up Archival Transmitting (PAT) satellite tags (Wildlife Computers) were applied to selected blacktip, bull, sandbar, and great hammerhead (*Sphyrna mokarran*) sharks during quarterly surveys targeting large coastal sharks (described in section 1a1). These tags archive light, depth and temperature data and were programmed to release from the shark after durations of one month or more depending upon circumstances. Once the detached tag reaches the surface,

summaries of archived data are transmitted via the ARGOS satellite system. Satellite tags were attached using a variety of configurations (see Satellite tag testing and development, section 1k). When downloaded light data from the tags were sufficient, daily estimates of the shark's location (longitude and latitude) were made. Additionally, Smart Position Only Transmitting (SPOT) tags (Wildlife Computers), which provide a position estimate whenever the fin on which the tag is mounted breaks the water's surface and a satellite is overhead, were experimentally deployed.

Outcomes and Significance. PAT tags were deployed on 30 large coastal sharks (9 bull sharks, 4 great hammerheads, 11 blacktip sharks, and 6 sandbar sharks). Success rates were very low during use of early versions of the PAT tags, prior to MK10's (see section 1k). Nine of the 30 (30%) PAT tag deployments resulted in the retrieval of some data. Data analysis in these cases is ongoing. In March of 2007, a PAT tag that was deployed on a blacktip shark in 2004 was fortuitously found intact by a beachgoer and returned to the CSR, enabling the downloading of all of its archived data. These data are undergoing analysis and should provide a detailed window into the life history of this animal. One great hammerhead captured on a drumline set off Siesta Key and held temporarily in captivity at MML was released with a SPOT4 satellite tag in July 2004. Multiple signals were received by the ARGOS satellite system but only in one case were sufficient signals received to provide an accurate position fix. This occurred two weeks after release and the signal came from offshore of the mouth of the Withlacoochee River, approximately 100 nautical miles north of the shark's release location.

1b3. Archival Tagging of Blacktip Sharks off South Carolina.

Objectives. Conventional tagging of blacktip sharks along the southeast Atlantic coast has revealed a great deal of information on the general movement patterns of this species. However, as one of the two most important species in the U.S. east coast shark fishery, detailed information is still needed to further define this species' migration pattern and habitat use. This study aimed to deploy archival tags on adult blacktip sharks as a means of gaining long-term, detailed data on the movement and habitat preferences of this species.

Methods. Sampling efforts were conducted between August 2000 and July 2002 off Cape Romain, South Carolina using a floating longline (~20 hooks) that was patrolled and checked continuously during the fishing period. Adult blacktip sharks that were captured alive were measured, sexed, externally tagged, and had an archival tag (Northwest Marine Technology, Inc. [NMT]) surgically implanted into the coelomic cavity. These archival tags record and store temperature, depth, and light level data via the tag's stalk/sensor that protrudes through a small hole in the body wall of the shark such that it is exposed to the outside environment. The archived data from these tags can only be retrieved if and when the shark is recaptured and the tag is returned to NMT, where a specialized reader can download the stored information.

Outcomes and Significance. A total of 13 blacktip sharks ranging in size from 138-180 cm TL were outfitted with archival tags between 2000 and 2002. None of these tags had been recovered until recently, when on August 15, 2007, a mature female blacktip shark with an archival tag was recaptured by a commercial fisherman off Edisto, South Carolina, after being at large for 1,897 days (5.2 yrs). The tag was returned to the CSR and subsequently sent off to NMT for data downloading. The tag was found to be in working order and a significant amount of data was recovered which is currently being analyzed by the CSR staff. Geolocation information for this shark presumably can be estimated from the recorded light level data. The recovered depth and temperature data from this tag will contribute to our understanding of the life history and behavioral ecology of this fisheries-relevant species.

1b4. Analysis of Juvenile Blacktip Tag Recapture Data.

Objectives. The CSR has conducted field surveys since 1991 in nursery areas of the blacktip shark (*C. limbatus*) to estimate relative abundance and investigate migration patterns and habitat preferences of this species along the southwestern coast of Florida. This study aims to utilize recapture data from these field efforts to increase our understanding of migration between nurseries areas and winter grounds, and also to understand habitat use of juvenile blacktip sharks.

Methods. The majority of the data for this analysis comes from blacktip sharks tagged during monthly, random stratified, fishery-independent sampling by gill net conducted in three Florida nursery areas, Yankeetown, Tampa Bay, and Charlotte Harbor. Additional sharks were tagged and released during various other project components using similar gill nets, longlines, drumlines, and rod and reel as means of capture. In all cases, the captured sharks were identified, sexed, categorized by stage of maturity, measured and weighed, and live sharks were tagged with CSR dart tags and/or rototags and released.

Outcomes and Significance. Between 1991 and 2007, a total of 4,508 blacktip sharks were tagged and released. The average size of animals tagged was 72.12 cm TL ($s = 23.52$ cm). Of these tagged individuals, 2,398 were females and 2,064 were males (sex ratio 1F:0.87M), and 46 were not sexed. The majority of the sharks were immature (juvenile) animals, an expected finding given that most of the data used for this analysis came from relative abundance studies targeting blacktip shark nurseries areas (Fig. 5).

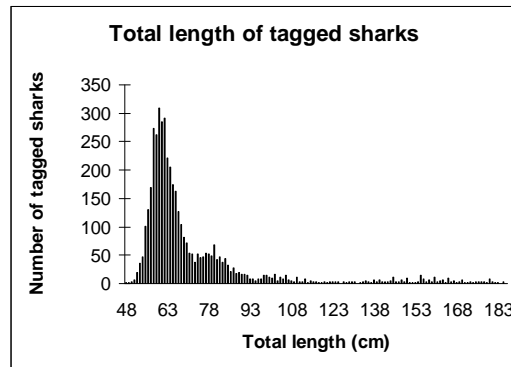


Figure 5. Frequency plot of total length (cm) of all blacktip sharks tagged by the CSR.

Of the 4,508 tagged and released blacktip sharks, 215 (4.8%) recaptures were reported. Of these 215 sharks, 80 were males, 129 were females, and 6 animals were not sexed. The maximum time at liberty was 8.64 years, and the maximum distance traveled recorded from a recaptured animal was 1,065 kilometers. Results support the hypothesis that this species demonstrates some form of philopatric behavior. When we evaluate blacktip shark recaptures that occur only during late spring and summer months (i.e. tagging and recapture both occur during the pupping season), there is evidence that blacktip sharks are returning to their natal nurseries, or adjacent areas, on annual cycles (Table 3). Conversely, other recaptures occurring during late fall and winter months show longer distances away from natal nurseries in those seasons. Our longest at-liberty recapture of a blacktip shark, a female that was a young-of-the-year (YOY) animal 66 cm TL at tagging, was recaptured only 37 kilometers from its nursery ground after 8.64 years at liberty. Given the >1,000 kilometers of overall movements of this species and the available coastline with suitable habitat in the Gulf, we believe these data indicate a trend towards philopatric behavior.

Sex	Size at Tagging (cm TL)	Tagging Date	Recapture Date	Time at Liberty (yrs)	Distance (km)
F	65	8/8/2002	6/25/2003	0.9	14.8
F	65	6/27/2000	6/14/2001	1.0	42.6
F	54	6/10/1993	6/5/1994	1.0	11.1
F	83	6/14/1995	6/13/1996	1.0	22.2
F	58	5/22/2003	5/27/2004	1.0	2.0
F	84	6/13/2003	7/3/2004	1.1	29.6
F	63	7/12/2001	8/20/2002	1.1	10.2
F	58	6/25/2004	7/8/2006	2.0	72.2
F	84	7/28/1993	6/12/1996	2.9	1.9
F	98	6/2/1994	7/3/2000	6.1	88.9
M	63	8/16/2000	5/12/2001	0.7	1.9
M	66	8/29/1996	7/5/1997	0.9	3.7
M	59	7/30/1999	6/27/2000	0.9	259.3
M	59	6/5/1999	5/31/2000	1.0	5.6
M	60	6/27/2002	8/9/2003	1.1	6.9
M	131	5/24/1995	5/30/1997	2.0	22.2
M	61	5/24/2002	7/7/2004	2.1	131.5

Table 3. Blacktip shark tag recaptures when both the tagging and recapture occurred in the summer months. Includes only examples where the recapture took place in a later year. Distance is the shortest at-sea distance between the tagging site and recapture site.

Analyses of these data are in progress and a number of manuscripts will result from this work. The study of migration and movements of juvenile sharks from their summer nurseries to winter feeding grounds, which have been poorly studied, is extremely important. Understanding these processes in sharks is crucial for the study of their biology and population dynamics as well as for their management and conservation.

1c. Essential Fish Habitat.

Overall Objectives. Environmental studies of shark nursery areas were conducted to further our understanding of the non-fishing impacts to federally designated shark Essential Fish Habitat (EFH). In particular, this research focused on determining if sharks residing in important nursery and breeding areas along the eastern U.S. coast are exposed to and affected by environmental pollutants capable of impairing growth, sexual maturation and/or reproduction. The compounds of interest in this research included organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), organometals, and pharmaceuticals and personal care products (PPCPs). The specific objectives were to: 1) determine and compare relative exposure levels and bioaccumulation of OCPs, PCBs, and PPCPs in small and large coastal shark species from major nursery areas on the eastern U.S. coast; and 2) develop and use biomarkers of pollutant exposure to identify EFH locations in which the physiology and health of resident shark populations are altered by environmental pollution.

1c1. OCP and PCB Exposure.

Objectives. The goal of this study was to determine and compare relative exposure levels and bioaccumulation of OCPs and PCBs in small and large coastal shark species from major nursery areas on the eastern U.S. coast.

Methods. Concentrations of OCPs and PCBs were examined in liver from juvenile bonnetheads, sandbar sharks and blacktip sharks sampled from 12 locations on the U.S. east coast: Delaware Bay; Virginia's Atlantic coast; Chesapeake Bay; Bulls Bay, SC; North Edisto River Estuary, SC; St. Simons Sound, GA; Biscayne Bay, FL; Florida Bay; Charlotte Harbor, FL; Tampa Bay, FL; Cedar Key, FL; and Apalachicola Bay, FL. All locations represent major sites of federally designated EFH for at least one of these species. Sharks were sampled using set gill nets and/or longline fishing by CSR scientists working alone or in collaboration with researchers from VIMS, NOAA Fisheries Service (Panama City and Narragansett Laboratories), South Carolina Department of Natural Resources, and the University of Georgia. Following capture and evaluation of gender, size, and weight, sharks were euthanized via anesthesia without revival by immersion in seawater containing 1 g/L tricaine methanesulfonate. Blood samples (5-10 mL) were obtained from most sharks via caudal venipuncture, transferred to sterile vacutainers, and stored on ice until returned to the laboratory. Blood samples were eventually centrifuged to separate serum, which was stored at 20°C until used for biomarker analyses. Liver samples (~5-10 g) were obtained from the lower third of the right hepatic lobe, transferred to 20-mL borosilicate glass vials, and stored on ice. These samples were eventually stored at -20°C until processed for pollutant analysis. Smaller (~1 g) liver samples were also obtained from most animals, flash-frozen in liquid nitrogen, and eventually stored at -80°C until used for biomarker analyses.

Lipid content of certain samples was measured gravimetrically, following the protocol described in Folch et al. (1957). Liver and yolk samples were analyzed for 25-30 OCPs and OCP metabolites, total PCBs, and (in some samples) 30 individual PCB congeners using gas chromatography-mass spectrometry (GC-MS), following methods modified from Holstege et al. (1994) and Schenck et al. (1994) and described more fully in Gelsleichter et al. (2007). Data were analyzed using one-way ANOVA followed by appropriate multiple comparison tests to determine if liver and/or yolk OCP and PCB concentrations differed among sites. Data sets that failed tests of normality and/or equal variance were log-transformed or analyzed using Kruskal-Wallis one-way ANOVA by ranks followed by Dunn's post test. In individual cases when one of these compounds was not detected or was present at levels below the limit of quantitation (LOQ), numerical values of zero or the midpoint between the limit of detection (LOD) and the LOQ, respectively, were assigned to permit statistical analysis. All results were presented as means \pm SE in both wet weight and lipid weight for comparison with other studies.

Outcomes and Significance. The results of our OCP and PCB screening indicate that sharks residing in mid- and south Atlantic estuaries and nearshore areas on the U.S. east coast generally accumulate greater levels of organochlorine contaminants than their Gulf coast counterparts. This is particularly the case for animals residing in the St. Simons Sound region in coastal Georgia, which exhibit liver PCB concentrations that are 2-10 times greater than those in Florida sharks. These differences were less pronounced in *S. tiburo* (Fig. 6) in comparison with *C. limbatus* (Fig. 7), which leads us to hypothesize that the unexpectedly high OCP and PCB concentrations in neonate blacktip sharks is likely to reflect a combination of both juvenile exposure and the maternal transfer of these pollutants to developing offspring via yolk. This may also occur in *C. plumbeus*, which exhibit liver pollutant concentrations generally comparable with those observed in Atlantic *C. limbatus* (Fig. 8).

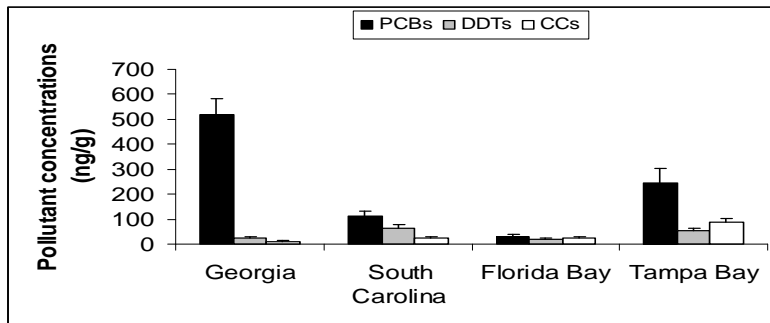


Figure 6. Total liver polychlorinated biphenyls (PCBs), DDT-related contaminants (DDTs), and chlorinated cyclodiene pesticides (CCs) in bonnetheads from St. Simons Sound, GA, North Edisto River, SC, Florida Bay, FL, and Tampa Bay, FL (N = 5 per site). Pollutant concentrations are expressed as means \pm SE.

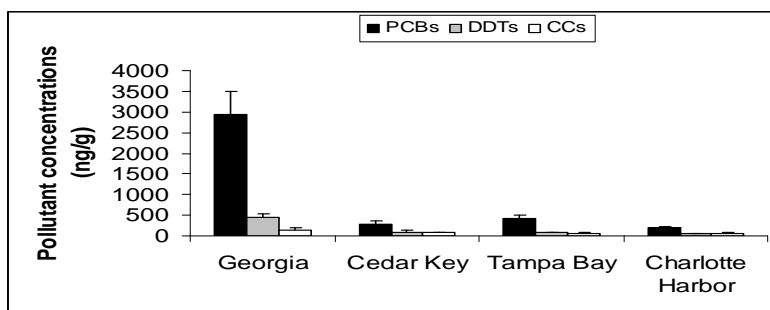


Figure 7. Total liver polychlorinated biphenyls (PCBs), DDT-related contaminants (DDTs), and chlorinated cyclodiene pesticides (CCs) in neonate blacktip sharks from St. Simons Sound, GA (N = 4), Cedar Key, FL (N = 9), Tampa Bay, FL (N = 5), and Charlotte Harbor, FL (N = 7). Pollutant concentrations are expressed as means \pm SE.

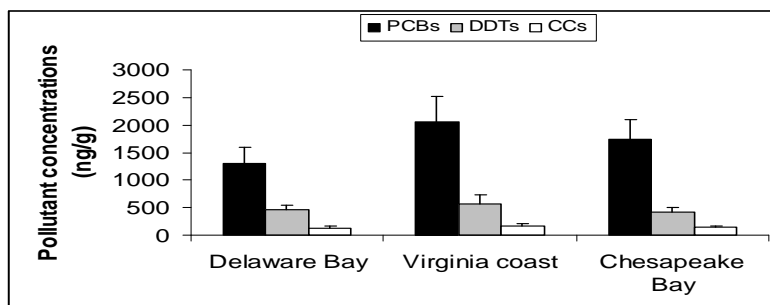


Figure 8. Total liver polychlorinated biphenyls (PCBs), DDT-related contaminants (DDTs), and chlorinated cyclodiene pesticides (CCs) in juvenile sandbar sharks from Delaware Bay (N = 4), Virginia's eastern shore (N = 9), and Chesapeake Bay (N = 10). Pollutant concentrations are expressed as means \pm SE.

Given concerns about PCB exposure in Atlantic coast sharks, the concentrations of individual PCB congeners were measured in liver of juvenile *C. limbatus* and *C. plumbeus* to determine if more harmful “dioxin-like” congeners constitute a sizeable proportion of overall PCB load. These data indicated that non- or less toxic PCBs make up a greater proportion of total PCB content in both Atlantic and Gulf sharks (Fig. 9). In fact, the highly toxic non-ortho chlorine substituted PCBs (PCBs 77, 126, 169) only accounted for 0.7-4% of total PCB load in Atlantic sharks and were completely undetected in Gulf coast specimens. Based on these data, the impact of PCBs on shark EFH on the U.S. east coast may be of only limited concern.

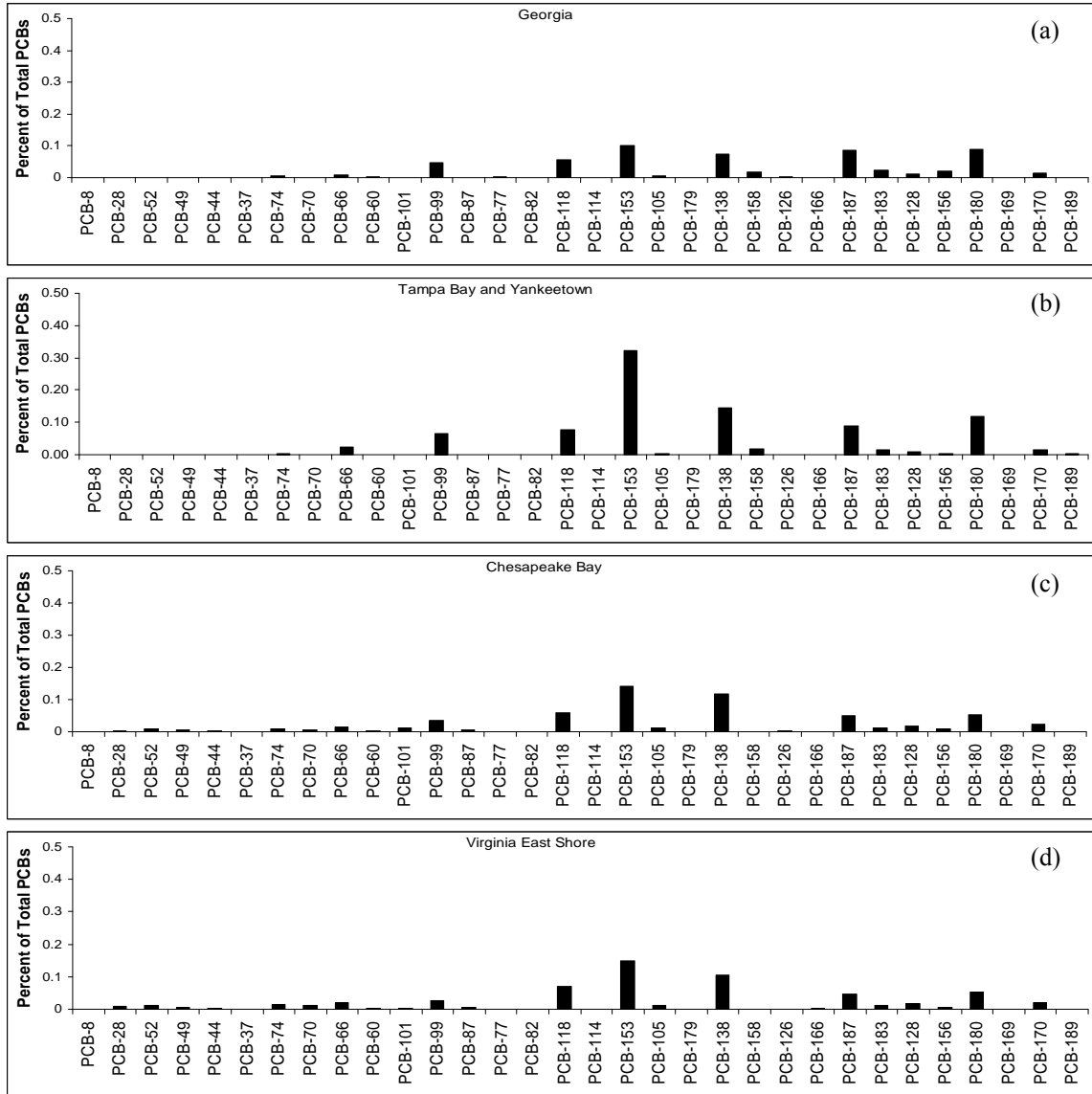


Figure 9. Percentage of total PCB concentrations made up by 30 individual PCB congeners in liver of (a) Georgia blacktip sharks (N = 4), (b) Florida blacktip sharks (N = 6), (c) Chesapeake Bay sandbar sharks (N = 5), and (d) Virginia eastern shore sandbar sharks (N = 5). PCBs 77, 126, and 169 represent highly toxic non-ortho chlorine substituted PCBs capable of impairing reproduction in aquatic vertebrates.

1c2. PPCP Exposure.

Objectives. The goal of this study was to determine and compare the relative exposure levels and bioaccumulation of PPCPs in large coastal shark species from a major estuarine nursery area on the U.S. Gulf coast.

Methods. Concentrations of PPCPs were examined in plasma from 10 juvenile bull sharks sampled from tidal portions of the Caloosahatchee River, an important nursery habitat for this species. Sharks were sampled using longline fishing and 5-mL blood samples were obtained from each specimen via caudal venipuncture. Following blood sampling, sharks were tagged and released live or euthanatized for other studies. Blood samples were transferred to sterile vacutainers containing anti-coagulant and protease inhibitor and stored on ice until returned to the laboratory. Blood was centrifuged to separate plasma, which was stored frozen at -80°C until processed for PPCP analysis.

Plasma concentrations of 17 α -ethynylestradiol (EE2), the synthetic estrogen commonly used in human contraceptives, was measured using GC-MS following a modification of the methods described by Croley et al. (2000). Plasma concentrations of the widely prescribed antidepressants citalopram, fluvoxamine, paroxetine, sertraline, venlafaxine, and fluoxetine (and its primary metabolite norfluoxetine) were measured using HPLC-UV-MS following solid-phase extraction, as described by Frahnert et al. (2003). Data obtained from this pilot study were analyzed using descriptive statistics.

Outcomes and Significance. Quantifiable levels of all antidepressants or their primary metabolites were detected in at least 1 of the 10 juvenile *C. leucas* examined in our pilot study on PPCP exposure in freshwater sharks. However, in most cases, these compounds were detected in only 1-4 animals. The sole exception to this was sertraline, the active compound in Zoloft, which was detected in 9 of the 10 samples. Average concentrations of sertraline in shark plasma were 0.43 \pm 0.5 ng/mL, which is 75-200 times lower than the circulating levels of this compound in humans taking Zoloft on a normal daily basis. Based on these observations, SSRI antidepressants appear to pose little risk to shark populations residing in the wastewater-impacted Caloosahatchee River. This also seems to be the case for EE2, which was not detected in any of the 10 samples examined. Nonetheless, it is important to note that these results are preliminary and the threshold level at which EE2 or SSRIs may impact shark physiology remain undetermined. Because of this, additional tests on exposure levels and effects of these and other PPCPs in freshwater sharks are ongoing.

1c3. Biomarkers for Cytochrome P450 1A1-Inducing Pollutants.

Objectives. To determine if PCBs and similarly acting contaminants are altering the physiology of sharks residing in federally designated EFH, biomarkers of exposure to these compounds were examined in *S. tiburo* collected from multiple locations on the southeastern U.S. coast.

Methods. Hepatic levels of cytochrome P-450 1A1 (CYP1A1), an enzyme induced by exposure to toxic “dioxin-like” PCB, as well as polycyclic aromatic hydrocarbons (PAHs) and dioxins, was measured in 107 *S. tiburo* collected from 8 sites in South Carolina, Georgia, and Florida. Hepatic CYP1A1 was determined using flash-frozen liver samples (see Methods for OCP and PCB exposure) and a modification of the ethoxyresorufin-O-deethylase (EROD) procedure described in Sepulveda et al. (2004). The EROD assay is a highly sensitive and well-established indicator of CYP1A1 activity in vertebrates, which measures the rate of the CYP1A-mediated deethylation of the substrate 7-ethoxyresorufin to form the fluorescent product resorufin. The production of

resorufin (hereafter referred to as “EROD activity”) is a reflection of the amount of CYP1A1 present in a homogenized liver sample and is generally expressed as the concentration of resorufin produced per mg protein per minute. To validate the use of this procedure for measuring pollutant exposure in *S. tiburo*, changes in EROD activity was assessed in captive sharks exposed to the known CYP1A1 inducer, beta-naphthoflavone (BNF). Differences in CYP1A1 activity in control and BNF-treated sharks were also compared via immunocytochemical detection of CYP1A1 in histological sections of liver using monoclonal antibody against scup CYP1A (provided by Dr. John Stegeman, Woods Hole Oceanographic Institution), which has been shown to cross-react with CYP1A1 in numerous vertebrates including sharks. Paraffin histology and immunocytochemistry was performed following the methods described in Nichols et al. (2003).

Outcomes and Significance. Increases in both the immunocytochemical localization of CYP1A1 (data not shown) and EROD activity (Fig. 10) were observed in the liver of bonnetheads exposed to the CYP1A1-inducer BNF. EROD activity was significantly greater in bonnetheads sampled from South Carolina and Georgia sites in comparison with all locations in Florida. These data suggested that sharks residing on the South Carolina and Georgia coast are exhibiting physiological signs indicative of elevated exposure to some CYP1A1-inducing compounds. Since prior data on PCB congeners in *C. limbatus* suggest that non-ortho chlorine substituted PCBs are unlikely to pose significant risks to Atlantic shark populations, future studies should examine possible exposure to other CYP1A1-inducing pollutants, such as dioxins and PAHs.

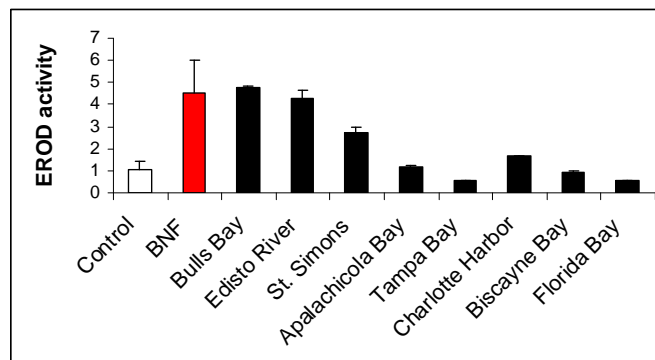


Figure 10. EROD activity (pmol resorufin per mg protein per minute) in liver of bonnetheads from Bulls Bay, SC (N = 15), North Edisto River, SC (N = 10), St. Simons Sound, GA (N = 8), Apalachicola Bay, FL (N = 13), Tampa Bay, FL (N = 19), Charlotte Harbor, FL (N = 19), Biscayne Bay, FL (N = 5), and Florida Bay, FL (N = 17). EROD activity from control captive sharks (N = 3) and BNF-treated sharks (N = 3) is displayed to demonstrate the validity of the EROD assay as a biomarker of CYP1A1 induction in *S. tiburo*. Values are means ±SE.

1c4. Biomarkers for Ecoestrogens.

Objectives. To determine if environmental pollutants capable of mimicking the natural hormone estrogens (such as certain OCPs, detergent surfactants, and EE2) are altering the physiology of sharks residing in federally designated EFH, a biomarker of exposure to these so-called “ecoestrogens” was examined in *S. tiburo* collected from multiple locations on the southeastern U.S. coast.

Methods. The presence of vitellogenin (Vtg), a commonly used biomarker of ecoestrogen

exposure in fish, was measured in 90 *S. tiburo* collected from 8 sites in South Carolina, Georgia, and Florida. Vitellogenin is the estrogen-regulated precursor to egg yolk in non-mammalian female vertebrates and is normally only present in mature females undergoing follicular development. However, males and immature females do possess Vtg genes and both Vtg mRNA expression and protein production can be induced in these animals via exposure to estrogenic substances. Little work has been conducted on vitellogenesis in sharks, thus, techniques for the detection of Vtg in *S. tiburo* were newly developed.

The production of Vtg was induced in captive male *S. tiburo* (N = 2) via single, 200- μ L interperitoneal injections of 17 β -estradiol in dimethylsulfoxide (DMSO) (dosage level of 5 mg/kg body weight). Control animals were also used in this study and each received single injections of DMSO alone. Following a 48-h period, animals were euthanized to obtain liver samples, which were immediately flash-frozen in liquid nitrogen and transferred to a -80°C freezer for long-term storage. A 450-bp cDNA clone was obtained from reverse transcribed liver RNA of estradiol-treated sharks using PCR with degenerate primers based on conserved regions of fish Vtgs (Bowman and Denslow, 1999). Similarity analysis of sequence data using NCBI BLAST demonstrated that this clone is homologous with most vertebrate Vtgs in the database. Furthermore, the identity of this clone as a Vtg has been verified by multiple alignment analysis using CLUSTAL W. A digoxigenin-labeled riboprobe for *S. tiburo* Vtg mRNA was prepared via *in vitro* transcription using the cloned cDNA template. Northern blot analysis of total liver RNA from estradiol-treated sharks demonstrated that full length *S. tiburo* Vtg is at least 5.5 kb, a size comparable with Vtgs from other vertebrates (~5.0-6.5 kb, Bowman et al., 2000).

Due to low expression of Vtg mRNA in even estradiol-treated sharks, it was determined that Northern blot or Bio-Dot procedures (which would make use of the riboprobe described above) would not be sensitive enough for detecting Vtg expression in small liver samples (generally used to conserve liver for use in other biomarker assays). Therefore, a more sensitive multiplex RT-PCR assay was developed using species-specific primers designed with Primer3 software. This assay results in the amplification of a 179-bp PCR product, which is only present when Vtg mRNA is actively expressed in *S. tiburo* liver. The assay also includes primers for the constitutively expressed 18S ribosomal RNA, which serves as a housekeeping gene for this procedure and can be used to normalize Vtg levels for comparison between samples. This assay has been validated via positive detection of Vtg mRNA expression in female *S. tiburo* undergoing follicular development and male *S. tiburo* treated with both estradiol and EE2.

Recently, we developed polyclonal antibodies against synthetic peptide corresponding to an antigenic fragment of bonnethead Vtg protein. The amino acid sequence of bonnethead Vtg was deduced using our gene sequence data. These antibodies cross-react with a 180-kDa protein present only in mature female *S. tiburo* undergoing follicular development (putative Vtg) and a single protein isolated from *S. tiburo* yolk, which has been tentatively identified as lipovitellin II (one of three yolk proteins derived from Vtg). Using these antibodies, we examined the presence of Vtg protein in plasma from all sharks previously screened for Vtg mRNA and 10 additional animals from the Apalachicola Bay estuary. These assays were conducted using SDS-PAGE and a modification of the immunoblotting procedure described in Piercy et al. (2003).

Outcomes and Significance. Both the hepatic expression of Vtg mRNA and the presence of Vtg protein in serum were observed in mature female *S. tiburo* undergoing follicular development (Fig. 11). Hepatic expression of Vtg mRNA was also observed in male *S. tiburo* treated with estradiol and EE2 (Fig. 11). Hepatic expression of Vtg mRNA and the presence of Vtg protein were not detected in immature female or immature/mature males collected from sampling locations. This suggests that ecoestrogen exposure in both Atlantic and Gulf coast sharks is below the threshold levels necessary to elicit physiological effects. Therefore, these compounds are unlikely to pose significant threats to the health of U.S. east coast shark EFH.

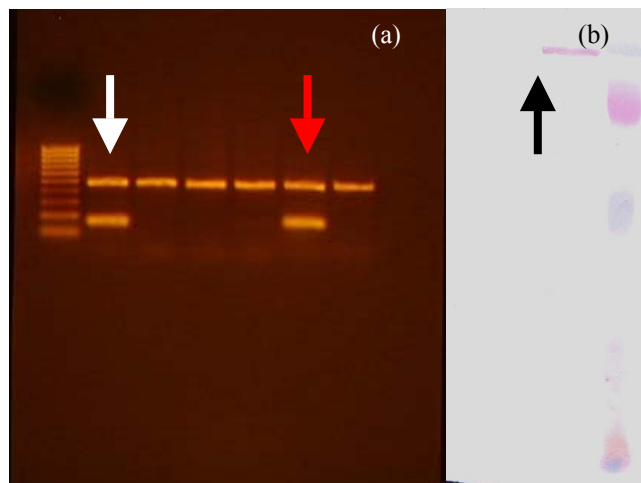


Figure 11. Examples of results from RT-PCR (a) and Western blot (b) analysis of Vtg mRNA and protein detection procedures. The expression of Vtg mRNA in *S. tiburo* liver is signified by the presence of two PCR products corresponding to Vtg (179-bp) and the constitutively expressed 18S ribosomal RNA (488-bp). Both products were only observed in male sharks exposed to natural and synthetic estrogens (white arrow) and mature female sharks undergoing follicular development (red arrow). The presence of Vtg protein in *S. tiburo* plasma is signified by the presence of an immunoreactive 180-kDa protein band (black arrow), which was only observed in the plasma of mature female sharks undergoing follicular development.

1c5. Biomarkers for Organometals.

Objectives. This portion of the study aimed to develop and use biomarkers of organometal exposure to identify EFH locations in which the physiology and health of resident shark populations are altered by this form of environmental pollution.

Methods. In a pilot study conducted in fall 2006, we injected bonnetheads and Atlantic stingrays (*Dasyatis sabina*) with $CdCl_2$ to determine if commercially available antibodies for metallothionein, a protein biomarker of metal exposure, would be suitable for use with elasmobranch species. The presence of metallothionein in these samples was analyzed using SDS-PAGE and a modification of the immunoblotting procedure described in Piercy et al. (2003). Initial plans to conduct dose-response experiments on captive *S. tiburo* and confirm that metallothionein expression rises with increased levels of exposure to organometals have been delayed due to a greater focus on other studies (particularly Vtg).

Outcomes and Significance. The presence of a 6-7 kDa band tentatively identified as metallothionein was observed in the liver of both control and $CdCl_2$ -treated bonnetheads. This indicates that commercially available tools for assessing metallothionein expression in fish are suitable for detecting its presence and abundance in shark tissues. As discussed previously, future research will be directed at comparing metallothionein expression in *S. tiburo* exposed to various levels of organometals. Once this research is completed, metallothionein expression will be examined in samples previously screened for CYP1A1 activity and Vtg expression.

1c6. Gene Chip Analysis.

Objectives. Gene arrays or “gene chips” have emerged as promising tools for screening pollutant effects in living organisms due to their capability of assessing changes in expression patterns of a large number of genes using a relatively small amount of RNA extracted from animal cells or tissues. This technique is particularly useful for examining the effects of hormone mimics and/or antagonists because the primary action of hormones is to alter levels of gene expression (Larkin et al., 2003). The use of gene array procedures is also appealing to conservation-minded biologists because they require only small amounts of biological material, which can be obtained using non-lethal procedures.

Methods. We partnered with EcoArray, Inc. (Alachua, FL), an industry leader in the development of custom gene arrays for fish and other aquatic vertebrates, to develop the first gene array procedure for detecting ecoestrogen responses in sharks. We generated a library of >120 cDNA sequences corresponding to genes differentially expressed in blood cells of estradiol-treated and control male *S. tiburo* (obtained as part of the Vtg study) via suppressive subtraction hybridization (SSH). The SSH procedure enables one to compare two populations of RNA in order to obtain DNA probes for genes that are expressed differently in one population compared to the other (Diatchenko et al., 1996). cDNA probes representing differentially regulated mRNAs were PCR amplified, cloned, and sequenced at the University of Florida Interdisciplinary Center for Biotechnology Research. Analysis of sequence data for the >135 probes that were differentially regulated in treatment and control group sharks was conducted using NCBI BLAST. We also cloned >140 gene sequences from the liver and testis of the same animals in a separate SSH experiment (many of these determined to be fragments of Vtgs). These genes would later be used to validate the selection of any blood cell genes that appeared to be suitable biomarkers of ecoestrogen exposure. Ideal biomarkers would only include blood cell genes that were differentially expressed in ecoestrogen-treated sharks in concert with more established biomarkers, such as Vtg.

Outcomes and Significance. Initial plans to validate and construct a gene chip using these sequences were put on indefinite hold due to the expense of conducting this research and our inability to obtain additional grant support. Proposed plans to conduct similar research on stingrays were also delayed for this reason.

1c7. Egg Infertility in *S. tiburo*.

Objectives. Previous studies have demonstrated that certain populations of *S. tiburo* on Florida’s Gulf coast exhibit high rates of an unexplained egg infertility, which may lead to an >20% reduction in population growth rate (Parsons, 1993; Cortes and Parsons, 1996). Since this phenomenon may be associated with environmental quality, we explored the occurrence of infertility in a greater number of *S. tiburo* populations and its potential relationship with pollutant exposure (as assessed via our measurements of OCP and PCB concentrations in shark liver and biomarker assays).

Methods. Infertility rate (expressed as the percentage of ovulated ova that do not give rise to viable offspring) was determined in pregnant female *S. tiburo* from a total of 5 bonnethead populations in South Carolina and Florida. We also measured OCP and PCB concentrations in yolk from the ovaries of mature female *S. tiburo* sampled from four of these locations in Florida to assess pollutant risks to embryonic development. Yolk samples were obtained from recently ovulated eggs present in uteri of pregnant *S. tiburo*, transferred to 1.5-mL cryovials, flash-frozen

in liquid nitrogen, and eventually stored at -80°C until processed for pollutant analysis.

Outcomes and Significance. As observed in earlier studies, egg infertility rates were lower in Florida Bay *S. tiburo* (1.5%) in comparison with their counterparts from the Tampa Bay estuary (11.5%). However, we also found egg infertility rates to be unexpectedly high in *S. tiburo* from other locations, such as Charlotte Harbor (7.5%), Apalachicola Bay (9%), and the North Edisto River (17%). The causative factors leading to this phenomenon remain unclear. Nonetheless, infertility is unlikely to be associated with exposure to OCPs or PCBs based on the results of our biomarker studies. This was also supported by measurements of OCPs and PCBs in *S. tiburo* yolks, which differed by site but were low in all Florida populations (Fig. 12).

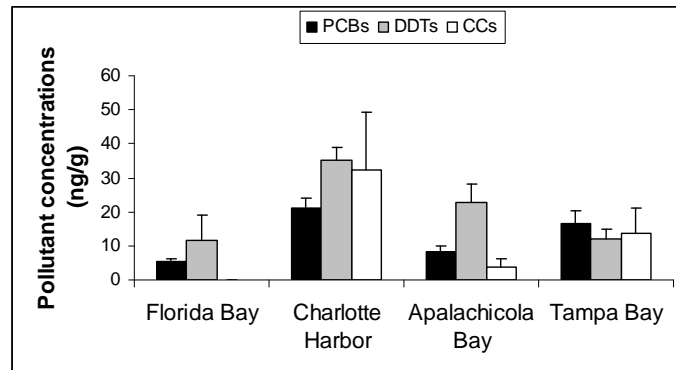


Figure 12. Total polychlorinated biphenyls (PCBs), DDT-related contaminants (DDTs), and chlorinated cyclodiene pesticides (CCs) in yolk from pregnant female bonnetheads from four Florida estuaries: Florida Bay, Charlotte Harbor, Apalachicola Bay, and Tampa Bay (N = 5 per site). Pollutant concentrations are expressed as means \pm SE.

1d. Ecosystem and Population Modeling.

1d1. Shark Population Modeling in Charlotte Harbor, Florida.

Objectives. Computer-based models can provide a significant amount of knowledge of the functioning and status of shark populations, and also of how changes in the ecosystem can affect sharks. This project aimed to develop an ecosystem model of Charlotte Harbor, Florida, an important nursery area for blacktip sharks and also an area utilized by a wide variety of other elasmobranch species (including bull and bonnethead sharks, cownose rays, stingrays and sawfish). This model was used to examine how changes within the environment affect shark populations, and how changes in shark populations can affect the ecosystem.

Methods. The Charlotte Harbor ecosystem model was developed in Ecopath/Ecosim, a powerful ecosystem modeling tool. The Ecopath/Ecosim model included 35 ecosystem components from primary producers to top-level predators, including sharks. A spatial component was added to the model using Ecospace. Spatial data were added at two scales – 1 km squares and 2 km squares. The spatial data included physical (temperature, salinity, nutrient loading, oxygen level) and biotic (seagrass coverage, animal movement rates, primary productivity rates and predator-prey interaction parameters) factors. The model also included information on trophic interactions,

population dynamics and fisheries. We ran the model with a variety of scenarios for the future (e.g. reduced freshwater flows, increased recreational fishing, decreased recreational fishing, etc.) to examine how these changes could affect the ecosystem and shark populations. To provide information on the distribution and abundance of fishes within the system (for inclusion in the spatial model), we performed hydroacoustic surveys of Charlotte Harbor. The surveys utilized a Biosonics DTX scientific echosounder and Visual Analyzer software.

Outcomes and Significance. Hydroacoustic surveys were used to validate the biomass estimates used in the initialization of the model. These surveys were conducted in four areas of the harbor to cover a wide variety of habitat types (seagrass [shallow estuary], medium depth estuary, deep estuary, river, inlet and river mouth). Analysis of the data indicated that during periods of hypoxia in the harbor the prey fish form smaller schools and inhabit shallower habitats. The hydroacoustic surveys, however, were unable to provide estimates of biomass by trophic group due to difficulties in the attribution of acoustic targets to specific species or species groups. Thus, although the model was run under a variety of assumptions about environmental and fishing strategies, there was considerable uncertainty about the validity of the results because of a lack of data on the abundance of several important trophic groups. The conclusions of the model were therefore limited to hypothetical results and were not further explored.

1d2. Evaluation of Localized Population Declines off Durban, South Africa.

Objectives. Understanding the dynamics of elasmobranch populations, and their role in ecosystems, is important to determining the sustainability of fisheries for these species. We have collaborated with the Natal Sharks Board in Durban, South Africa, to investigate how shark populations respond to localized fishing pressure, and if this leads to localized depletion. The results of this work have important implications for understanding how localized surveys measuring catch rate, as are used in U.S. shark stock assessments, represent population abundance.

Methods. In collaboration with the Natal Sharks Board, we examined catch rate data from long-term gillnet fishing off the KwaZulu-Natal coast of South Africa. These data cover the period from the 1950's to present, with catches recorded separately for 42 beach locations along the coast. We examined the catch rate data for trends in abundance at a broad scale, and at local scales. If differences exist between broad and local scale patterns, this would indicate that small-scale surveys might be more reflective of localized stock effects rather than overall abundance. The results of these analyses were interpreted in light of the surveys used to measure abundance of sharks in U.S. stock assessments.

Outcomes and Significance. Studies of shark populations and how they respond to localized fishing were initiated in December 2004. With Dr. Sheldon Dudley of the Natal Sharks Board, the Mote CSR analyzed data from 14 species of sharks that have been fished since the 1950's in the waters off South Africa. Analysis of data from this project demonstrated that even in situations where regular fishing occurs on shark populations, they are able to reach equilibrium yield levels. This result demonstrates that all shark species, even those with K-selected life histories (e.g. dusky sharks), have density-dependent population processes and so can sustain some level of fishing. Analysis of beach-by-beach catch rates of commonly captured sharks also indicated that localized depletion of large shark species occurs at scales less than 50 km, indicating that even these larger sharks may have relatively high site fidelity. The work was published in a peer-reviewed journal (Dudley, S.F.J. and C.A. Simpfendorfer. 2006. Population status of 14 shark species caught in the protective gillnets off KwaZulu-Natal beaches, South Africa, 1978-2003. *Mar. Freshw. Res.* 57:225-240). The results of this work contribute to the

understanding of how shark populations function and will improve the management of shark fisheries.

1d3. Status of Batoid Populations off the Florida Gulf Coast.

Objectives. We began analyzing data on the species composition and abundance of batoid populations on the Gulf coast of Florida to investigate potential long-term changes. It has been suggested that the decrease in abundance of larger sharks in this region may have released batoid populations from a significant source of predation, resulting in an increase in batoid abundance. Hence, this study aimed to investigate changes in the species composition and abundance of batoids on the Gulf coast of Florida.

Methods. Data on the batoid catches off the Gulf coast of Florida collected by Dr. Eugenie Clark in the 1950's and 1960's were examined to investigate historic species composition and abundance. These data were first transferred from paper records to electronic form for analysis. The historic data were then compared to similar data from recent times (e.g. Mote sawfish longline data or Florida Wildlife Research Institute [FWRI] fisheries-independent monitoring data) to determine if changes in the species composition or abundance have changed over time. Size composition data were examined to investigate whether there had been any changes between the 1950's and present.

Outcomes and Significance. Analysis of historic catches of batoids on the west coast of Florida was examined in early 2005. Data from 1950's and 1960's catches made by Dr. Eugenie Clark and collaborators were extracted from original logbooks and entered into a spreadsheet for analysis. However, comparison of the location data in the historic data to the contemporary Mote sawfish and FWRI fishery-independent databases revealed that there was insufficient geographic overlap to permit valid qualitative analysis. Given this finding, no further analysis was undertaken.

1e. Advances in Shark Distribution and Abundance Assessment Methodology.

1e1. Use of Baited Underwater Video Cameras.

Objectives. As a means of enhancing our assessments of shark distribution and abundance, baited underwater video cameras were incorporated into our fishery-independent sampling regime. This technology enabled us to record sharks as they approach the baited camera rig thus providing a visual record of the number and species composition of sharks in an immediate area. If these cameras captured usable data, these methods could be used to complement field sampling efforts using conventional gear, as an alternative to conventional gear in restricted areas, or be useful in documenting sensitive or protected species where capture is not an option.

Methodology. A steel frame was constructed using re-bar and pipe to support and protect the Sony digital video recorder and its housing. The camera/frame was also fitted with a PVC pipe that terminated with a plastic mesh basket containing bait (primarily herring and mackerel). The pipe extended straight out from the camera such that the bait was in the camera's field of view. The baited cameras and frames were deployed concurrently and in the same general vicinity as drumline and longline gear deployed as part of the CSR's quarterly large shark surveys (see section 1a1). A total of 46 cameras were deployed (each with 80 min of video time) during these surveys from April-December 2006.

Outputs and Significance. The only shark species observed with certainty on camera was the blacknose shark (*C. acronotus*) despite at least six other shark species being captured in the vicinity by drumline and longline (a total of 77 sharks were captured during the associated surveys). In several instances, blacknose sharks approached the baited frames and bit the bait baskets. The tapes also recorded several large but blurred images in the distance but they could not be identified. Additionally, loggerhead sea turtles (*Caretta caretta*) were observed to approach the cameras and consume bait from the baskets.

The underwater cameras used in this pilot study produced only marginal results. It is possible that the low number of sharks captured on camera is related to poor water visibility, potential electromagnetic interference from the camera and housing, or from the short duration of recording time. Further, the angle of the camera limited the field of vision making it difficult at times to discern the presence of a single shark versus multiple sharks around the camera. Given the limited results of this pilot effort, this method was discontinued during routine surveys.

1e2. Experimental Use of Aerial Surveys.

Objectives. We explored the use of aerial surveys parallel to the coastline to estimate the presence and abundance of coastal shark species.

Methods. A trial survey was conducted in June 2007, using a PA-30 Piper Twin Comanche airplane that was provided free of charge by LightHawk, a volunteer-based environmental aviation organization. The survey was conducted along the Florida Gulf shoreline from the mouth of Tampa Bay to the north tip of Sanibel Island, a straight-line distance of about 140 km. The flight crew consisted of a pilot, two observers and one photographer. The observers had a view from either side of the plane and recorded any sharks, rays, or other large marine life that was identifiable. The low wing plane flew at an altitude of ~200 ft but was able to fly as low as 100 ft when more detailed observations were necessary. When animals were spotted, the pilot was able to circle back to assist the observers in identifying the sharks when possible.

Outcomes and Significance. Despite some limitations that can be encountered with this type of survey technique (e.g. water visibility, weather, etc.), this pilot study was successful. We were able to identify a number of sharks and pinpoint a few areas of high abundance. The highest concentration of sharks was observed along the Gulf side of passes. For example, 13 bull sharks were identified in an area no greater than ~200 square meters in Boca Grande Pass. In addition to sharks, spotted eagle rays (*Aetobatus narinari*, a protected species), bottlenose dolphins (*Tursiops truncatus*), and several groups of manatees (*Trichechus manatus*) were easily identified during the course of the survey. The information and experience gained from this trial survey will be applied in future studies of the movement patterns and habitat utilization of bull sharks and other large coastal species occupying coastal waters in the Florida Gulf.

1f. Reproduction of Shortfin Mako and Pelagic Thresher Sharks.

Objectives. This study component investigated the reproductive biology and cycles of shortfin mako (*Isurus oxyrinchus*) and pelagic thresher (*Alopias pelagicus*) sharks. These two species are commercially important in the United States and Mexico but their reproductive biology is poorly known. Questions about their reproductive cycles can only be answered by examining gravid females, and the most efficient and cost-effective method is to examine specimens taken by commercial fisheries. The only area in the U.S. where there is a possibility of obtaining gravid shortfin mako sharks is the Gulf of Mexico in winter. Gravid pelagic thresher sharks can be examined in the fishing camps of Baja California, Mexico during the summer.

Methods. For these reproductive studies, two large shortfin mako females and about 60 pelagic thresher females were examined.

Shortfin mako: Only one of two 300 cm females was gravid. This female was in the early stages of pregnancy, carrying 5-6 embryos of 30-40 mm each in each uterus. Each uterus contained a large number of feeding egg cases destined to be eaten by the embryos. This is one of only two shortfin mako females seen at this early stage of pregnancy known to biologists.

Pelagic thresher: Females in all stages of gestation were examined, from blastodisc stage to term. Examination revealed that pelagic threshers ovulate only two fertilized eggs, one going to each uterus in large eggcases. Unlike the makos and other lamnoid females, thresher females continue to ovulate during the entire gestation period. Therefore, thresher embryos do not acquire the large yolk stomachs of other lamnoid sharks. Pelagic thresher embryos may appear externally ready for birth when they are only 60 cm in length although they will grow to at least 140 cm before birth.

Outcomes and Significance. The shortfin mako sample was very small, and only limited conclusions could be made. Only about six or seven pregnant shortfin makos have ever been examined by scientists, so our knowledge of this species' reproductive cycle is very meager. Further specimens were not received from Brazil as planned, mostly due to restrictions placed on biological specimens by the Brazilian government.

By contrast much was learned about the pelagic thresher. Females in various stages of gestation were encountered in summer, indicating that females of the species are not synchronous in their reproductive cycles and that reproduction occurs year round. Given that only gravid females were encountered during the entire sampling, it is likely that all the adult females are gravid each year, indicating a yearly reproductive cycle, although the specific time of gestation is variable. Although the pelagic thresher reproduces yearly, it produces a maximum of two young per birth, so its reproductive potential is limited and the thresher fisheries must be managed conservatively.

Ig. Nursery Population Assessment.

Objectives. CSR research has demonstrated that Terra Ceia Bay, a small bay adjacent to Tampa Bay, Florida, is a critical nursery habitat for neonate and juvenile blacktip sharks. Previous studies have shown that neonate and YOY blacktip sharks use this nursery continuously for up to 6 months and some individuals return to use the nursery ground as 1- and 2-year olds. Although we have developed an understanding of how young sharks use this habitat via acoustic monitoring techniques, it is unclear how large the population is within this nursery. This project aimed to address this question by defining the size of the population of neonate and YOY blacktip sharks utilizing Terra Ceia Bay as a nursery area and then estimating the number of adult females pupping in the region.

Methods. Mark-recapture experiments were conducted within Terra Ceia Bay during several days in each month from June-September 2003. This survey was modeled after the random-stratified method used in other CSR nursery ground relative abundance surveys and utilized a standardized CSR gill net (10 ft depth with 4⁵/₈" SM weighted gill net). The procedure entailed capturing and tagging a large portion of the population over a short period of time. All captured sharks were identified, sexed, measured and weighed. After allowing a couple of days for the sharks to redistribute, re-sampling of the population was conducted to check for tagged individuals. The size of the population at the time of tagging was then estimated from the proportionality of tagged vs. untagged animals during the two sampling phases (Peterson method).

Outcomes and Significance. The June mark-recapture efforts entailed 13 gill-net sets and resulted in the capture of 165 sharks including 128 blacktip sharks. Based on these results, we estimated the number of first-year blacktip sharks in Terra Ceia Bay to be approximately 200. Assuming a mean fecundity of 3.85 pups per litter for blacktip sharks, we estimate approximately 50 gravid females use this region as a pupping area. The combined efforts from July through September (22 gill-net sets) resulted in the capture of 23 sharks with only 7 blacktip sharks. The latter results were uncharacteristically low and thus not sufficient to estimate absolute abundance. A localized toxic algal bloom (red tide) occurred during the study period, which likely affected the distribution of sharks in the immediate area and resulted in lower catch rates during latter months. However, a large number of sharks were still found to use this region early in the summer (when pupping would have occurred) and further influx of young sharks (after pupping had ceased) is unlikely. Therefore Terra Ceia Bay appears to act as an important nursery supporting pups from about 50 females. This result is even more significant if adult females are philopatric and rely on this nursery for their entire reproductive life. Thus, the data from this population estimate provides information on the number of animals relying on this habitat and can be used to define the dynamics of shark nursery areas and the critical nature of this habitat.

1h. Elasmobranch Freshwater Habitat Use.

Objectives. The overall aim of this study was to examine the utilization of southwest Florida freshwater habitats as nursery grounds for bull sharks (*C. leucas*). Specifically, this work used acoustic tracking methods to define bull shark habitat utilization by examining the length of time individuals remained exposed to various salinity levels and individual depth preferences. Further, the project examined the response of sharks to changes in salinity levels within the nursery ground. Freshwater releases from Lake Okeechobee via the Caloosahatchee River are very common and can cause dramatic changes in salinity level. Knowledge of freshwater release dates in combination with continuous monitoring provided information on whether these abrupt changes alter the habits of young bull sharks.

This project also had two secondary objectives. The first was to examine stable isotopes for individual sharks caught in various salinity regimes to define physiological stress associated with residence in those conditions. This work further examined trophic structure and paths of carbon flow in the river via stable isotope analysis. The second was to examine blood parameters for individuals caught in various salinity regimes to define physiological stress associated with residence in those conditions.

Methods. An array of 20 acoustic receivers was deployed along the length of the Caloosahatchee River covering the entire range of the river from the estuarine mouth region to the fully freshwater reaches of the upper river. Receivers were used to monitor and record the movements of neonate and juvenile bull sharks fitted with acoustic transmitters. Bull sharks were collected via longline fishing within the river system. All sharks captured were sexed, measured, weighed and tagged with plastic tags for future identification. Transmitters have an approximate battery life of one year, allowing long-term tracking to be conducted. Acoustic receivers were downloaded every second week to recover relevant data on the movement patterns of sharks. These data were examined to calculate the length of residence within the river, response to freshwater flow and home range size. Detailed analyses of animal association, habitat use and potential feeding areas were conducted.

In addition to passive monitoring of individual movements, bull sharks were also actively tracked for periods of 24 hours. Active tracking transmitters also report the depth the individual

shark is swimming in. During the track, water quality measurements (temperature, salinity, turbidity, dissolved oxygen) were recorded.

Tissue samples from bull sharks were collected for stable isotope analysis by Ph.D. candidate Jill Olin under the guidance of collaborator Dr. Aaron Fisk of the Great Lakes Institute of Environmental Research at the University of Windsor, Canada. Blood samples were collected for analysis by Dr. James Sulikowski of the University of Florida.

Outcomes and Significance. The space utilization and distribution of young (< 2 yr old) bull sharks within a 27 km stretch of the Caloosahatchee River estuary in Southwest Florida was examined using the array of acoustic monitors to define influences of environmental variables. Fifty-six young sharks from three cohorts (2003, 2004, 2005) were fitted with acoustic tags and monitored for up to 379 days. Sharks did not remain within the estuary continuously, but on average approximately one-third was present at any one time from each cohort. Salinity and flow showed greatest influence on shark distribution with temperature appearing to play a limited role. Individuals occurred in salinities from 0.1 to 34.0 ppt, but electivity analysis indicated that they avoided areas with salinity less than 7 ppt and had an affinity for areas with salinities from 7 ppt to at least 20 ppt. There were significant relationships between the mean location of a cohort within the estuary and salinity, with sharks occurring further up river when the river was more saline. These relationships were more pronounced for youngest animals, and decreased with increasing age. Bull sharks are euryhaline and these results suggest that they may select environmental conditions via movement possibly to reduce energetic costs associated with osmoregulation.

Acoustic telemetry was used to examine home range size, small-scale movement patterns, and water quality preferences of juvenile bull sharks. Eight sharks were fitted with acoustic, depth-sensing transmitters and manually tracked for periods up to 24 hours with water quality parameters recorded every 15 minutes. Analysis of movement patterns focused on home range size, rate of movement, swimming depth, linearity of path, direction of travel, tidal influence, and diel patterns. Home range size varied from 1.2 to 4.3 km² and showed significant diel differences. Animals grouped into two sets based on movement characteristics. The first group utilized larger spaces at night accompanied by an increased rate of movement (day = 10.9 m/min, night = 18.8 m/min) and distance traveled during the night. The second group had an increased average speed (day = 10.8 m/min, night = 8.2 m/min), distance traveled, and larger space utilization during the day. All individuals remained closer to the surface during the night (mean = 0.6 m) and utilized deeper depths during the day (mean = 1.5 m). Sharks swam downriver and slightly deeper in the water column (mean = 1.1 m) during a falling tide and upriver and shallower during a rising tide (mean = .9 m). Water quality parameters displayed significant variation between tracks and diel differences within tracks. Tracks 1 and 2 showed a significant relationship between shark location and salinity ($p < .000$), temperature ($p < .000$), and dissolved oxygen ($p = .012$), and the model accounted for .609 of the sample variation. Tracks 4 through 7 were significantly driven by temperature ($p = .017$), DO ($p < .000$), turbidity ($p < .000$) and pH ($p < .000$), with .560 of the sample variation explained.

Tissue and blood samples were collected from bull sharks in 2006 for stable isotope and blood parameter analysis in collaboration with consortium-funded sampling in the Caloosahatchee River for the acoustic monitoring project. Additional tissue samples were collected in 2007 by Ph.D. student Jill Olin and analysis of samples is due to begin in 2008.

Ii. Population Genetics.

Objectives. Examination of the population genetics of blacktip and bull sharks in Florida waters is needed to understand stock structure of these important migratory species in U.S. fisheries. In

collaboration with geneticists at Southern Illinois University (Dr. Ed Heist and students), this study addressed the lack of genetic data shedding light on blacktip and bull shark life history, movement patterns and natal philopatry, information that is needed for federal and state fisheries management. Data collected during this study examined whether blacktip and bull sharks are wide-ranging and genetically mixed, particularly between the Gulf of Mexico and Atlantic Ocean, or if subpopulations and separate breeding stocks exist.

Methods. Small clips of the first dorsal fins of blacktip and bull sharks captured during field work were taken and stored in a buffer solution. Fin clips were collected from 238 blacktip sharks within Florida waters during the course of the project. These samples came from the following locations: the Florida panhandle, 25; Yankeetown, 88; Terra Ceia Bay, 50; Pine Island Sound, 53; Ten Thousands Islands, 4; and the Florida Everglades, 18. An additional 138 fin clip samples were collected from juvenile and adult bull sharks along the Florida coast. These samples included: offshore collections from the Gulf of Mexico, 6; Pine Island Sound, 13; the Caloosahatchee River, 62; the Florida Everglades, 47; and the Indian River Lagoon (courtesy of NSRC partner University of Florida), 10. Captured sharks were sexed, measured and weighed whenever feasible, and in the case of live animals, were tagged and released. Location data were collected at each capture point to help define subpopulations or stock separation. All samples were transferred to the geneticists at Southern Illinois University, where the genetic analyses were performed.

Outcomes and Significance. This effort resulted in the publication of two significant papers on the population genetics of the blacktip shark (Keeney et al., 2003, 2005). In the first study, genetic population structure of this species was investigated using mitochondrial DNA control region sequences. Neonate blacktip sharks were sampled from three nurseries, Pine Island Sound, Terra Ceia Bay, and Yankeetown, along the Gulf of Mexico coast of Florida and one nursery, Bulls Bay, on the Atlantic Ocean coast of South Carolina. Sequencing of the complete mitochondrial control region of 169 neonates revealed 10 polymorphic sites and 13 haplotypes. Overall haplotype diversity and percent nucleotide diversity were 0.710 and 0.106%, respectively. Haplotype frequencies were compared among nurseries to determine if the high mobility and seasonal migrations of adult blacktip sharks have maintained genetic homogeneity among nurseries in the Atlantic and Gulf. Based upon the dispersal abilities and seasonal migrations of blacktip sharks, the genetic results supported the presence of philopatry for nursery areas among female blacktip sharks. Our data also supported the treatment of Atlantic and Gulf blacktip shark nursery areas as separate management units. These results helped form the basis for NMFS to implement separate management of Gulf and Atlantic stocks of the blacktip shark in U.S. waters.

In the second study, the genetic structure of blacktip shark continental nurseries was investigated in the northwestern Atlantic Ocean, Gulf of Mexico, and Caribbean Sea using mitochondrial DNA control region sequences and eight nuclear microsatellite loci scored in neonate and young-of-the-year sharks. Significant structure was detected with both markers among nine nurseries, and sharks from the northwestern Atlantic, eastern Gulf of Mexico, western Gulf of Mexico, northern Yucatan, and Belize possessed significantly different mitochondrial DNA haplotype frequencies. Differences in the magnitude of maternal vs. biparental genetic differentiation supported female philopatry to northwestern Atlantic, Gulf of Mexico, and Caribbean Sea natal nursery regions with higher levels of male-mediated gene flow. It was concluded that philopatry has produced multiple reproductive stocks of this commercially important shark species throughout the range of this study. These results have broad significance for the management of highly migratory shark species that exhibit philopatric behavior.

Genetic analyses of the fin clips from bull sharks are being conducted at the Southern Illinois University laboratory and results have not been published as of the writing of this report.

1j. Elemental Analysis.

Objectives. Bull sharks use low salinity and freshwater areas as nursery grounds. To investigate the amount of time these animals spend in low salinity areas, we attempted use elemental analysis of vertebrae to detect changes in the ratio between strontium and calcium. There is a strong inverse relationship between strontium and salinity, and therefore periods when bull sharks are in low salinity areas will be characterized by high strontium levels in their vertebrae. By taking sequential measurements of elemental composition across vertebrae, time-series of Sr/Ca ratios can be developed. This project strived to develop methods to measure Sr/Ca ratios in bull shark vertebrae as a means of evaluating the nursery habitat use patterns of this species.

Methods. Vertebrae from bull sharks collected in southwest Florida coastal waters were excised and stored frozen. To process the samples further, the vertebrae must be cleaned and dried, before being sectioned and polished. Electron-probe microanalysis (EPMA), laser ablation ICPMS, or Proton microprobe analysis (PIXE) must then be used to determine Sr and Ca levels on transects along the corpus calcarium of the vertebrae. Matching vertebrae are then prepared and aged using traditional shark ageing techniques to provide a time-line for the changes in Sr/Ca ratio. Sr/Ca ratios can then be plotted to identify periods of low salinity and high salinity habitat use.

Outcomes and Significance. To date, vertebrae from three bull sharks have been collected in southwest Florida coastal waters. Samples were frozen and archived at Mote Marine Laboratory. This project is ongoing as samples must be collected opportunistically.

1k. Satellite Tag Testing and Development.

1k1. Experimental PAT Tag Attachment Methods.

Objectives. In recent years, Pop-up Archival Transmitting (PAT) tags have been commonly used to track the large-scale movements and behavior of sharks. However, users of this relatively new technology have commonly experienced less than perfect results. A workshop of satellite tag users held at Mote Marine Laboratory in December 2003 (see section 11) identified tag attachment and tag design as two of the most important challenges facing users of this technology. The impacts of different tag attachment methods, and the impact of the tags on shark behavior, have never been formally assessed. The goal of this work was to assess the behavioral responses and effects of different forms of satellite tag attachment on sharks in a captive environment.

Methods. In 2004, a “dummy” PAT tag was attached to an adult bull shark using a plastic “Christmas tree” tag head to test this form of attachment. This shark was maintained in Mote Aquarium’s 140,000-gallon display tank and observations were made for a total of 4 days. At that point, the shark died as a result of a heavy infestation of monogenean parasites. A similar tag with this same type of tag head was attached to an adult female blacktip shark at the base of the first dorsal fin and maintained in a 40,000-gallon tank within the CSR’s recently constructed Marine Experimental Research Facility (MERF). In September of 2006, PAT tag attachment experimentation resumed using a sandbar shark in a 60,000-gallon tank within MERF. Two tag attachment methods were tested in this trial: 1) a PAT tethered to the first dorsal fin by a rototag and plastic coated wire; and 2) a PAT tethered to the body using a metal-headed dart tag and

plastic coated wire. To determine if the sharks may be removing the PAT tags through contact with the substrate, two 50 kg artificial reef balls were placed in the center of the tank prior to tag attachments. This permitted the shark time to adjust to the new structures and provided a hard, rough surface for the shark to rub against, should it be so inclined. Video cameras were positioned in front of the tank windows to record the shark's behavior around the reef balls for the first eight hours after tagging. Each tag was left on the shark for four weeks and daily observations were made of the tagging wound and the shark's behavior.

Outcomes and Significance. Although the 2004 bull shark trial ended after just 4 days, a necropsy of the shark indicated that tagging had caused a large area of edema around the point of tag penetration, but no other adverse signs were identified. Signs of infection were observed at the point of tag entry on the subsequent blacktip shark trial. Based on these results, it was concluded that the "Christmas tree" tag head was not suitable for PAT tag attachment on these shark species. During the September 2006 trials, there was no attempt by the shark to remove the tag during the first eight hours. In general, the PAT tags wobbled continually when the shark was swimming, creating excessive drag on the attachment area. Infection at the tag attachment area was observed in both trials. This resulted in premature detachment within the four-week trial periods. Overall, these tag retention trials provided evidence that tag loss could be attributed to infection of the attachment location. Consequently, alternative attachment methods have been developed and utilized in subsequent field-testing trials, which have yielded improved results (reported in section 1k3).

1k2. SPOT Tag Attachment Development.

Objectives. Since 2003, a number of PAT tags have been deployed on whale sharks (*Rhincodon typus*) off the Yucatan Peninsula to gain an understanding of the large-scale movements and behavioral ecology of this species. In the early stages of this work, a significant proportion (>50%) of failures resulted from these deployments. A viable alternative for real-time tracking of these surface swimming sharks is a Smart Position Only Transmitting (SPOT) tag. The SPOT can be mounted high on the shark's dorsal fin such that the tag is in the air (and able to communicate with satellites overhead) when the shark is swimming at the surface. However, before these tags could be field-tested, a suitable attachment method had to be devised such that a SPOT could be easily and securely deployed to a free-swimming shark while at the same time causing minimal trauma to the shark's fin.

Methods. The design of the SPOT attachment required accurate measurements of a whale shark's first dorsal fin. The Georgia Aquarium in Atlanta provided the shape and the thickness of the fin measured from one of their captive whale sharks. Based on these measurements, an attachment clamp was designed in a manner similar to a torsion spring that when closed pinches onto the dorsal fin of the shark. Small teeth welded to the spring arms provided stability and a secure connection to the shark. The SPOT tag was attached to a small plate welded to the clamp arm creating a flat durable surface for its long-term deployment.

Outcomes and Significance. A prototype SPOT tag attachment device has been developed and preliminary testing has taken place. Testing has been conducted on a simulated dorsal fin but further measurements regarding the dorsal fin shape is needed to ensure the tag will attach properly to the fin and not cause undue trauma. Furthermore, the thickness and contour of the entire dorsal fin of a whale shark is needed to ensure the tag can remain fixed in an orientation that permits long-term communication with satellites. Field trials will begin once the prototype can be adjusted to address all of these concerns.

1k3. Field Testing of New PAT Tag Attachment Methods.

Objectives. The results of experimental PAT tag attachment on captive sharks were utilized to develop a more reliable method of field attachment for this type of satellite tag. The objective of this project component was to apply these new attachment methods to sharks in a field situation in which the researchers had a high likelihood of observing the sharks and their tags days or weeks post-tagging.

Methods. The alternative attachment method consisted of a metal-headed barb measuring 33 mm x 9 mm (Hallprint Pty. Ltd.) attached to a 6 cm section of nylon-coated wire (120 lb test). A loop was created at the non-barb end of this wire enabling its attachment to a 9 cm section of monofilament (200 lb test). The other end of mono was threaded through the RD1800 (a release device in case of extreme depth) and secured around the corrosible attachment link of an Mk10-PAT (Wildlife Computers). All wire and mono were covered in uniquely colored shrink tubing to protect the attachment, reduce fouling and abrasion, and permit quick visual identification of an individual shark/PAT. A total of 10 PAT tags with this type of attachment were deployed using a pole spear on whale sharks off the Yucatan Peninsula, Mexico.

Outcomes and Significance. All tags were attached easily and securely using the described attachment methods. Because these sharks reside in the Yucatan feeding area for weeks or months, tagged animals can be occasionally observed by collaborating biologists and ecotourism operators in the area. There have been no reports to date of any of these PAT tags causing obvious trauma or necrosis at the point of entry into the shark. Of the 10 tags deployed, two were removed from the shark prematurely by human interference. However, six of the eight remaining tags functioned properly and transmitted archived data after being at large for as long as 157 days and diving to at least 1,376 m. Some of this recent success may be due in part to the newer PAT versions (Mk10), but improvements to the attachment method are believed to be also contributing to these enhanced results.

II. Satellite Tag Workshop.

Objectives. In recent years, satellite telemetry has increasingly been used to track the large-scale movements and behavior of sharks and other large marine vertebrates. However, users of this relatively new technology have reported a significant proportion of tag failures where little or no data has been returned to the user. As a step toward improving this methodology, a forum was organized to bring researchers and tag manufacturers together to discuss the problems and work toward solutions.

Methods. An international workshop to discuss satellite telemetry methodology and its application to the study of sharks was hosted at Mote Marine Laboratory on 3-5 December, 2003. A total of 22 participants attended from four countries and 18 institutions, including representatives from all four NSRC institutions. Participants included researchers from all levels (graduate students to experienced senior scientists) and also included tag manufacturers. The meeting involved detailed discussions about problems with the current technology and discussed ways of solving or avoiding the problems many users are experiencing when deploying this technology on shark species. A detailed survey of user experiences and results was conducted among the workshop participants prior to the meeting. This information was used to lead discussions and make recommendations for current and future use of this technology on elasmobranchs.

Outcomes and Significance. The group made recommendations for development of new tag designs and plans were made for tag testing in laboratory facilities. A summary report from this workshop, including the list of recommendations, was compiled and distributed to all contributors and was published as a Mote Technical Report in early 2004 (Heupel, M.R. and R.E. Hueter. 2004. Sharks and satellite tagging: achieving the potential. Mote Marine Laboratory Tech. Rpt. No. 962, 42pp; available at <https://dspace.mote.org:8443/dspace/handle/2075/224>). This workshop was significant as one of the first to discuss both theory and logistics of satellite tagging in detail with shark researchers and tag manufacturers together. The workshop led to improved communications between research and manufacturing communities.

Im. Mortality Rates of Sharks within a Coastal Nursery Area.

Objectives. Examination of the mortality rates and residency of juvenile sharks within a coastal nursery, Crooked Island Sound, Florida, were conducted in collaboration with the National Marine Fisheries Service Southeast Fisheries Science Center (NMFS SEFSC). This project built upon previous CSR research that showed high mortality rates of young blacktip sharks within a local nursery area. The study site provided a unique opportunity to examine a nursery utilized by multiple species simultaneously, blacktip and Atlantic sharpnose sharks. The main objectives were to define the movement patterns, residency times and mortality rates of coastal sharks utilizing a confined nursery area, and to explore whether mortality rates varied among species.

Methods. An array of 12 acoustic receivers was deployed within St. Andrews Bay (Crooked Island Sound) on the Florida panhandle. These receivers were used to monitor the extent of movements of neonate and juvenile sharks within this nursery area. Sharks were collected by gill net via NMFS SEFSC sampling. All sharks captured were sexed, measured, weighed and tagged with NMFS plastic tags for identification. Up to 25 sharks of each species were fitted with coded acoustic transmitters. Transmitters have an approximate battery life of 2 years, allowing long-term tracking. Acoustic receivers were downloaded once per month to recover relevant data on the movement patterns of sharks. These data were examined to calculate the length of residency, home range size, and mortality rate for each species.

Outcomes and Significance. Blacktip and Atlantic sharpnose sharks in Crooked Island Sound did not respond to environmental factors (i.e., tidal or lunar phase, temperature, salinity, and dissolved oxygen) but appeared to exit and enter the study site randomly. When sharks were present in the bay, spatial use varied by species. Atlantic sharpnose sharks occupied the center of the bay, adjacent to the opening, 53.3% of the time, while spending 24.8% and 21.9% at the shallower extremes, respectively. Conversely, blacktip sharks occupied the center of the bay 33.1% of the time, and the western and eastern extremes 46.1% and 20.8% of the time, respectively. These results differ from those previously obtained for blacktip sharks for a similar-sized bay (Terra Ceia Bay, Florida) where sharks spent over 100 days in residence, and they contradict commonly held theory that sharks remain in distinct coastal areas throughout summer months. Mortality estimates have not yet been calculated for either species, but data analyses are currently ongoing.

Results of this study will be displayed via computer animations of shark movement patterns as in other CSR telemetry projects. This study will provide unique data about young shark mortality rates that will be directly relevant to stock assessments for all of these species. This project strengthened the relationship between MML CSR staff, who handled most of the data analysis, and NMFS SEFSC staff, who performed most of the field effort for this project.

In. Nurse Shark Reproduction, Life History, Genetics and Habitat Studies.

Objectives. The nurse shark, *Ginglymostoma cirratum*, is a common resident of U.S. Atlantic and Gulf of Mexico waters and is frequently caught by commercial and recreational fishermen. It is the shark species most seen by recreational divers and is an important component of the reef environment, competing with lobsters and bony fishes for reef space. In spite of its prevalence and accessibility, information on life history and ecology is limited for this species, which is one of 39 species of large coastal sharks managed by NMFS. The goal of this study was to reveal the reproductive habits, nursery grounds and use of Essential Fish Habitat of the nurse shark, to provide for successful management. Primary objectives were: 1) determine the temporal and spatial movement patterns and habitat preferences of individual reproductively active male and female nurse sharks; 2) document the annual and biannual sexual cycle of adult nurse sharks; 3) determine relatedness as it pertains to reproductive functions (paternity, dominance, avoidance, cooperative behaviors and mating site fidelity) by collecting genetic samples from neonate, juvenile and adult nurse sharks from the Dry Tortugas and the Lower Keys; and 4) investigate habitat preferences of mating and birthing female sharks and their offspring (mating and nursery grounds). Because all stages of the nurse shark are resident in the Dry Tortugas archipelago, the area is a natural laboratory for studying reproduction in the nurse shark. This study extended cooperative research conducted since 1991, with colleagues from Albion College, University of Southern Illinois, Sea World of Florida, NMFS NEFSC and SEFSC, National Geographic Society, Florida International University and other institutions.

Methods. Year-round sampling and observations of all life stages of the nurse shark were conducted in the Dry Tortugas and the Lower Florida Keys. Surveys were aided by private and commercial charter boats usually in January, March, June, July, October and November of each year. Over the three years of this study from 2004-2007, a total of 179 days were spent on-site in the Dry Tortugas. Animals were caught with specially designed dip nets and seines to minimize disruption. June and October trips coincided with important reproductive events in the lives of the adults. Neonates and other juveniles were sought, captured and tagged during other trips. Daily observations of activities and habitat preferences were made from temporary towers while on station. Instruments to record shark activities and environmental data were permanently placed and serviced in January, May, and October of each year. Tagging was used to study the seasonal movements of the nurse sharks inhabiting the Lower Keys and Tortugas. All captured live sharks were tagged with MML conventional fin tags (such as rototags) before release; some sharks were double-tagged with NMFS tags. Mature nurse sharks were tagged with Vemco R-coded transmitters to investigate the reproductive movements in and out of mating habitats. An array of twelve hydrophone listening stations was deployed around Garden, Bush, Long and East Keys. "Cittercam" underwater cameras were used to view the *in situ* activities of mating sharks. Examination of the population genetics of nurse sharks in the Lower Keys has been underway since 2000. During this project, genetic samples were provided to Southern Illinois University (Dr. Ed Heist).

Outcomes and Significance. Over the course of the full study from 1993-2006, a total of 249 nurse sharks (109 adults and 140 juveniles) in the Dry Tortugas population were tagged. Eighty-six different sharks have been recaptured by hand net to date (34.5% recapture rate). Several were physically recaptured seven or more times. Of the identified adults, 35 were subsequently sighted (visually recaptured or netted) at least once, and some much more frequently. Tag retention studies on five nurse sharks held captive at the Mote Summerland Key Lab and at Mote's Key West facility showed that aquarium tag retention is as high as in the field. Adult 2.5m sharks mate on the shallow grass flats of the Dry Tortugas in June and July. Hundreds of courtship events were observed and documented by the CSR biologists. Pregnant females use the

shallow flats in the study site as late as mid-November while they await parturition. In past years we have counted over 60 large, rotund “egg case-passing” females in the reproductive refuge. Our hypothesis is that June-mated sharks return to the sunny fall shallows for environmental thermoregulation. Adult males depart after the June-July mating season and some return to the study area in the winter months. Observations from tagging and natural markings indicate that most adult males visit the study site faithfully every mating season. The reproductive significance of these philopatric movements and the site fidelity of both sexes to our identified research area are unprecedented in any species of recorded shark and underscore the uniqueness of this work. In July 2005, Hurricane Dennis was the first of four hurricanes to directly impact the Dry Tortugas. We placed 16 coded transmitters on mating sharks just before this storm hit the Tortugas. Data from these transmitters showed that shallow water mating by the sharks was only delayed, not stopped, by the storm. Mature female sharks arrived in the fall after hurricane Katrina and stayed in the shallow refuge through the fall of 2005, making brief departures for the passing of hurricanes Rita and Wilma.

10. Whale Shark Research in the Gulf of Mexico and Caribbean Sea.

Objectives. In Gulf of Mexico waters northeast of Mexico’s Yucatan Peninsula, a large gathering of whale sharks (*Rhincodon typus*) occurs every spring and summer from May through September. Given the highly migratory nature of this largest of all fishes, the paucity of data on this protected species, and its emerging value in the Gulf through ecotourism, a whale shark research and conservation project was initiated by scientists from Mote’s CSR in collaboration with Mexican scientists and resource managers with the Comisión Nacional de Áreas Naturales Protegidas (CONANP) of the federal Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT) in Cancun, Quintana Roo. This project is the first scientific effort to document the size and characteristics of this aggregation and study these animals up close. The goals of this study are several: 1) to better understand the basic biology of this largest of shark species in its natural environment; 2) to explore the connectiveness of Gulf, Caribbean and other oceanic habitats in the life history of whale sharks; 3) to develop resource management and ecotourism plans for conservation of this unique species in Mexico and the U.S. (This field research was partially funded through the NSRC; the majority of support came from other sources.)

Methods. Study methods for this project have included aerial surveys, on-water surveys, oceanographic measurements, shark size/sex determination, spot pattern photo ID, feeding behavior analyses, plankton tows, genetic tissue sampling, and both visual and electronic tagging. Part of the CSR’s role in this collaboration is to provide expertise in the shark-tagging component. A prototype visual tag was first developed in 2003 and was used through 2007 with only minor modifications. Acoustic telemetry was used in a pilot effort to examine the home range size and small-scale movement patterns of these animals. Pop-up Archival Transmitting (PAT) satellite tags (Wildlife Computers) were used to track the large-scale movements and behavior of these sharks. These efforts have benefited by the CSR’s work to improve the attachment methods used for these types of satellite tags (reported in sections 1k1 and 1k3).

Outcomes and Significance. Whale sharks ranging in size from 1.5-13 m TL are present at the site north of Isla Holbox, Cabo Catoche and Isla Contoy from May through September each year. The aggregations comprise both immature and mature animals of both sexes. They do not appear to be feeding ephemerally on reef fish or coral spawn as in other areas but more generally on plankton for long periods. Plankton tows conducted at the site have revealed relatively common species of calanoid copepods, crab zoeae, sergestids and chaetognaths. During the period of this report, 568 whale sharks were visually tagged; preliminary estimates of the total size of the

summer aggregation range between 500-1,500 sharks. Two of the visually tagged animals have been reported off Utila, Honduras, about 600 km south in the western Caribbean.

A total of thirteen PAT satellite tags were deployed on whale sharks ranging from 4.5 to 8.5 m in total length. Detailed data reports have been received from eight of these tags demonstrating movements from the tagging site into the western Gulf of Mexico near the Texas/Mexico border, south into the Caribbean, and eastward into the Florida Straits between the Florida Keys and Cuba. These results indicate that the Yucatan site serves as a summer feeding ground for whale sharks ranging broadly over the Gulf and western Caribbean. Analysis of the depth data logged on these tags has demonstrated that these sharks perform regular dives to significant depths. One PAT tag recorded a dive to at least 1,376 m, possibly the deepest dive for a whale shark on record. Acoustic telemetry was used to actively track one shark for a period of 4½ hrs; the shark moved 8.6 km at an average speed of 1.9 km/hr. Additionally, efforts to develop attachment methods for Smart Position Only Transmitting (SPOT) tags on whale sharks have been undertaken. This type of tag is a viable option for the real-time satellite tracking of these surface swimming sharks (see section 1k2).

Mote scientists also participated in an integrative, multinational workshop on the conservation and management of whale sharks in the Gulf and Caribbean (29-31 August 2005, Isla Holbox, Mexico). This meeting brought together resource managers, scientists and ecotourism operators to develop management strategies to ensure the regional, multilateral protection of this species.

1p. Shark Navigation and Orientation Experiments.

1p1. Translocation Studies in Terra Ceia Bay, Florida.

Objectives. Evidence of philopatric behavior in diverse species of sharks is accumulating through various sources of data including studies of shark behavior, genetics and fisheries. If sharks display natural tendencies to return to specific home areas, birthplaces or other adopted localities during portions of their life cycles, as opposed to roaming and dispersing throughout their overall ranges, the impact of fisheries removals and habitat alterations on shark populations and stocks could be profound, and the use of shark catch data to assess stocks could be complicated. The goal of this study was to examine the degree of fidelity of individual sharks to remain in, or return to, critical habitats.

Methods. Neonate and YOY blacktip sharks in Charlotte Harbor, Florida, were captured and transported with established methods of live animal maintenance, and introduced into an equivalent habitat and documented blacktip shark primary nursery in Terra Ceia Bay, approximately 145 km north of Charlotte Harbor. Transported sharks were outfitted with acoustic transmitters according to standard CSR methods successfully used on this species and size/age for many years. Control experiments were conducted by collecting YOY blacktips from Terra Ceia Bay, holding them in a captive maintenance tank for a time equivalent to the transport time between Charlotte Harbor and Terra Ceia Bay, fitting them with transmitters and then re-introducing them back into the bay. Movements of acoustically tagged animals' were passively tracked using a system of underwater acoustic receivers in Terra Ceia Bay. Transmitters had an approximate battery life of two years, allowing long-term tracking to be conducted. Acoustic receivers were downloaded every second week to recover relevant data on shark movement patterns.

Outcomes and Significance. In May of 2005, a series of 24 acoustic hydrophones were deployed within Terra Ceia Bay to monitor the presence and movements of sharks from both nurseries. A

total of 9 blacktip sharks were successfully transported from Charlotte Harbor to Terra Ceia Bay. After transmitters were attached, sharks were released within the study site. Six blacktip sharks were captured within Terra Ceia Bay and treated as control animals for translocation experiments. All 15 sharks survived transport and surgery procedures and were released within the study site. All sharks were monitored via the acoustic monitoring network and none suffered mortality immediately post-release. Individual sharks showed varying movement patterns with translocated sharks tending to use more of the study site than native sharks. However, only two weeks worth of data were collected with all sharks present within the study site, when an intense toxic red tide algal bloom moved into the study site causing large-scale mortality of sharks, rays and teleosts. All individuals fitted with transmitters died during this event and no further data were collected that year.

Hydrophones were again deployed in May of 2006 and were similarly used to monitor the presence and movements of sharks from both nurseries. A total of 10 local blacktip sharks (control animals) were released into the northern end of the study site while 5 individuals were successfully transported from Charlotte Harbor to Terra Ceia Bay. All 15 sharks survived transport and surgery procedures and were released within the study site. Data from acoustic receivers were gathered to examine the movement and behavior patterns of resident and translocated individuals. However, as was the case in 2005, the study was impacted by a toxic red tide algal bloom which caused mortality and/or immediate departure of the sharks from the study site. The resulting analyzed movement data were inconclusive.

The data gathered from this study were intended to be compared with published accounts of YOY blacktip shark patterns of association, habitat use and migratory movements in Terra Ceia Bay, to determine the impact of the translocation from the natal nursery to a “foreign” nursery. The brief glimpse of movement data collected prior to algal blooms revealed introduced sharks were using more of the study site than native animals, suggesting some differences in behavior and habitat use were occurring. Although this study’s limited data were not useful to address the questions posed, the successful translocation of individuals indicates that this type of study can be successfully carried out and useful results obtained.

1p2. Sensory Capabilities of Captive Sharks.

Objectives. This study investigated the tendency of sharks to return to specific home areas using laboratory studies of mechanisms underlying philopatry in sharks, to establish the physiological and behavioral basis for this behavior. Recent studies by CSR scientists and others have established that sharks are sensitive to such environmental cues as barometric pressure and day-length, and these cues help to stimulate short-term movements and long-term migrations. Specifically, this project investigated the sensory capabilities of sharks to orient and navigate in the sea in response to external cues from the environment.

Methods. Using the CSR’s Marine Experimental Research Facility (MERF), captive sharks were tested for their ability to orient to, and localize the source of various sensory stimuli that may be involved in important behaviors including prey detection, avoidance of obstacles and navigation to specific habitats. Multisensory integration (olfaction, audition, vision, mechanoreception and electroreception) in sharks was tested in MERF using large and small coastal species available in the area. The large MERF tanks are outfitted with several acrylic observation windows for photographic and video documentation of animal behavior in the tanks. An overhead camera system was used to document animal responses to sensory stimuli. Experiments have been designed to provide statistically testable results. These experiments have been conducted in collaboration with several scientists from other institutions, including: Jelle Atema, Marine Biological Laboratory, Woods Hole (olfaction); David Mann, University of South Florida (audition); and Stephen Kajiura, Florida Atlantic University (electroreception).

Outcomes and Significance. For captive experimental studies on shark navigation and orientation, collecting trips were conducted to obtain ten blacktip sharks and ten bonnetheads. These animals were collected from local waters around the Tampa Bay area using standard CSR fishing gear and transported to MML in coolers with oxygenated seawater. To provide an experimental tool for studying the sensory capabilities of various shark species, a 50ft x 8ft x 6ft research flume was constructed within the large research tank of MERF. This flume is designed to create a laminar flow of water and constructed using non-metallic materials to eliminate sensory interference. In addition, the flume is equipped with observation windows for photographic and video documentation of animal behaviors. Researchers use this flume to examine the sharks' abilities to integrate information from multiple senses during important behaviors of prey detection and navigation, and to determine the spatial scale at which the different senses come into play. Currently our understanding of sensory biology is largely due to studies of individual senses, studied in isolation from one another. This project is ongoing and represents groundbreaking research that will elucidate thus far unknown sensory solutions to complex navigation problems in marine animals.

Iq. Habitat Utilization by Large Predatory Sharks.

Objectives. This study aimed to examine the short- and long-term location and movement of bull sharks along the Gulf of Mexico coast of Florida in order to help identify migratory corridors, depth preferences and essential habitats of this species. Defining the habitat utilization of bull sharks within coastal waters is essential for the management of this species, which is both the target of shark fisheries and is implicated in rare attacks on humans.

Methods. Adult *C. leucas* were collected via drumline fishing off Sarasota, Florida during CSR quarterly surveys targeting large coastal sharks. The gear was set at least twice each day in at least two depth strata at four sites. Hooks were baited with teleost, shark or ray bait. Lines were soaked for approximately four hours before being retrieved. Sharks caught were identified, measured, sexed, tagged and released. All live sharks were tagged with an appropriate external tag. Bull sharks greater than 150 cm TL were fitted with PAT tags that archive light, depth and temperature data. Satellite tags were attached by anchor through the skin, muscle and cartilage below the first dorsal fin. Summaries of archived data were transmitted via the ARGOS satellite system after tags detached from the animals at a user-specified date and time. Tags were programmed to release from animals at intervals of one month or more depending upon circumstances. Light data downloaded from the tags via satellite were used to provide estimates of longitude. Depth and temperature data summaries were examined.

Outcomes and Significance. Two mature bull sharks, one of each sex, were captured via drumlines off Longboat Key, FL on September 26, 2006, and outfitted with Wildlife Computers Mk10 PAT tags. As programmed, the tags released from the sharks after one month and transmitted summaries of their archived data to ARGOS satellites where this information was then downloaded by CSR biologists. Preliminary analyses revealed movements of at least 200 km from the tagging site and dives down to 32m.

Ir. Publications and Conferences. During the five years of this project, MML CSR scientists produced 102 scientific publications, 44 technical reports and three theses (see Appendix). CSR staff participated in 51 international, national and regional conferences and workshops and seven NMFS Highly Migratory Species Advisory Panel meetings.

**MOSS LANDING MARINE LABORATORIES
PACIFIC SHARK RESEARCH CENTER**

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2a. Eastern North Pacific Chondrichthyan Life History Data Matrix.

Objectives. One of the primary objectives of the PSRC over the past five years was the creation of a comprehensive Life History Data Matrix covering the entire body of literature on all chondrichthyans (sharks, rays, and chimaeras) from the eastern North Pacific (ENP). The LHDM was created as a queriable web-based tool that would serve as a valuable resource and source of information on ENP chondrichthyans for interested biologists, students, and the general public. The matrix was design to provide comprehensive information on individual species and clearly indicate which aspects of their life history and distribution remain unknown. A review of the matrix, therefore, allows NSRC personnel, fisheries biologists, and students to quickly assess the gaps in biological knowledge for an individual species and to better develop studies that address this lack of critical information.

Methods. The LHDM was modeled after a previous version, completed in 2001, on the life histories of 124 common nearshore fish species of California for the California Department of Fish and Game, with support from the Pacific States Marine Fisheries Commission. PSRC researchers surveyed, among others resources, the electronic databases of Aquatic and Fisheries Science Abstracts, Biosis, and Zoological Record for published references pertinent to chondrichthyans known from the ENP. Bound volumes of the Science Citation Index and literature cited sections of accumulated references were examined to obtain references published prior to 1970. Details pertaining to longevity, fecundity, gestation period, growth, age and size at maturity, size at birth, mortality, feeding habits, distribution, movement patterns, habitat use, maximum recorded size, and population structure were obtained from the literature and entered into a queriable database.

Outcomes and Significance. The resulting LHDM was completed in 2004 and made available via the world wide web on the PSRC web site for state and federal fisheries management agencies and the general public (<http://psrc.mlml.calstate.edu>) and can be downloaded. The LHDM was compiled, organized, and edited, using all pertinent regional literature to determine what is known and, more importantly, what is not known about the life history, distribution, and population biology of ENP chondrichthyans. The LHDM includes 106 species of chondrichthyans reported to occur in the ENP. The ENP as defined here includes the area ranging from the eastern Bering Sea to the southern tip of the Baja California peninsula. The chondrichthyan data matrix covers 22 families, 56 species of sharks, 11 families and 46 species of batoids, and two families and four species of chimaeras. The LHDM serves as an important resource tool for PSRC scientists to develop research projects, many of which are carried out as Masters Theses by graduate students, and to develop additional grant-funded research projects. PSRC scientists and graduate students are now working on research projects to fill gaps uncovered by this extensive literature search. The LHDM will be updated over the next five years as new information becomes available. Given the success and valuable resource that the LHDM has become, we envision expanding this

tool over the next five years to eventually include all chondrichthyan species known to occur in North American waters.

2b. Age, Growth, and Demographic Studies.

Objectives. The objectives of this research component are to initiate studies on the age and growth of rays and skates that are common direct or indirect components of eastern North Pacific fisheries. We will determine if vertebrae, or other structures such as caudal thorns, serve as reliable ageing structures for the roughtail, *Bathyraja trachura*, and California, *Raja inornata*, skates. The population dynamics of commercially exploited rays will be further examined using age-based demographic models. Net reproductive rates, intrinsic rates of increase, generation times, and stable age distribution will be calculated for the diamond stingray, *Dasyatis dipterura*. A demographic comparison of myliobatoid and rajoid rays will be initiated.

Methods. Samples for age and growth studies were collected from commercial fisheries, NMFS and Alaska Department of Fish and Game survey cruises. Each specimen examined were sexed, measured to the nearest centimeter, and weighed to the nearest 0.1 kg. A minimum of eight vertebrae were excised from the region posterior to the cranium between the 5th and 20th vertebral elements. Caudal thorns were removed from the tail. Confirmation of the periodicity of growth increment deposition for all size classes was attempted using marginal increment and centrum edge analyses. Age-at-size estimates based upon microscopic examination of vertebrae and caudal thorns were compared using the average percent error index, index of precision, and tests of symmetry to determine the reproducibility and similarity of estimates between structures. Multiple growth models (including von Bertalanffy, Gompertz, and logistic) were fit to age-at-size data to determine the most appropriate model for each species and sex. Using the information provided from the above life history studies, we will begin to model demographic parameters, including net reproductive rates, generation times, intrinsic rates of increase, and stable age distributions of Pacific sharks using life history table analysis and age-based stochastic matrix demographic models. We will also conduct sensitivity and elasticity analyses to determine the most sensitive life stages of select species of Pacific sharks. These approaches are ideally suited to sharks and rays due to the lack of or uncertainty in life history parameters available for most species. Deterministic and stochastic demographic models will be developed in an effort to best predict the population status and future trends of Pacific sharks.

Outcomes and Significance. Age determination and validation studies are continuing to fill some of the gaps in the life history of poorly studied species. To date, the roughtail study has been completed and published. Other studies completed and currently being written up for publication include the sandpaper (*Bathyraja kincaidii*), California, and longnose (*Raja rhina*) skates. Age estimates have been calculated for all of the aforementioned species with ages at 50% maturity estimated for the roughtail skate at 13 years for females and 14 years for males, sandpaper skate at 6.8 years for females and 7.3 years for males, California skate at 8 years for females and 7 years for males, and longnose skate at 16 years for females and 14 years for males. Roughtail skates were found to reach a maximum age of 17 years for females and 20 years for males. The best model fit for describing roughtail skate growth was the two parameter von Bertalanffy ($TL_{\infty} = 101$ mm, $k = 0.09$); there was no statistical difference between female and male growth rates. Age estimates from vertebral centra for the sandpaper skate indicates a minimum longevity of 17 years for males and 18 years for males. Females ($TL_{\infty} = 537$ mm, $k = 0.237$, $t_0 = -1.629$) grew faster but males ($TL_{\infty} = 580$ mm, $k = 0.185$, $t_0 = -2.530$) reached a larger size. There was no significant difference found between the growth rates of female and male sandpaper skates. The maximum estimated age for the California skate was 13 years. Female and

male California skates grow at similar rates which were best described by the Gompertz growth model ($TL_{\infty} = 726$ mm, $g = 0.18$, $TL_0 = 239$ mm). Indirect methods of age validation were inconclusive for this species. In comparison to other elasmobranchs, these life history characteristics indicate a moderate growth rate and longevity for these species.

The growth characteristics of the California skate were estimated using three ageing structures and seven different growth models. The suitability of neural arches and caudal thorns as alternative ageing structures was examined. Neural arches appear to be poorly calcified and lack patterns that could be consistently interpreted for age estimation. Our analysis suggests that caudal thorns of this species grow at differential rates or are replaced and therefore also do not appear to be a suitable ageing structure for the California skate. Age estimates using caudal thorns for the roughtail and sandpaper skates also appear to underestimate age relative to band counts taken from vertebral centra. Of the three skate species whose caudal thorns we have examined to date this structure has been found to be unsuitable for these species.

The sandpaper skate, roughtail skate, and longnose skate age and growth studies were completed as Masters Theses. The longnose skate was part of a collaborative effort between PSRC personnel and researchers at Oregon State University. Results from the roughtail skate study were presented at an international symposium on the “*Biology of Skates*” and was published as part of a special volume that resulted from this symposium. The sandpaper skate paper has been accepted for publication and the California and longnose skate studies are presently being written up for publication.

Age-structured demographic models were developed for the diamond stingray which incorporated uncertainty in life history parameters. Projections generated through the incorporation of Monte Carlo simulations produced mean population growth rates of 1.05-1.06 (5-6% increase) per year, net reproductive rates (R_0) of 2.3-2.4, and generation times (\bar{A}) of 14.9-16.5 years. The introduction of a low fishing mortality into probabilistic models produced mean population growth rates of 1.01 yr^{-1} . Elasticity analysis indicated that population growth rates of diamond stingrays are much more strongly influenced by the survival of juvenile and adult stages than by survival of neonates or changes in fecundity. Deterministic and probabilistic demographic analyses indicate that diamond stingrays have a low intrinsic growth potential and a limited resilience to fishing pressure. Localized depletion or population collapses are therefore likely to occur in response to unrestricted, unmonitored fishing effort on these stingrays. Demographic analyses of California, roughtail, and sandpaper skates based on vital rates will be calculated in the future by PSRC personnel. Results from this portion of the study will provide critical baseline information necessary for the effective management of these skates.

Training in age and growth techniques was provided to federal and foreign fisheries biologists and student researchers. This technical support enabled a publication on the growth of big skate (*Raja binoculata*) and longnose skate from the Gulf of Alaska by NMFS Alaska Fisheries Science Center personnel and two Masters Theses by students at Oregon State University and the University of Washington.

2c. Age Validation Studies.

Objectives. The objectives of this study were to provide validated growth rates and estimates of longevity for the white shark, *Carcharodon carcharias* and shortfin mako, *Isurus oxyrinchus*. We collaborated with Dr. Lisa J. Natanson (NMFS, Rhode Island), George Burgess, and Dr. Frank Snelson (University of Florida) on radiocarbon age validation of several other east coast species, including the tiger (*Galeocerdo cuvier*) and sandbar (*Carcharhinus plumbeus*) sharks. Questions to be addressed by PSRC personnel through the course of next year’s research were: 1) do the vertebrae of *C. carcharias* and *I. oxyrinchus* record and preserve a record of marine

radiocarbon (^{14}C) pulse and 2) do the ^{14}C assay results support previous age estimates of *C. carcharias* and *I. oxyrinchus*?

Methods. The interpretation of vertebral growth bands to estimate growth and life history parameters is a subjective process that requires validation to determine the temporal nature of growth zone formation. The accuracy of age estimates often remains unconfirmed and may lead to inappropriate management decisions. The PSRC will continue to advance a novel technique for age validation through the analysis of radiocarbon isotopes from vertebral centra. Radiocarbon assays were conducted for all four species to provide a basis for definitive growth models. Samples for radiocarbon age validation studies were obtained from archived vertebrae collections, commercial fisheries, and NMFS personnel. Vertebrae were transversely sectioned using the thin-section technique and independent age estimates were made from microscopic examination. A growth band pair was defined as one translucent and one opaque growth band. Selected individual growth band pairs were sampled from thin-sections of the corpus calcareum using a New Wave micro-milling machine with a small-scale end mill. The width of growth band pairs was used to guide extraction and minimize the amount of older or younger material incorporated in the sample. Hence the amount of material extracted decreased as growth slowed and band width decreased. The first growth band pair past the birth band (estimated as the first year of growth after birth) and one to three subsequent growth band pairs further up the corpus calcareum were extracted for analysis from each vertebrae. The last band pair, corresponding to the last year of growth, was targeted to provide a sample where time of formation was constrained by the collection date. In some cases, the penultimate growth band pair was targeted because the width of the last growth band pair provided insufficient sample size. Extraction of the sample with the micro-mill resulted in a solid sample of material that was weighed to the nearest 0.1 mg. Radiocarbon levels were determined using accelerator mass spectrometry at the Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory. The ^{14}C values were reported as D^{14}C . A qualitative comparison of the shark D^{14}C record was made with existing marine records including two otolith-based records, the yelloweye rockfish, *Sebastes ruberrimus*, and Pacific halibut, *Hypoglossus stenolepis*, and three vertebra-based shark records, the western North Atlantic porbeagle, *Lamna nasus*, western North Atlantic shortfin mako, and the western South Pacific school shark. The validity of dates of growth increment formation assayed to differing degrees of success for each species using the appropriate regional radiocarbon chronology, with a comparison to other global radiocarbon record sources as well.

Outcomes and Significance. Results have been published or are in press for three of the four species studied to date. The findings for each is listed here specifically as the abstract from the publication.

White shark: The white shark has a complex life history that is characterized by large scale movements and a highly variable diet. Estimates of age and growth for the white shark from the eastern North Pacific Ocean indicate they have a slow growth rate and a relatively high longevity. Age, growth, and longevity estimates useful for stock assessment and fishery models, however, require some form of validation. By counting vertebral growth band pairs, ages can be estimated, but because not all sharks deposit annual growth bands and many are not easily discernable, it is necessary to validate growth band periodicity with an independent method. Radiocarbon (^{14}C) age validation uses the discrete ^{14}C signal produced from thermonuclear testing in the 1950s and 1960s that is retained in skeletal structures as a time-specific marker. Growth band pairs in vertebrae, estimated as annual and spanning the 1930s to 1990s, were analyzed for $\Delta^{14}\text{C}$ and stable carbon and nitrogen isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). The aim of this study was to evaluate the utility of ^{14}C age validation for a wide-ranging species with a complex life history and to use stable isotope measurements in vertebrae as a means of resolving complexity introduced into the ^{14}C chronology by ontogenetic shifts in diet and habitat. Stable isotopes provided useful trophic

position information; however, validation of age estimates was confounded by what may have been some combination of the dietary source of carbon to the vertebrae, large-scale movement patterns, and steep ^{14}C gradients with depth in the eastern North Pacific Ocean.

Shortfin mako: Age estimation is an issue for the shortfin mako because of disagreement on vertebral band-pair deposition periodicity. In the 1950s–1960s, thermonuclear testing released large amounts of radiocarbon into the atmosphere, which diffused into the ocean through gas exchange. This influx created a time-specific marker that can be used in age validation. Annual band-pair deposition in the Atlantic porbeagle was validated in a previous study and indicated preliminary annual deposition in the shortfin mako, using four samples from one vertebra. In the present study, age estimates from 54 shortfin mako vertebrae collected in 1950–1984 ranged 1–31 years. Ageing error between readers was consistent, with 76% of the estimates ranging within 2 years. Twenty-one $\Delta^{14}\text{C}$ values from eight shortfin mako vertebrae (collected in the western North Atlantic in 1963–1984) ranged –154.8 to 86.8. The resulting conformity with the $\Delta^{14}\text{C}$ timeline for the porbeagle supported annual band-pair deposition in vertebrae of the shortfin mako.

Tiger shark: Age and growth estimates for the tiger shark were derived from band counts of 238 sectioned vertebral centra. Bomb radiocarbon analysis of ten band pairs extracted from four vertebral sections suggested band pairs are deposited annually up to 325 cm FL. Males and females were aged to 20 and 22 years, respectively, although longevity estimates predict maximum ages of 27 and 29 years, respectively. Two-parameter von Bertalanffy growth functions fit to length at age data demonstrated that growth rates were similar for males and females up to around 200 cm FL after which male growth slowed. Both sexes appear to reach maturity at age 10. Von Bertalanffy parameter estimates were $L_{\infty} = 331$ cm FL, $k = 0.129$ for males and $L_{\infty} = 352$ cm FL, $k = 0.119$ for females, with L_0 set at 62 cm FL.

Sandbar shark: The distribution of radiocarbon data for the sandbar shark in this study was very similar to that of the white shark. It was difficult to come to any kind of conclusion about age relative to the radiocarbon findings; however, it implies that radiocarbon sources for sandbar shark were similar in terms of ontogeny and/or trophic position. Further study is recommended, perhaps with stable isotopes, to determine more about these findings.

2d. Reproductive Biology.

Objectives. Knowledge of the reproductive biology of most eastern North Pacific skates and rays is either unknown or extremely limited. Of the more than 15 skate species that are reported from the region, limited information exists on only two; the big skate and the longnose skate. We intend to complete a detailed study of the reproductive cycle of the sandpaper, California, and longnose skates and determine maturity and reproductive patterns for these skates in relation to season, size, and depth of occurrence. In addition, we examined and compared the reproductive biology of a suite deep-living skates, *Bathyraja* spp., from the eastern Bering Sea.

Methods. Members of the PSRC in collaboration with the NMFS Santa Cruz Laboratory, Northwest Fisheries Science Center, the Alaska Fisheries Science Center, and the Alaska Department of Fish and Game collected and examined the reproductive tracts of skates taken during research cruises in the eastern Bering Sea and off the California, Oregon, and Washington coasts. Specimens were sexed, measured to the nearest centimeter, and weighed to the nearest 0.1 kg. Oviducal glands, ovaries, uteri, and testes were measured and rated based on gross morphology. Egg cases, when present, were examined and preserved. The relationship between total length and clasper length was determined for males. Temporal variation in gonad and liver weights was assessed in relation to size, sex, and reproductive condition. The number of ovarian eggs, uterine eggs, and oviducal gland width was plotted against total length and season to

determine the maturity and the reproductive cycle. Size-at-first, 50%, and 100% maturity was also examined.

Outcomes and Significance. The reproductive cycles of the sandpaper, rougtail, California, and longnose skates were completed during this report period. The reproductive cycle of the big skate from off central California is still ongoing, while a study on this species in the Gulf of Alaska was completed. Median size at maturity for female and male skates, respectively, were estimated for sandpaper skate at 467 mm TL and 492 mm TL, rougtail skate at 770 mm TL and 720 mm TL, California skate at 563 mm TL and 502 mm TL, and longnose skate at 802 mm TL and 696 mm TL. In addition, we examined a suite of skate species from the eastern Bering Sea and reported on their reproductive biology. Eight softnose (*Bathyraja* spp.) skate species, Aleutian (*B. aleutica*), Bering (*B. interrupta*), Commander (*B. lindbergi*), white-spotted (*B. maculata*), whitebrow (*B. minispinosa*), Alaska (*B. parmifera*), mud (*B. taranetzi*), and rougtail, were collected along the eastern Bering Sea Continental Slope and examined for reproductive information. The sex ratio for six of eight species was approximately 1:1. Five of eight species examined approximately equaled or exceeded their previously reported maximum length. Maximum length for three species revealed that the largest individuals were males and that in three other species both females and males grew to approximately the same size. Both females and males matured at approximately the same L_T for seven of eight species. Only female Aleutian skates matured at a much larger size than males. All eastern Bering Sea skate species matured within a narrow size range following a protracted juvenile stage and once mature these skates appear to grow very little. First maturity for all species occurred at $\geq 80\%$ of their maximum L_T . The number of oocytes did not significantly differ between the left and right ovary for any species. The total number of oocytes increased with L_T for six of eight species examined. The number of oocytes for one species increased in number until 145 cm in length, and then declined in those individuals greater in length. The egg cases of all species were described, of which four species had never been previously reported. Evidence was presented that suggest skates may be limited to as little as one to as many as 13 spawning seasons, depending on the species, and that senescence may occur in older individuals. Based on our overall findings from these various studies it appears that in many cases female and male skates mature at a similar size and in most species that we have studied they do not appear to have a defined reproductive cycle, but rather deposit egg cases throughout the year. This is contrary to most viviparous elasmobranchs.

A study on the reproductive biology and distribution of eastern North Pacific deepsea catsharks (Scyliorhinidae) was completed as a Masters thesis. Information and results on the distribution and reproduction of these catsharks were generated from specimens collected by fishery-independent survey cruises from June 2001 through October 2004 between northern Washington and San Diego, California. Longline catches consisted mainly of filetail catsharks (*Parmaturus xaniurus*), with gravid female brown catsharks (*Apristurus brunneus*) occasionally. Conversely, trawl catches consisted mainly of *Apristurus* species. Brown catsharks were typically found between 300 and 942 m, whereas longnose catsharks (*A. kampa*) occurred $> 1,000$ m depth. Filetail catshark were caught between 300 and 550 m depth. Total length at first, 50% and 100% maturity were determined for males and females of all three species. At higher latitudes, brown and filetail catsharks reached sexual maturity at larger sizes. Brown and filetail catsharks reproduce year-round, based on the temporal occurrence of gravid females and the lack of seasonal variation in gonadosomatic (GSI) and hepatosomatic indices (HSI) for both males and females. Gravid longnose catshark females were found from July through December. The egg case of the longnose catshark has been described and its morphology compared to the egg cases of the brown, filetail and other *Apristurus* species.

Two studies, one each on the rougtail and sandpaper skates, were completed as Masters Theses, and along with studies on the California and longnose skates are being written up by PSRC staff for publication. Additional studies on the reproductive biology of other eastern North

Pacific chondrichthyans, including the big and starry (*Raja stellulata*) skates and white-spot chimaera (*Hydrolagus colliei*), are continuing.

2e. Feeding Ecology.

Objectives. Chondrichthyans play important roles in marine ecosystems, often occupying the highest trophic levels as apex predators. Despite the fundamental importance of trophic relationships in understanding food web dynamics, community structure, and energy transfer in marine systems, quantitative dietary information is unavailable for most chondrichthyan species in eastern North Pacific waters. The objectives of this research component were to initiate food habit studies of the skates (*Bathyraja* and *Raja* spp.) and catsharks (*Apristurus* and *Parmaturus* spp.) most commonly taken in the central California groundfish fishery. The diets and dietary overlap of four co-occurring rays in Mexican waters—the diamond stingray, California butterfly ray (*Gymnura marmorata*), giant electric ray (*Narcine entemedor*), and shovelnose guitarfish (*Rhinobatos productus*)—were also examined.

Methods. In collaboration with NMFS NWFSC and Santa Cruz labs, skates and catsharks were collected opportunistically from fishery-independent surveys along the west coast of the contiguous United States and Monterey Bay throughout 2002-2007. Additional specimens from California waters were obtained from California Department of Fish and Game fisheries observers. Specimens of the four studied ray species were collected from artisanal fishery landings in Bahía Almejas, Baja California Sur, Mexico. Each specimen was sexed, measured to the nearest centimeter, and weighed to the nearest 0.1 kg. Foreguts were excised and stored in formalin (whenever possible) or frozen. All prey items were identified to the lowest possible taxon. Diet composition was reported using several common indices: percent number, percent weight, percent frequency of occurrence, and percent index of relative importance. Cumulative species curves were generated to determine if sample sizes were large enough to adequately represent the diet of each species. The feeding habits and dietary overlap of rays, skates, and catsharks were examined in relation to depth, size, sex, habitat, and season (whenever possible) to determine their trophic roles in demersal fish assemblages. Power analyses were conducted prior to dietary comparisons to ensure adequate sample sizes. Multivariate statistics (e.g.; MANOVA, Principal Components Analysis), traditional indices (e.g., Morisita's, Bray-Curtis) and/or randomization tests were used for comparisons of dietary overlap.

Outcomes and Significance. Dietary analyses of sandpaper, big, California, and longnose skates feeding ecology have been completed with three manuscripts accepted for publication thus far. A pending publication on standardized diet compositions and trophic levels of skates also utilized dietary information from these four species. Results from both the sandpaper and longnose skate studies were presented at an international symposium on the “*Biology of Skates*” that was held in conjunction with the annual American Elasmobranch Society meetings in July 2006. Longnose and sandpaper skate diet studies were each completed as part of Masters Theses. Manuscripts detailing the diets of big and California skates are in preparation by PSRC staff. The big skate feeds primarily on demersal teleosts, crustaceans, and cephalopods, whereas the California skate feeds mainly on benthic shrimp, crabs, and demersal teleosts. A comparative study of these four skate species is *in press* and showed considerable overlap in general prey composition at similar sizes, but varied diets between large (> 60 cm TL) and small (\leq 60 cm TL) skates at both continental shelf (\leq 200 m) and continental slope (ca. 200-500 m) depths. Dietary analysis of the brown and filetail catsharks and of the roughtail skate are underway as part of two thesis projects. Among ray species from Mexican waters, the shovelnose guitarfish preyed on a diverse assemblage of primarily epibenthic crustaceans (e.g., mysids, crabs, and shrimps), the giant

electric ray mainly consumed polychaetes and naticid gastropods, the diamond stingray had a specialized diet, primarily ingesting pea crabs and razor clams, and the California butterfly ray consumed demersal fishes almost exclusively. Similarities in temporal and intergender diet composition were detected for all species except the shovelnose guitarfish; however, secondary prey composition of California butterfly ray males and females also was markedly different. Diet composition of all species was largely dissimilar, potentially facilitating their coexistence.

2f. Stable Isotope Analysis.

Objectives. PSRC staff expanded the original research of the variation in stable isotope signatures recorded in the vertebral centra of Pacific sharks. We examined the composition of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) in vertebrae and white muscle tissue of the blue shark, *Prionace glauca*. Questions to be addressed through the course of this research are: 1) How does isotopic composition of adult and juvenile blue sharks vary within the vertebral centra and 2) does variation in the composition of stable carbon, nitrogen, and oxygen isotopes within banding of the vertebral centra support on-shore-off-shore movement patterns indicated by tag-return studies? In addition, stable isotopes were used to better understand the radiocarbon distribution that was measured from white shark vertebrae. These data were compared to existing stable isotope records, as well as the blue shark data.

Methods. Stable isotopes have proven to be powerful markers in other ecological studies, but have not yet been used to discern movement patterns in elasmobranchs. PSRC staff collected vertebrae and white muscle tissue samples from blue sharks in cooperation with fisheries agencies and commercial fishers from the eastern and western Pacific. Sub-samples were collected from individual vertebrae using a computerized micromilling technique that were removed from specific growth increments. This provided detailed information pertaining to movements, feeding, and growth during specific life stages. Carbon, oxygen, and nitrogen stable isotope composition were determined from white muscle and vertebral samples using mass spectrometry. Assessment of the isotopic composition of discrete vertebral growth increments were employed to compare patterns of ontogenetic and sex-specific movement patterns of blue sharks in the Pacific.

Outcomes and Significance. The primary species for this segment objective, the blue shark, was changed to the white shark because of the application of those data to understanding the bomb radiocarbon levels in the cartilage at different ages. The white shark has a complex life history that is characterized by large-scale movements and a highly variable diet. Estimates of age and growth for the white shark from the eastern North Pacific Ocean indicate they have a slow growth rate and a relatively high longevity. Age, growth, and longevity estimates useful for stock assessment and fishery models, however, require some form of validation. By counting vertebral growth band pairs, ages can be estimated, but because not all sharks deposit annual growth bands and many are not easily discernable, it is necessary to validate growth band periodicity with an independent method. Radiocarbon (^{14}C) age validation uses the discrete ^{14}C signal produced from thermonuclear testing in the 1950s and 1960s that is retained in skeletal structures as a time-specific marker. Growth band pairs in vertebrae, estimated as annual and spanning the 1930s to 1990s, were analyzed for D^{14}C and stable carbon and nitrogen isotopes (d^{13}C and d^{15}N). The aim of this study was to evaluate the utility of ^{14}C age validation for a wide-ranging species with a complex life history and to use stable isotope measurements in vertebrae as a means of resolving complexity introduced into the ^{14}C chronology by ontogenetic shifts in diet and habitat. Stable isotopes provided useful trophic position information; however, validation of age estimates was confounded by what may have been some combination of the dietary source of carbon to the

vertebrae, large-scale movement patterns, and steep 14C gradients with depth in the eastern North Pacific Ocean. Results from this segment have been published.

2g. Habitat Associations and Nursery Grounds.

Objectives. The PSRC built upon the pilot studies initiated during 2002-03 that will help to determine which marine and estuarine habitats might serve as Essential Fish Habitat for Pacific sharks. With increased knowledge of movement patterns and spatial utilization gained from this study, proper steps can be taken to identify critical habitat types and lead to the identification of Essential Fish Habitat for these species. Questions to be addressed through the course of these investigations are: 1) What are the spatial and temporal patterns of habitat utilization of female leopard sharks, *Triakis semifasciata*, in Elkhorn Slough, CA; 2) What habitat features are skate and catshark egg cases associated within Monterey Bay, CA across large (km) and small (m) spatial scales? and 3) What are the depth and habitat associations of the dominant skate species within Monterey Bay, CA across large (km) and small (m) spatial scales?

Methods. The PSRC examined spatial and temporal patterns in habitat utilization and movements of sharks inside Elkhorn Slough National Estuarine Reserve (ESNER), CA using acoustic telemetry techniques. A mixture of both active and passive tracking techniques allowed us to collect data on short- and long-term movements and habitat use by transient and resident sharks. Leopard sharks were captured with longline fishing gear and monofilament gill nets set for periods of less than 30 minutes. Sonic transmitters were surgically implanted in sharks to collect data on size- and sex-specific movement patterns in ESNER. Receiving stations were established at key locations throughout the slough to track movements within and outside the embayment. A plastic coded anchor tag was inserted into the musculature near the dorsal fin of the shark for long-term identification purposes. Additional monitoring was conducted continually over 24 hour periods from an MLML research vessel using a directional hydrophone and acoustic receiver to determine the location of study animals and to validate the details obtained from passive monitoring. The resulting data were related to detailed habitat maps made available for ESNER. In addition, site-specific information on the abundance and distribution of skates collected in the NMFS Santa Cruz labs fishery-independent monthly surveys were overlaid onto geo-referenced habitat maps of the Monterey Bay National Marine Sanctuary. Species-specific occurrence was examined in relation to habitat features, depth, season, size, and sex. In cooperation with the Monterey Bay Aquarium Research Institute and Moss Landing Marine Laboratories Center for Habitat Studies, archived video footage recorded from remotely-operated and manned submersible dives in Monterey Bay was reviewed to determine possible nursery locations for oviparous chondrichthyans including skates, catsharks, and ratfishes.

Outcomes and Significance. From May 2003 to February 2005, 20 female leopard sharks (78 – 140 cm TL) were tagged with acoustic transmitters in Elkhorn Slough, California, and their movements and habitat use were examined using acoustic tracking techniques. Nine sharks were manually tracked for 20-71.5 h, and 11 sharks were monitored for 4-443 d using an array of acoustic receivers. Use of different regions in Elkhorn Slough by tagged sharks changed seasonally and was associated with changes in temperature, salinity, and dissolved oxygen. Sharks used Elkhorn Slough National Estuarine Research Reserve (ESNERR) extensively throughout the year, but especially during the spring and summer. ESNERR appeared to be important as both a foraging and nursery area, likely due to the abundance of intertidal mudflats. Movements and habitat use were tidally influenced, and likely were related to the distribution of important prey items in Elkhorn Slough. Leopard sharks therefore are seasonally abundant in Elkhorn Slough during the spring through fall, and generally leave in the winter when

temperature and salinity levels decrease. Once they are in the slough, females are largely residential and do not frequently move between the slough and coastal areas. Within the slough, there is a high degree of sexual segregation. Females are more abundant year round in ESNERR, whereas males are more abundant in the main channel in the spring, although sex ratios in the summer and fall in the main channel are nearly 1:1, which is possibly related to mating.

ESNERR is an important region of Elkhorn Slough that is used extensively by female leopard sharks throughout the year. Tidal creeks used to be important habitats for leopard sharks, but their utility has diminished as a result of habitat alteration. However, while intertidal habitats in the main channel were lost, new intertidal habitats were created during the formation of ESNERR, and leopard sharks shifted their habitat use accordingly. ESNERR is important as a foraging area and as a primary and possibly secondary nursery area. This is likely because ESNERR has an abundance of intertidal mudflats and prey, in particular fat innkeeper worms, is shallow, warm, and protected.

At low tidal levels, sharks are restricted to channels, but as tidal levels increase they move out of the channels to forage on the intertidal mudflats. Sharks use low intertidal mudflats almost exclusively when they are available, indicating that these habitats are of particular importance as foraging and possibly pupping areas. Leopard sharks use of ESNERR diminishes in the late summer or fall when dissolved oxygen is decreased and temperatures are increased, which causes sharks to spend more time in the main channel, which is more environmentally stable. When in the main channel, sharks exhibit different patterns of movement and habitat use. In particular, the foraging ecology of leopard sharks changes as they primarily use subtidal habitats and forage over larger areas. Use of intertidal mudflats is not as extensive in ESNERR, likely due to the absence of fat innkeeper worms in the upper main channel, which is where most of the intertidal mudflats are located in the main channel.

Sharks also use the tide to assist their movements, which allows sharks to increase the area over which they can forage while minimizing the energetic costs of moving. Thus, they usually move during periods when the tidal currents are greatest, which allows them to move faster and further. These tidal movements up and down the main channel often occur with a high degree of periodicity, often every day around lower low tide. By using the tides they are more easily able to forage over the entire length of the main channel and gain access to the different prey items that are abundant in different areas of the main channel.

In the late fall, usually in October or November, use of the upper region and ESNERR diminishes as sharks spend more time in the lower regions of the main channel. This is likely because temperature and salinity decreases faster in the upper regions, and ESNERR to a lesser extent, than in the lower regions of the main channel, which are more stable due to their proximity to the ocean. Once temperature and salinity levels drop to lower levels, male and female leopard sharks generally leave the slough for the winter, although juveniles may remain throughout the winter.

This research segment was expanded to include a study on prickly shark (*Echinorhinus cookei*) movement and habitat use in the Monterey Canyon. It served as a Masters thesis for one student. Results of this study were as follows: Between March and August 2005, fifteen subadult prickly sharks, from 170 – 270 cm TL were tagged with acoustic tags in the Monterey Canyon. The movements and activity patterns of 10 female and 5 males were examined using manual tracking and acoustic monitoring techniques. One female and two male sharks were tracked manually for 51.8, 61.0, and 62.8 h. Occurrence of those sharks and one other female was recorded for 101.2 – 123.6 d. An array of non-overlapping receivers extending 3.5 km offshore recorded the occurrence of five females and 3 male sharks for 400 – 561 d. Also, 3 female sharks were tagged with archival transmitters. All tagged sharks demonstrated a pronounced diel movement pattern, moving offshore to discrete areas during day and moving inshore along the axis of the canyon and actively swimming off the bottom at night. Subadult prickly sharks tagged in this study were present in the upper reaches of the Monterey Canyon during all four seasons.

2h. Population Genetic Studies.

Objectives. The PSRC investigated the population structure of the common thresher shark, (*Alopias vulpinus*); a project that was expanded to study all three thresher shark species including the pelagic (*Alopias pelagicus*) and big-eye (*Alopias superciliosus*). The results of this investigation will provide details on the genetic diversity among Pacific and Atlantic regions and aid in determining the degree of exchange between these groups. The objectives of this research were to determine: 1) If microsatellite loci will serve as appropriate, independent markers of gene flow for thresher sharks; and 2) The degree of genetic relatedness among thresher shark species within the Pacific and between the Atlantic and Pacific oceans.

Methods. The population structure and extent of movement of highly mobile, pelagic species has proven difficult to discern. However recent advances in molecular genetic techniques have been successful in determining population structure of these species by quantifying the degree of genetic relatedness between geographically separated populations. Population studies at the PSRC included specimens and genetic data made available by NMFS Southwest Fisheries Science Center and from collaborators in Japan, Taiwan, Mexico, Australia, New Zealand, South and Central America. Molecular genetic data were obtained by analysis of the mitochondrial DNA (mtDNA) d-loop and microsatellite loci. The increased variability typically observed in microsatellite loci may provide an improved resolution for determining interpopulational variation. Multiple microsatellite markers will be examined. Genetic variability, population divergence, and rates of mutation were assessed from mtDNA. Genetic diversity of the study species was described using haplotype (h) and nucleotide (B) diversity measures. Tajima's D statistic was used to test for neutrality of mtDNA mutation. Population structure was investigated using pairwise comparisons (F_{ST}) of population genetic variation.

Outcomes and Significance. Despite growing conservation concerns, genetic relatedness among thresher shark populations is poorly known. The phylogeographic structure of all three species of thresher sharks was examined using DNA sequences from the mitochondrial control region. Thresher shark species samples were collected from the East and West coasts of the United States, Gulf of Mexico, Gulf of California, Clipperton Atoll, Guatemala, Ecuador, Hawaii, Taiwan, Indonesia, New Caledonia, New Zealand, South Africa and France.

Despite their high dispersal potential, our data indicate limited gene flow among thresher shark populations, but phylogeographic patterns differ among species. Gene flow in *A. pelagicus* is limited across the Pacific Ocean, but is extensive among locations in both the eastern and western Indo-Pacific. In *A. superciliosus*, shallow population structure was detected among Indo-Pacific and Atlantic populations, but not among populations spanning the entire Indo-Pacific Ocean. Results for *A. vulpinus* indicate genetic heterogeneity among almost all sampled populations, both within and between the Atlantic and Indo-Pacific oceans. Taken together, our data indicate that intraspecific biological and ecological differences among thresher sharks are sufficient to cause variable patterns of interspecific genetic population structure. This study also highlights the need for international cooperation for the conservation and management of thresher sharks. This project was carried out as part of a Masters thesis project that has now been completed and is currently being written up for publication.

2i. Shelf, Slope and Pelagic Surveys.

Objectives. The objectives of this research component were to provide west coast National Marine Fisheries Service (NMFS) scientists with onboard support during pelagic, shelf, and slope surveys and to utilize specimens collected during these cruises to maximize the biological

information that can be obtained from these surveys for chondrichthyan species.

Methods. NMFS scientists conduct regional annual and bi-annual surveys of pelagic, shelf, and slope environments to assess fishery stock status, species size composition, species abundance, and geographic distribution. PSRC participation in NMFS research cruises include pelagic longline surveys in the California Bight, central California slope trawl and longline surveys, and a portion of the slope surveys conducted off Washington, Oregon, and California, and in groundfish surveys in Alaskan waters, including the eastern Bering Sea and Gulf of Alaska. PSRC staff assisted NMFS researchers with the identification, enumeration, and measurement of elasmobranchs collected during these surveys. Vertebrae, reproductive tracts, stomachs, and tissue samples were also collected from selected elasmobranchs and chimaeras.

Outcomes and Significance. In cooperation with NMFS Alaska Fisheries Science (AFSC), Northwest Fisheries Science Center (NWFSC), Southwest Fisheries Science Center (SWFSC), and the Alaska Department of Fish and Game (ADFG), PSRC personnel participated in, and continue to participate and assist in the annual shelf and slope groundfish surveys off Alaska, Washington, Oregon, and California. Measurements, wet weights, reproductive tracts, tissue samples, parasites, vertebrae for age and growth estimation, and/or stomachs for feeding analyses have been collected from nearly 10,000 specimens to date. The primary elasmobranchs observed in NMFS SWFSC Santa Cruz Lab groundfish surveys were the big, California, longnose, starry, and sandpaper skates, spiny dogfish (*Squalus acanthias*), brown and filetail catsharks, and white-spotted chimaera (*Hydrolagus colliei*). The specimens collected and examined have resulted in five completed Masters Thesis projects to date and one Sea Grant Internship for an undergraduate student. PSRC scientists have actively participated in the NWFSC Fisheries Resource Analysis Monitoring program surveys for the past five years providing critically needed volunteer personnel to assist NMFS fisheries biologists during these surveys. The primary species collected and studied from these cruises include the deepsea (*Bathyraja abyssicola*), Aleutian, sandpaper, and rougtail skates. Data collected from these survey cruises have resulted in five completed Masters Thesis projects with several more still in progress. Our participation in the AFSC and ADFG survey cruises in Alaskan waters has provided much needed personnel support to both agencies and has resulted in some of the first studies on the life history of many Alaskan skate species. The primary species studied to date include the big, longnose, Aleutian, Bering, Commander, mud, whitebrow, and white-spotted skates. At the present time at least five students are currently working on Masters Thesis projects from data that they have collected during survey cruises conducted in Alaskan waters by the AFSC and ADFG. PSRC scientists have also participated in several pelagic longline survey cruises with the SWFSC La Jolla lab. Participation by PSRC scientists has in these survey cruises proven to be an excellent, mutually beneficial relationship between the various NMFS labs, ADFG, and the PSRC. The resulting information generated from Masters Theses and peer-review publications has helped NMFS in identifying individual skate species, and in filling in missing gaps in the life history of the chondrichthyan species commonly encountered. Given the budgetary cuts among most of these agencies in recent years, the participation and assistance provided by the PSRC through its staff and students to each of the aforementioned agencies has been invaluable resource in terms of support personnel in allowing them to carry out their surveys. Without the assistance of PSRC personnel many of these surveys would have been inadequately staffed.

2j. Eastern Pacific Shark Fisheries Analysis.

Objectives. PSRC personnel previously compiled available catch statistics and developed a comprehensive database containing state- and species-specific landings information on

elasmobranchs from Alaska, Washington, Oregon, and California. The objective of this research segment is to continue to maintain and update this centralized database for staff and collaborative researchers. Our investigations will also be expanded to include analyses of historic records of recreational and commercial elasmobranch landings from California and Hawaii.

Methods: Commercial landings data were compiled from NMFS (1950–present) and PacFIN (1980–present) for Washington, Oregon, and California. Source data differs slightly between these organizations, and data from both were included to provide a comprehensive account of landings and for comparative purposes. Recreational fishing data was compiled from RecFIN (1980–present) for these states. AKFIN and Alaska Department of Fish and Game data were used as sources for commercial and recreational landings taken in Alaskan waters since 1980. State and federal regulations information was compiled from fisheries agencies and legislative records. Time and a lack of resources did not allow us to expand this segment as indicated in the objectives.

Outcomes and Significance. The resulting Chondrichthyan Fisheries Database (CFD) was recently completed and will be made available via the world–wide web on the PSRC web site (<http://psrc.mlml.calstate.edu>) during autumn 2007. It is intended for use by interested students, researchers, fishers, and resource managers and can be downloaded or queried. The CFD is comprised of Microsoft Excel® workbooks for Washington, Oregon, and California, each containing four spreadsheets (i.e., commercial regulations, commercial landings, recreational regulations, recreational landings), and a workbook for Alaska containing two spreadsheets (i.e., commercial landings, recreational landings). State–specific landings data were also graphically depicted for all chondrichthyan taxa. The CFD will serve as an important resource tool for PSRC and other scientists to develop research projects concerning long–term trends in eastern North Pacific chondrichthyan fisheries and targeted populations. It can also be used by commercial and recreational fishers to check regulations. The CFD will be updated yearly as new information becomes available.

2k. Taxonomic Studies.

Objectives. Most eastern Pacific rays and skates that are taken in fisheries are poorly described, resulting in an extremely limited understanding of the species composition that is directly impacted by fishing activities. Without adequate guides or keys, these species cannot be properly identified. PSRC research personnel continued to review and revise the large, complex skate genus *Bathyraja* (Family: Arhynchobatidae) to provide improved keys to onboard fisheries observers and fisheries management agencies along the U.S. west coast. Taxonomic concerns to be addressed include: 1) The status and validity of the butterfly rays, *Gymnura crebripunctata* and *G. marmorata* and 2) A review and revision of the skate genera *Bathyraja*.

Methods. PSRC personnel surveyed commercial vessels that target or incidentally capture sharks, skates, and rays in Moss Landing, Monterey, Santa Cruz, and Half Moon Bay, CA. Specimens were also obtained from NMFS surveys and on-board observers. Investigators examined museum specimens from the California Academy of Science, Harvard Museum of Comparative Zoology, Los Angeles County Museum of Natural History, Scripps Institution of Oceanography Vertebrate Museum, Smithsonian National Museum of Natural History, and the University of Washington. Detailed measurements of external features and counts of internal characters (e.g.; spiral valves, vertebrae, and teeth) were made. Members of the PSRC collaborated with Dr. Leonard Compagno (South African Museum) and Dr. John McEachran (Texas A & M University) on the complicated issues surrounding ENP skate taxonomy. Genetic

analyses were conducted to complement traditional morphometric approaches to determine the degree of genetic similarity between *G. crebripunctata* and *G. marmorata*. The 5' end of mtDNA cytochrome b gene was amplified using the polymerase chain reaction. Mitochondrial DNA analysis of white muscle and red muscle tissue was conducted in conjunction with researchers at Nova Southeastern University, Florida. The use of multiple techniques will allow us to definitively determine if the two butterfly rays are valid species or male and female morphs of the same species.

Outcomes and Significance. A revision of the softnose skate genus *Bathyraja* (Family: Arhynchobatidae) is continuing. Colleagues at the NWFSC, with assistance from PSRC personnel, have been and are continuing to collect and save skates during their annual slope cruises. A revised key to the skates and a description with a key to the skate egg cases of the ENP was published during this report period. A description and key to the skate egg cases of Alaskan waters was also completed. The description and keys to skate egg cases is an invaluable tool as skate nursery grounds can be identified to individual species by knowing how to associate egg cases to specific skate species. In addition, skate specimens from the aforementioned museum collections will continue to be examined. The revision of ENP bathyrajid skates has proven to be somewhat more complicated and molecular tools are now being incorporated to understand the species complexes that appear with some of these species. Also, examination of video tapes from deepsea exploratory dives conducted by the Monterey Bay Aquarium Research Institute's (MBARI) ROV's has revealed at least two additional skate species that are either range extensions or may represent species that are entirely new to science. Continued studies into the deepsea chondrichthyan fauna in the ENP is continuing in collaboration with MBARI. PSRC personnel are continuing to consult and collaborate on this project with leading experts at the Shark Research Center, Cape Town, South Africa, CSIRO, Hobart, Tasmania, Australia, and at Texas A & M University, Galveston, Texas.

The taxonomic uncertainty surrounding eastern Pacific butterfly rays (family: Gymnuridae) was also investigated. The extent of morphological and nucleotide variation between *Gymnura crebripunctata* and *G. marmorata* was assessed using multivariate morphological and mitochondrial DNA analyses in the framework of these characters from established congeneric species. Discriminant analysis of 21 initial morphometric variables successfully distinguished *Gymnura* species. Sexual dimorphism was evident in several characters that have previously been relied upon to distinguish *G. crebripunctata* and *G. marmorata*. Phylogenetic and genetic distance analyses based on 698 base pairs of the mitochondrial cytochrome b gene indicated that *G. crebripunctata* and *G. marmorata* form highly divergent lineages, supporting their validity as distinct species. Reliable characters for distinguishing these species in the field are presented. The closely related western Pacific batoid *Aetoplatea zonura* clustered within the *Gymnura* clade, indicating that it may not represent a valid genus. The first phylogenetic hypothesis for the Gymnuridae was introduced. Strong population structuring (overall $\Phi_{ST} = 0.81$, $P < 0.01$) was evident between *G. marmorata* from the Pacific Baja coast of México and Gulf of California, supporting the designation of distinct management units in these regions.

The *Taxonomic Studies* research segment was expanded considerably from its original objectives as the need for expertise on a variety of international systematic issues came about during the course of this component. The expertise provided by PSRC researchers was invaluable as resource managers tried to accurately identify species for management and conservation reasons. Among the more notable projects that PSRC personnel were involved with included describing at least nine new Pacific Ocean species of chondrichthyans; six sharks, two rays, and two chimaeras. Among the new sharks species described were two species of swell sharks (genus *Cephaloscyllium*) and a revised key to the species of this genus from the North Pacific. Two species of gulper sharks (genus *Centrophorus*) were described in collaboration with Drs. William White (CSIRO, Hobart, Australia) and Leonard Compagno (South African

Museum, Cape Town, South Africa). The gulper sharks are listed as vulnerable to near-threatened by the IUCN Shark Specialist Group as populations of these sharks have declined by up to 99% in some regions. This is a problematic genus with identification being quite difficult between species. The research efforts to date have been crucial in identifying individual species such that they can be managed and where necessary protected from regional extirpation. Two new species of chimaeras (genus *Hydrolagus*) were described from the Galapagos Islands, considered a biodiversity hotspot. Both species descriptions were the result of collaborative efforts between PSRC researchers and colleagues Dr. Dominique Didier (Millersville State) and Dr. Douglas Long (California Academy of Sciences). Another important product of this segment was a review and revision of North Pacific angel sharks (*Squatina* spp.) that was recently published. This particular group of sharks has life history characteristics that make them vulnerable to over-exploitation. However, these rather morphologically undiverse sharks can be difficult to identify between individual species, thus making identification much less management decisions problematic. The aforementioned project re-described four species and developed a key to identify each. Other systematic projects undertaken during this report period include descriptions of two new species of electric rays from the North Pacific, a new lanternshark (genus *Etmopterus*) species from the western Pacific with a revised key to the etmopterid sharks from that region, and a project still in progress revising the lanternsharks from the Hawaiian Islands. Identification of individual species within these latter groups is critical as many of these sharks and rays are taken as bycatch in other fisheries. Finally, PSRC personnel have developed international collaborative projects with researchers in Australia, Brazil, Mexico, and South Africa.

21. Publications and Conferences.

Members of the PSRC attended and presented results from our research at 29 conferences, including the Alaska Marine Science Symposium, American Elasmobranch Society, American Fisheries Society, CalCOFI, Committee of Age Reading Experts Working Group, Conservation and Management of Deepsea Chondrichthyan Fishes, Cowshark Conservation Workshop, Foro de Intercambio Científico Sobre Tiburones y Rajas, Gilbert Ichthyological Society, Monterey Bay National Marine Sanctuary Currents Symposium, 7th Indo-Pacific Fish Conference, Society of Integrative and Comparative Biology, 3rd International Symposium on Fish Otolith Research and Application, Western Groundfish, and Western Society of Naturalists. In total 95 presentations were given. PSRC students received approximately 20 conference awards for their presentations including outstanding presentation awards at the American Elasmobranch Society, American Fisheries Society, Western Groundfish, and Western Society of Naturalists. The PSRC web site “Featured Elasmobranch” that highlights a different species each month continues to attract attention from the general public, especially educators, who visit the web site on a regular basis to find out more about our ongoing research efforts. This series has been running since February 2004 with contributors committed through September 2008. A total of 12 PSRC graduate students completed Masters Theses projects during this report period. Another seven students are currently working on their Masters Thesis projects. PSRC staff and students attended a total of five IUCN Shark Specialist Group workshops and contributed at 103 Red List species assessments. PSRC staff produced a total of 76 papers during this report period that are published, in press, or in review.

**VIRGINIA INSTITUTE OF MARINE SCIENCE
SHARK RESEARCH PROGRAM**

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3a. Relative Abundance and Distribution Studies.

3a1. Coastal Population Monitoring.

Objectives. VIMS has conducted a coastal longline survey since 1973 to track population abundance, species composition, size and age distributions and sex ratios of sharks in the coastal ocean from Maryland to North Carolina out to ca. 200m depth. The time series of catch rates is used in stock assessments and management plans by federal, regional, and state management agencies.

Methods. These surveys entailed standard longline sets with 100 hooks baited with menhaden and fished for 4 hours. Approximately ten stations were sampled each month from June to September. Additional cruises were conducted during May and October as funding allows. Most sharks were tagged and released to investigate long-term movement patterns. However, samples needed for studies of age, growth, reproduction, and genetics were collected as needed.

Outcomes and Significance. Between 01 July 2002 and 30 June 2007, 486 longline sets fished 20,699 standard J-hooks and 16,980 alternative circle hooks. In total 3,886 sharks from 25 species and nearly 1,000 batoids belonging to eight species were captured. The most common species captured was sandbar sharks (*Carcharhinus plumbeus*), which composed nearly 60% of the catch, followed by smooth dogfish (*Mustelus canis*), Atlantic sharpnose sharks (*Rhioprionodon terraenovae*), and dusky sharks (*Carcharhinus obscurus*). Approximately 75% of all sharks captured were tagged and released.

Nominal and standardized catch rates and other data from this fishery-independent survey were used by the National Marine Fisheries Service in the stock assessments for the Large Coastal Shark Complex (2006), Sandbar Shark (2006), Dusky Shark (2006), and Atlantic Sharpnose Shark (2007). In addition, data from this survey were used in the redlist assessments for numerous species by the IUCN Shark Specialist Group. Catch rate and distribution data from this survey were also published in four peer-reviewed papers during the contract period.

3a2. Virginia Nursery Grounds.

Objectives. VIMS continued its long-term monitoring of recruitment and relative abundance of juvenile sandbar sharks in Chesapeake Bay. In addition, sampling was expanded in the inlets, lagoons, and tidal creeks along Virginia's Eastern Shore where only ancillary studies have been conducted in the past to investigate use of this area by various species of elasmobranchs, especially juvenile sandbar sharks. The longline monitoring program in the nursery grounds dove-tailed with the long term (1973- present) coastal longline monitoring program.

Methods. Longline sampling included the standard VIMS longline gear as well as alternative gear using smaller circle hooks to increase catchability of juvenile sandbar sharks. Gill net sets were also included following the specifications of NMFS COASTSPAN program (Narragansett, RI). As part of the standard VIMS survey, two standard stations in the lower Chesapeake Bay have been sampled monthly during summer months since 1996. During 2003 and 2004, twenty fixed longline stations distributed in four inlet/lagoon systems along Virginia's Eastern Shore were sampled monthly from May through October to delineate spatial and temporal use of this area for comparison with previous work completed in Chesapeake Bay. In addition, sonic tracking was conducted to define daily activity space and Essential Fish Habitat.

Outcomes and Significance. Patterns of temporal and spatial use of Chesapeake Bay and lagoons along Virginia's Eastern Shore were elucidated for juvenile sandbar sharks and both areas were determined to include essential fish habitat (EFH) for this species. In Chesapeake Bay, relative abundance of sandbar sharks was highly correlated with abiotic factors such as salinity, depth, and dissolved oxygen. In the lagoons along Virginia's Eastern Shore, where these factors were much less variable, these correlations were not observed. Biotic factors such as predation risk and prey availability may exert greater influence on distribution patterns in this area. The results of this research were included in four peer-reviewed publications (Conrath and Musick 2007a, b; Grubbs and Musick 2007; Grubbs et al. 2007). The results from the study using a stratified-random design for longline sampling are currently being analyzed to examine the distribution patterns of several species of sharks in the region.

3a3. Abundance and Distribution of Salmon Sharks in Alaska.

Objectives. This study used line transects with side scan sonars coupled with mark-recapture techniques to study distribution and estimate the number of salmon sharks (*Lamna ditropis*) using the Port Gravina fjord adjacent to Prince William Sound, Alaska.

Methods. Sonar study: Preliminary studies in summer 2000 using sonically tagged sharks (known targets) suggested that a sonar survey using a line transect design would be possible. Exploratory cruises were conducted in 2002, 2004, and 2005, but discontinued due to funding restrictions. The Port Gravina fjord was divided into three geographic strata; outer, middle and inner. Line transects in each stratum ran across the fjord (shoreline to shoreline) and were spaced ~½ nm apart. Transect lines were selected by a random draw within each stratum. A CTD cast was done at the beginning, in the middle, and at the end of each transect line run to look for possible temperature and/or salinity preferences in salmon sharks.

Tag & recapture study: VIMS and Alaska Department of Fish and Game (ADF&G) scientists collaborated to attach streamer tags to salmon sharks. We are able to tag numerous sharks over short periods of time by luring them to the surface using rod and reel with a herring bait clipped to the end of the line (no hook). Once the shark's dorsal surface was near or broke the surface of the water, the tag was applied via a billfish tagging tip on the end of a dowel pole.

Sharks were also tagged when brought aboard the research vessel for other purposes (e.g. acoustic transmitter tagging) and released.

Outcomes and Significance. In 2004, we used the acoustic identification technique to estimate local salmon shark numbers in Port Gravina. Fourteen transects were run; 44 salmon sharks and 40 schools of fish were counted on these transects. In 2005, 20 transects were run; 48 salmon sharks and 26 schools of fish were identified. We also conducted CTD casts at several places in Port Gravina where we encountered large numbers of salmon sharks. We found salmon sharks all the way to the head of the fjord in milky glacial silt waters with salinities between 10 and 12. These data are under further analysis.

As of mid-summer 2007, approximately 750 salmon sharks had been tagged and 64 individuals have been recaptured. This does not account for sharks that have been double-tagged, which we know occurs (infrequently). ADF&G is considering applying simple tag-recapture models to the data in a preliminary attempt to model population size. Distribution and abundance estimates are critical to managing fish populations. This is especially true for seasonal localized populations of fishes that are easily targeted by fisheries. The data collected in this study will be critical to monitoring and regulating the developing sportfish fishery for salmon sharks.

3a4. Seasonal Distribution and Abundance of Batoids in Coastal Lagoons of Virginia.

Objectives. Assessment of elasmobranch biology and population dynamics are generally focused on sharks because they are targeted by recreational and commercial fisheries. Batoid fishes have not been studied as thoroughly in the United States because they are typically discarded as bycatch. However, many species of batoids may have significant impacts on predator-prey dynamics and overall ecosystem energy budgets as being some of the largest predators in their ecosystems. Knowledge of their role in ecosystem function is critical as fisheries science shifts to ecosystem-based management plans. Furthermore, recent interest in developing fisheries for rays and skates in the U.S. increases the importance of garnering information on these species. This study will determine the spatial and temporal distribution, and abundance of the various batoids that inhabit the coastal lagoons of Virginia by examining catch data and acoustic tracking data from the Hogg Island Bay coastal lagoon ecosystem. The results from this study should provide insights into factors important in determining Essential Fish Habitat for batoids many of which have vulnerable life history characteristics.

Methods. Specimens were collected using a longline gear on VIMS vessels. Machipongo and Sand Shoal inlets and the adjacent lagoons and coastline were sampled monthly beginning in May (before arrival) and ending when sampling indicates the animals had left the lagoons. Proportional sampling was conducted within shoals, channels, inlets, and coastline of the region in order to determine specific habitat preferences. Standard sets included 75 gangions baited with menhaden spaced approximately 20 meters apart. These were attached to 1.5 kilometers of monofilament and soaked for two hours.

Outcomes and Significance. Intensive longline sampling (>200 stations) was conducted during 2006 and 2007 using monofilament gear and a stratified random design. Stations were stratified by habitat defined as 1) tidal channels, 2) shoals/flats, 3) nearshore coastal areas, and 4) inlets. Seven channel stations, seven shoal stations, ten coastal stations, and three inlet stations were sampled per month. During 2006 and 2007, 401 batoids from eight species were caught within this ecosystem. Disc width and sex were recorded for all species of rays. Specimens were tagged with a dart tag and also tagged with a Petersen disk through the spiracles.

This is the first study to try to quantify the fine scale differences of habitat use by batoids within Virginia's coastal lagoons. Marked differences have been observed both temporally and

spatially in habitat use by many of the species. For instance, the bluntnose ray, *Dasyatis say*, migrates into and out of the lagoons earlier than its closest relative within the lagoon, the southern stingray, *Dasyatis americana*. The information garnered from this study will help identify batoids' influences within the coastal lagoon ecosystem.

3a5. Seasonal Distribution and Abundance of Sharks in Coastal Lagoons of Virginia.

Objectives. The purpose of this study was to determine the fine scale habitat use of the sharks that seasonally migrate into the coastal lagoons of Virginia and the adjacent coastline. It has been determined that this is an essential nursery ground for the fisheries-targeted sandbar shark, *Carcharhinus plumbeus*, though little is known about the importance this region plays for various other shark species.

Methods. Specimens were collected using a longline gear on VIMS vessels. Machipongo and Sand Shoal inlets and the adjacent lagoons and coastline were sampled monthly beginning in May (before arrival) and ending when sampling indicates the animals had left the lagoons. Proportional sampling was conducted within shoals, channels, inlets, and coastline of the region in order to determine specific habitat preferences. A total of 75 gangions baited with menhaden spaced approximately 20 meters apart were attached to 1.5 kilometers of monofilament. Lines soaked for two hours.

Outcomes and Significance. Sampling was the same as in 3a4 above. During 2006 and 2007, 1,435 sharks from 12 species were caught. Standard length, total length, and sex were recorded for all species of sharks. Specimens were tagged with dart tags and then released. There is little information on the habitats that sharks prefer within Virginia's coastal lagoons. Understanding these preferences is necessary in determining their essential habitat. Preliminary results show the lagoons and adjacent coast is frequented by numerous shark species (Table 1) many with different habitat preferences. The federally recognized Species of Concern, the dusky shark, *Carcharhinus obscurus*, and sandtiger shark, *Carcharias taurus*, reside outside of the lagoons making them susceptible to commercial fishing pressure whereas the sandbar shark, *Carcharhinus plumbeus*, the blacktip shark, *Carcharhinus limbatus*, and others spend a considerable amount of time within the lagoons where commercial fishing is limited. Information from this study should provide valuable insight on habitat preferences of these vulnerable species.

3b. Age, Growth and Demographic Studies.

Knowledge of age, growth and demographic parameters are essential to informed management of any elasmobranch species. Elasmobranch age and growth studies have been ongoing at VIMS since 1973. These data have been essential to modeling efforts by VIMS scientists as well as NMFS scientists at the NMFS Shark Evaluation Workshops which have formed the basis for NMFS Shark FMPs.

3b1. Sandbar Sharks.

3b1a. Comparison of the age, growth, and maturity of the sandbar shark, *Carcharhinus plumbeus*, in the Northwest Atlantic between samples obtained over thirty years.

Objectives. This study examined age and growth of the sandbar shark in the Northwest Atlantic to provide updated growth parameters for stock assessment purposes and compare past and present growth parameters in search of compensatory growth.

Methods. Vertebral centra were obtained from sandbar sharks landed by the VIMS longline survey, Commercial Shark Fishery Observer Program (CSFOP), and NMFS longline survey. The VIMS longline survey operated in Chesapeake Bay, Virginia coastal waters and North Carolina coastal waters. CSFOP and NMFS surveys operated from North Carolina south to Florida and into the Gulf of Mexico along Florida's western coast. Samples were collected during 1980-1983, 1990-1992, and 2000-2004. Vertebral centra were cut using an isomet saw, mounted on slides then polished using a metaserve grinder. Ages were then assigned multiple times by multiple readers. Models were then fit to these data and the most appropriate model was selected using statistical comparisons.

Outcomes and Significance. Over the time period of 2000-2004, 464 sandbar sharks were sampled. Of these, 250 were females that ranged in length from 38 cm to 167 cm PCL and 206 were males that ranged from 40 cm to 162 cm PCL. The oldest estimated age for a female shark was 25 years at a length 150 cm PCL and for males was 22 years at a length on 148 cm PCL.

Growth parameters estimated for sharks sampled between 2000 and 2004 were significantly different than the earlier time period (1980-1981) suggesting slightly faster growth at present. This difference may represent density compensation.

3b1b. Growth model estimated from tag recapture data for the sandbar shark in the Northwest Atlantic.

Objectives. This study aimed to develop length-based growth model for the sandbar shark in the Northwest Atlantic from VIMS tag recapture data for comparison with growth rates from vertebral aging studies.

Methods. Sandbar sharks were caught on longlines within Chesapeake Bay, coastal lagoons of Virginia's eastern shore and coastal Virginia waters fished by the VIMS longline survey. Sharks were measured then tagged with nylon, double-return dart tags (Hallprint) inserted into the musculature at the base of the first dorsal fin and released (Grubbs et al. 2007). A modified version of Faben's method (Francis 1988b) for analyzing tag-recapture data was used. Inputs to the model included length at initial capture (L_1), change in length between captures (ΔL), time at initial capture (T_1), time at recapture (T_2), and time at liberty in years (ΔT). The model was fit to the data using likelihood methods. Model parameterization was completed by fitting the least complex model then adding additional model parameters. Model comparisons were made using likelihood ratio tests to determine optimal parameterization of the model. The model that provided the best fit to the data was then bootstrapped 5000 times and corrected for 95% confidence intervals. Model parameters were estimated using AD Model builder.

Outcomes and Significance. Over the time period 98 sandbar sharks were recaptured from New York south to Port Aransas, Texas. Time at liberty ranged from 26 to 3,561 days at liberty. Measurement information was obtained from 43 of these recaptures. Growth increments ranged from 0.10 to 66 cm. Of these recaptures, 22 were females and 15 were males.

Growth model estimates were in close agreement to those estimated through age-based methods discussed in the previous section. The length based model suggested slightly slower growth than the age based models. These results are being prepared for publication.

3b1c. Age and growth of the sandbar shark in the central Pacific Ocean.

Objectives. The sandbar shark is the most common large coastal shark in the Main Hawaiian Islands, yet little data exist on their life history in this sub-tropical region. The primary objective

was to estimate growth parameters for sandbar sharks in the central Hawaiian Islands through vertebral analyses.

Methods. We collected sandbar sharks using demersal longlines outside of Kaneohe Bay, Hawaii at depths between 70 and 100 meters. Gangions consisted of a stainless-steel snap-clip attached to three meters of monofilament followed by a one-meter stainless-steel leader that was attached to a circle hook. We used two sizes of gangions terminating in 14/0 circle hooks and 18/0 stainless-steel circle hooks. Hooks were allowed to fish for 3 hours before being retrieved. At least five male and female sharks within each 5 cm size class between 45 cm PCL and 150 cm PCL were euthanized and vertebral samples were removed from below the first dorsal fin. Once the required vertebral samples had been collected, subsequently caught sharks were injected with oxytetracycline (OTC, 25 mg kg body weight⁻¹), tagged with Hallprint dart tags, and released for age-validation purposes. Collected vertebrae were sectioned sagittally and aged using well-published methodologies.

Outcomes and Significance. We captured 320 sandbar sharks as part of this study and vertebral samples were collected and aged from 194 of these. We fit four growth models to length-at-age data for male and female sharks (two forms of the von Bertalanffy growth model, a modified version of the Gompertz growth model, and a logistic model). The data suggested males and females matured at eight and ten years of age, respectively. The form of the von Bertalanffy growth model using length-at-birth provided the best biological and statistical fit to the data and gave parameter estimates of $L_{\infty} = 138.5$ cm PCL and $k = 0.12$ year⁻¹ for males and $L_{\infty} = 152.8$ cm PCL, $k = 0.10$ year⁻¹ for females.

Sandbar sharks in Hawaii were found to grow slightly faster, reach maturity at an earlier age, and attain smaller maximum size than sandbar sharks in the Northwest Atlantic. Size at maturity for males and females was also lower than sandbar sharks in the Northwest Atlantic. These results were published in a peer-reviewed journal (Romine et al. 2007).

3b2. Barndoor Skates.

3b2a. Barndoor skate life history characteristics.

Objectives. The barndoor skate declined by more than 95% during the later twentieth century because it occurs as bycatch in the ground fisheries in Canada and New England. NMFS Office of Protected Resources has placed this species on the Candidate List under ESA. A lack of basic life history information has hampered assessment and management efforts. The objective of this study was to estimate the age and growth of the barndoor skate by using vertebral ring analysis. This work was conducted in cooperation with scientists at the NMFS Northeast Fishery Science Center (NEFSC).

Methods. Barndoor skates were collected onboard commercial scallop vessels fishing in the southern portion (south of 41°30"N) of Georges Bank Closed Area II. All skates were measured and maturity states were assessed. Vertebral samples were collected from a sub-sample of these and frozen. Reproductive tracts were preserved in 10% formalin. Vertebrae were sectioned sagittally and banding patterns were similar to those observed in other skate species.

Outcomes and Significance. Data were collected from 2,310 barndoor skates and vertebral samples were collected from 639 of these. Length at 50% maturity was estimated to be 116.3 cm TL (N=142) for females and 107.9 cm TL (N=148) for males. An analysis of 118 vertebrae indicated faster growth ($k = 0.14$ to 0.18) and younger female maturation (6.5 to 7.2 yr) than previously believed.

The life history parameters estimated here, combined with the observed recovery from NMFS survey indices, suggest that the barndoor skate is more resilient to fishing pressure than previously believed. Results of this study were published in a peer-reviewed journal (Gedamke et al. 2004), have been used in population modeling (Gedamke 2007), and have been used for management by NMFS, NEFSC, and Office of Protected Resources, as well as to the New England Fishery Management Council.

3b2b. Barndoor skate mortality estimation.

Objectives. Barndoor skates were reported in 1998 to be on the brink of extinction in the northwest Atlantic. A critical component to assessing the threat status of a population is an estimation of the current mortality rate. The objective was to estimate mortality using variants of commonly used methods.

Methods. Catch data were obtained from the NMFS Northeast Fisheries Science Center's fall bottom trawl surveys conducted from 1963 to 2005. The spring and fall surveys are the primary indices used by the New England Fisheries Management Council for assessing populations of the NW Atlantic skate complex. We developed two mortality estimators, one based on mean lengths and another based on catch rates.

Outcomes and Significance. Data from between 167 and 374 trawls were available for each year. Both mortality estimators provided similar results suggesting that overall mortality was very high in the 1960's ($0.89-1.0 \text{ yr}^{-1}$). However, overall mortality was reduced to much lower levels in the late 1990's.

The results of this analysis and our earlier work on the life history parameters, combined with the observed recovery from NMFS survey indices, suggest that the barndoor skate is more resilient to fishing pressure than previously believed. A re-analysis of NMFS survey data also indicates that the recovery of barndoor skate populations began in the mid 1980's, long before the concern about their extinction began. These results have been accepted for publication in a peer-reviewed journal (Gedamke et al. *in press*).

3b2c. Maximum population growth in barndoor skate.

Objectives. In this study, the objective was to determine if a spawner-recruit relationship exists for barndoor skates and to estimate the maximum population growth rate for this species.

Methods. Data from the NMFS Northeast Fisheries Science Center's fall bottom trawl surveys were used to assess the relationship between spawners and recruits. A relationship would allow estimation of maximum population growth rate using standard stock-recruit models.

Outcomes and Significance. Recruitment was closely tied to spawner abundance. Results from Ricker and Beverton-Holt stock recruitment models allowed the estimation of an instantaneous rate of maximum population growth of 0.35 yr^{-1} . Maximum population growth was estimated to be between 0.36 and 0.48 yr^{-1} using the Leslie matrix demographic model.

The results of this analysis also suggest that the barndoor skate is more resilient to fishing pressure than previously believed. These results have been submitted for publication in a peer-reviewed journal.

3b3. Deep-sea Sharks.

Deep-sea sharks are among the most vulnerable to stock collapse or even extirpation because of

very low intrinsic rates of increase, yet age, growth, and reproductive parameters have only been described for a few species. Globally, fisheries are expanding ever deeper onto the continental slopes and sea mounts. This presents an increasing threat to deep sea sharks.

3b3a. Community structure of deepwater chondrichthyans collected by longlines in the Gulf of Mexico and along the Mid-Atlantic Ridge.

Objectives. This study sought to describe the deepwater chondrichthyan assemblages of the Mid-Atlantic Ridge (MAR) and Gulf of Mexico and to compare the shark community structure from stations located on the MAR with stations located near the MAR. Furthermore, this work compared the species composition for elasmobranchs collected during the 1986 MARE expedition to the Azores to those collected during the 2004 MAR-ECO expedition to the Azores.

Methods. In the summer of 2004, a VIMS scientist participated in the MAR-ECO expedition (www.mar-eco.no) to the Mid-Atlantic Ridge. The goal of this expedition was “to describe and understand the patterns of distribution, abundance and trophic relationships of the organisms inhabiting the mid-oceanic North Atlantic, and identify and model the ecological processes that cause variability in these patterns.” During the cruise, data were collected (length, weight, sex, maturity) from approximately 5,500 deep-sea sharks (12 species) captured by longlines. VIMS scientists continue to work within the MAR-ECO Taxonomy Group to verify the identification of all deep-sea elasmobranch voucher specimens. An annotated list of fish species captured along the Mid-Atlantic Ridge is in preparation. The involvement of VIMS scientists in the MAR-ECO expedition has led to several productive collaborations with scientists at the Russian Federal Research Institute of Fisheries and Oceanography (VNIRO) and Norwegian scientists at the University of Bergen, Institute of Marine Research (IMR), and Moere Research Section of Fisheries.

DATA from MAR-ECO will be used to describe and compare communities on and off the MAR. In addition, data collected during MAR-ECO will be compared to unpublished data collected during the 1986 MARES expedition to the same regions. Finally, data collected during several longline cruises to the edge of the continental shelf in the eastern Gulf of Mexico by NMFS scientist Dr. José Castro will be used to describe the species assemblage in this region. Communities will be described and compared in terms of CPUE (# per 1000 hooks), total catch (# and weight), sex ratios, maturity stages, diversity, richness, evenness, and comparison of assemblages (multidimensional scaling or cluster analysis).

Outcomes and Significance. Data have been collected from all of the various sources and expeditions. Analyses are currently underway. The final product of the comparison of species assemblages will resemble that of Musick et al. (1996) or Fossen et al. (in press).

Little data exist on the structure of deep-sea communities, especially for larger predators such as elasmobranchs. An understanding of species distribution patterns and community structure is critical for management of marine resources. Two manuscripts resulting from the MAR-ECO project have been coauthored by a VIMS scientist (Orlov et al. 2006, Fossen et al. *in press*)

3b3b. Suitability of fin spines for age determination in deepwater squaloid sharks and holocephalans.

Objectives. The objectives of this research were to investigate the utility of fin spines for age-determination in ten species of squaliform sharks and two species of chimaeras.

Methods. VIMS scientists collaborated with colleagues in Norway, Australia, Mote Marine Laboratory, and the NMFS Northeast Fisheries Science Center to study the ecology of deep-sea

sharks to better understand their demographics and habitat use. Collaborative research has yielded hundreds of fin spines from 12 different species of squaloid sharks and two species of chimaeras. In this study we determined best method of cleaning tissue from fin spines: bleach, hot water, or decay and try to elucidate external banding patterns evident on the stem of the fin spines using a 10% (v/v) alizarin red and 1% potassium hydroxide solution or other staining techniques (crystal violet, cobalt nitrate + ammonium sulfide, or silver nitrate). We plan to validate the periodicity of band formation on fin spines by using the decay rate of the naturally occurring radioisotope ^{226}Ra . The decay rate of ^{226}Ra to its daughter product ^{210}Pb is known quite accurately such that the relative proportions of these two isotopes within an "annual" band can be used as a proxy for time elapsed since the incorporation of ^{226}Ra into the fin spine. Five samples will be analyzed per species by a nationally accredited Thermal Ionization Mass Spectrometry (TIMS) lab. We anticipate validating age estimations for 10 species of squaloid sharks.

Outcomes and Significance. During the MAR-ECO expedition, fin spines were collected for age and growth analysis from approximately 500 squaloid sharks and chimaeras (*Centrophorus squamosus*, *Centroscymnus coelolepis*, *C. crepidator*, *C. owstoni*, *Etmopterus princeps*, *Deania calcea*, *Centroscyllium fabricii*, *Hydrolagus affinis*, and *H. pallidus*). These fin spines are currently being evaluated for their utility in age determination. To date, fin spines have proven to be useful structures for age determination in six of the species examined.

Due to the severe vulnerability of deep-sea sharks to fishing pressure, these data will be crucial for the future management of these long-lived species as more deepwater fisheries continue to exploit this resource.

3b3c. Age and growth of *Etmopterus princeps* and *Centroselachus crepidator*.

Objectives. The objectives were to determine age using dorsal fin spines and generate growth models for *Etmopterus princeps* and *Centroselachus crepidater*.

Methods. *Etmopterus princeps* and *Centroselachus crepidater* are the only two species of deep-sea chondrichthyans collected during the MAR-ECO expedition in sufficient numbers for age and growth. Aging is being conducted as described in 3b3a above.

Outcomes and Significance. Fin spines from 128 male and 68 female *Etmopterus princeps* and from 59 male and 16 female *Centroselachus crepidater* have been collected. These spines are currently being prepared for ageing.

Little data exist on the life history of deep-sea sharks. The few studies to date indicate that most of these taxa are very slow growing, mature late, and have limited fecundity. As deepwater fisheries continue to grow, data on the life history of the species involved are critical to proper management.

3b3d. Reproductive biology of three species of squaloid sharks, *Centrophorus c.f. niaukang*, *Etmopterus princeps*, and *Centroscymnus coelolepis*.

Objectives. The objectives of this study were to determine maturity stages and fecundity and investigate possibility of muroid histotrophy in each of these species.

Methods. During routine sampling on all cruises, data on sex, maturity, stage and fecundity were recorded and reproductive tracts were collected from a sub-sample. These data will be reported. In addition, ash-free dry weights of freshly fertilized ova and near term embryos will be compared for each species to determine if histotrophy exists in these species, and if so, the

relative maternal contribution to the developing offspring.

Outcomes and Significance. Sufficient samples of reproductive tracts and embryos in various stages of development for *E. princeps* and *C. coelolepis* were collected during the MAR-ECO expedition to investigate the usage of histotrophy by these species. In addition, six adult female *Centrophorus* c.f. *niaukang* have been collected from Norfolk Canyon in the northwest Atlantic during the VIMS Longline Survey in 2006 and 2007.

Basic information about reproductive biology of shark species is important for understanding the life history of these animals. The mode of embryonic development employed by a given species offers insight into the energetic requirements of the reproductive process.

3b3e. Life history of Squalus mitsukurii from the Main Hawaiian Islands.

Objectives. The shortspine spurdog is a common slope-water species in the Main Hawaiian Islands and is often caught as bycatch in the dropline bottom-fisheries. The objectives of this study were to investigate the age, growth, and reproductive biology of this population.

Methods. Demersal longlines were set at depths ranging from 200 meters to 400 meters off the windward coast of Oahu. All spurdogs captured were retained for samples including fin spines, vertebrae, reproductive tracts, livers, and stomachs. In the future, the samples will be examined using the standard methodology for determining growth rates and maturity schedules.

Outcomes and Significance. Samples from approximately 150 spurdogs have been collected, but about 80% of these are from females. Preliminary examination and past studies suggest the second dorsal spine will serve as a useful hard part for age determination. Additional samples will be obtained over the next year depending on funding.

This species is common bycatch in bottom-fisheries in the central Pacific Ocean. It is likely to be very slow-growing and late to mature like most squaliforms. Estimation of life history parameters is critical to determining the impact these fisheries and future fisheries may have on this population.

3c. Habitat Use and Migration Studies.

Resolving and understanding movement patterns and habitat use in coastal sharks and rays (especially the sandbar shark) has been a primary objective of VIMS scientists for more than 15 years. Many different techniques have been used to investigate long-term and short-term movement patterns including conventional tagging, manual telemetry, and satellite tagging.

3c1. Movements of Juvenile Sandbar Sharks Using Conventional Tagging.

Objectives. Using conventional tagging, we investigated short-term movements, migration, and philopatry of juvenile sandbar sharks to Virginia waters.

Methods. VIMS scientist have been collaborating since 1981 with NMFS scientists with the Cooperative Tagging Program at Narragansett, RI to use NMFS tags on several species of sharks off Virginia. In addition, in 1995 we began to tag juvenile sandbar sharks with nylon dart tags to investigate movements and migration patterns of juveniles. These are the same tags that are used by Mote scientists on the Gulf Coast and by the NMFS scientists COASTSPAN program. Our juvenile tagging program is an integrated part of a NMFS Atlantic and Gulf Coasts study of shark nursery habitats.

Outcomes and Significance. Using conventional tagging methods, more than 5,000 juvenile sandbar sharks have been tagged. Data from more than 100 recaptures were used to define important wintering grounds near the Outer Banks of North Carolina and to verify that most juveniles return to Virginia waters during summer months for several years. Juveniles return to Chesapeake Bay and nearby estuaries for the first three summers of life and return to Virginia coastal waters for the first six years. These data also verified the timing of migration. Most sandbar sharks leave Virginia estuarine waters by mid-September and arrive in North Carolina by mid-October. They leave North Carolina waters by early May and return to Virginia waters in June.

We found that while Chesapeake Bay is a critical summer nursery, the nearshore waters of North Carolina are important wintering grounds. These results were used to define federal Habitat Areas of Particular Concern. The results of this research were published in two peer-reviewed papers (Conrath and Musick 2007b, Grubbs et al. 2007).

3c2. Vertical Movements and Migration of Large Juvenile Sandbar Sharks Using Satellite Telemetry.

Objectives. Although standard tags have provided a great deal of information so far on shark movements and migration, they provide basically only two pieces of geographic information: point of release and point of recapture. The objectives of this study were to deploy pop-off satellite transmitters on large juvenile and adult sandbar sharks to further define migration patterns and habitat use.

Methods. Large juvenile sandbar sharks were captured in coastal lagoons along Virginia's Eastern Shore during the summer of 2003. Pop-up archival transmitters (PAT) were attached to the first dorsal fin. These tags were programmed to collect light, temperature, and depth data at hourly intervals and were scheduled to release from the sharks between December 2003 and February 2004.

Outcomes and Significance. In total, 21 large juvenile sandbar sharks (121-142 cm TL) were fitted with pop-off satellite transmitters. Four of these failed to report, 12 released earlier than scheduled and 5 released when scheduled. The sharks inhabited colder and deeper waters during the wintering period (19.9°C and 20.8 m) than during summer (24.0°C and 4.3 m). Pop-off locations confirmed conventional tagging data that suggest warmer waters off the Outer Banks of North Carolina represent important wintering grounds for juvenile sandbar sharks. These results have been published in a peer-reviewed journal (Conrath and Musick 2007a).

3c3. Reproductive Periodicity and Philopatry of Adult Female Sandbar Sharks Inferred from Telemetry.

Objectives. This study investigated residence time and philopatry in adult female sandbar sharks captured in pupping areas along the eastern shore of Virginia

Methods. An array of VR2 stationary receivers has been in place during summer months for various other projects (see 3c5 below). These include receivers placed at the primary inlets allowing access to the coastal lagoons. Adult female sandbar sharks were fitted with multi-year sonic transmitters. These transmitters will be detected by the inlet receivers when the sharks emigrate from the lagoon system or return to this system. This will allow an estimation of residence time within the estuary following pupping as well as detection if they return to the same pupping area during subsequent pregnancies, to allow an estimation of reproductive periodicity.

Outcomes and Significance. Nine pregnant or post-partum sandbar sharks were outfitted with transmitters during the summers 2006 and 2007. Most females left the Hog Island lagoon system within a couple of weeks of tagging. However, one female remained in the system through September. Deployment and monitoring of the receivers over the summers of 2008 and 2009 will be required to determine if the females return to the system. If they do, this will provide strong evidence of the reproductive periodicity since only pregnant adults inhabit this region.

It has been generally accepted that sandbar sharks in the Northwest Atlantic reproduce biennially. However, some researchers have recently proposed that the periodicity may be triennial. If true, this would have profound effects on demographic models currently in use. Data from these tagged females could resolve this issue.

3c4. Alaskan Studies: Movements and Thermal Physiology of Salmon Sharks.

Objectives. This study used multiple tagging methodologies to investigate thermal physiology as well as patterns of movement and migration in salmon sharks.

Methods. VIMS scientists have opportunistically collaborated with scientists from Alaska Fish and Game and Hopkins Marine Station, Stanford University to attach satellite tags to salmon sharks in Prince William Sound. A subset of those sharks caught and tagged with streamer tags as well as those tagged with acoustic transmitters for short-term temperature tracking and fine-scale movement delineation were also tagged with pop-off and SPOT (Smart Position Only Tags) satellite transmitters. All sharks caught for tagging with either sonic or satellite transmitters were also tagged with ADF&G streamer tags.

In order to better quantify the ability of salmon sharks to physiologically thermoregulate, VIMS collaborated with Dr. Kenneth Goldman (ADF&G) to obtain a record from the deep red and epaxial musculature along with ambient water temperature and swimming depth. Sharks were caught via hand-lines and brought to the side of the vessel where an ultrasonic transmitter (that transmits temperature and depth) is attached to the dorsal musculature and the thermistor wire placed into the deep red muscle. A second transmitter, which records temperature, was attached to the first transmitter. These two transmitters allow simultaneous readings of muscle temperature, swimming depth and ambient water temperature. Sharks were tracked from a skiff during the day and the larger ADF&G vessel at night using a VEMCO acoustic hydrophone system.

Outcomes and Significance. Direct satellite telemetry from the dorsal fins of salmon sharks reveals subarctic-to-subtropical migrations over multiple years. Therefore salmon sharks have a subarctic-to-subtropical niche, ranging from 2°C to 24°C, and some may spend winter periods in waters as cold as 2°C to 8°C. Data (ranging from 4 hrs to 22 hrs) on muscle temperature of salmon sharks were gathered in 2004 and 2005. These data continue to be analyzed with the goal of producing an in-depth publication on the thermal physiology of this species. The deep red muscle temperature fluctuated in response to changes in ambient water temperature, as expected, and preliminary results from these data show a low rate of heat loss through the body wall. This indicates that salmon sharks are extremely efficient at conserving their metabolically generated heat.

The combination of tag types (streamer, acoustic and satellite) is helping to define local and seasonal movements and habitat utilization for this species, which is an important recreational species and which may be capable of supporting a modest commercial harvest in Alaska. Satellite tracking technologies can be used to rapidly map shark habitats worldwide, an objective that is critical to their future protection.

3c5. Movement and Migration of Batoids in Coastal Lagoons of Virginia.

Objectives. The goal of this study was to examine the daily and seasonal movements of the various rays that inhabit Virginia's coastal lagoons and determine the physical parameters influencing the patterns seen.

Methods. Currently there are 42 stationary VEMCO acoustic receivers strategically placed throughout the lagoon system. Five YSI sondes are placed throughout the lagoon system to assess the physical parameters affecting movements. Vemco multi-year sonic transmitters are implanted in the most common species of gymnurid and dasyatid rays.

Outcomes and Significance. During the summer of 2006, 5 bluntnose stingrays and 5 southern stingrays were implanted with Vemco acoustic transmitters to examine their migration patterns. All five bluntnose stingrays emigrated in late July and early August while the 5 southern stingrays emigrated during late September to early October. In 2007, 3 bluntnose stingrays returned to the array in early June while no southern stingrays returned. In 2007, seven southern stingrays, *Dasyatis americana*, and 12 spiny butterfly rays, *Gymnura altavela*, were implanted with acoustic tags and their movements are currently being tracked with stationary receivers in the Wachapreague lagoon system.

Little is known about the movement of rays that inhabit the coastal lagoons of Virginia or the factors that influence the patterns seen. This experiment is ongoing so the results are not complete. Preliminary data suggest that bluntnose stingrays return to the estuaries annually but southern stingrays may not. Current findings also suggest spiny butterfly rays and southern stingrays occupy different niches within the ecosystem. The spiny butterfly ray spends a large amount of time outside of the lagoons, whereas the southern stingray spends its time almost exclusively within the lagoon system. Also, the spiny butterfly ray's movement appears to be tidally influenced, while the southern stingray's movement is more complex.

3c6. Vertical Movements of Adult Bluntnose Sixgill Sharks (*Hexanchus griseus*) in the Northwest Atlantic Ocean.

Objectives. The bluntnose sixgill shark is an apex predator in deep waters along island and continental slopes. Very little is known about the ecology of this species. The objective of this study was to investigate the vertical movement patterns of this very large demersal shark.

Methods. Sixgill sharks were caught on demersal longlines fished in the vicinity of Norfolk Canyon off the Virginia coast. Sets were deployed at depths ranging from 250 meters to 1,200 meters. Healthy sixgill sharks were fitted with Microwave Telemetry High Rate Pop-off Satellite Transmitters (PSAT) to gain detailed information about diel patterns of vertical movement.

Outcomes and Significance. Two adult bluntnose sixgill sharks were captured in June 2006 and one of these was outfitted with a PSAT. This female was 355 cm TL and weighed ~500 kg. Following a nine-day deployment, this tag released as scheduled and transmitted its data to the satellite. The data from this animal suggested there was a recovery period of about 60 hours following the trauma of capture. The shark's depth was as great as 600 meters during this time. Following the proposed recovery period, a distinctive diel pattern was observed with the shark spending daylight hours at depths around 300 meters and nighttime at depth around 200 meters. A second sixgill shark (female, 372 cm TL) was outfitted with a 30-day High Rate PSAT in August 2007. Data have not been received from this animal.

Prior to this study, the only telemetry data for adult bluntnose sixgill sharks were obtained from manual tracking of two animals captured at 500 meters depth off Bermuda. These tracks

only lasted 24 hours and may have only represented behavior during the recovery period. Future plans include tagging additional bluntnose sixgill sharks in the western Atlantic and central Pacific oceans.

3d. Shark Energetics and Osmoregulatory Studies.

3d1. Trophic Ecology of Juvenile Sandbar Sharks.

Objectives. The goal of this study was to describe and quantify the diet of juvenile sandbar sharks by region in Virginia waters.

Methods. Stomach samples were obtained from 232 sandbar sharks. Samples were sorted and identified to the lowest taxa. Differences based on sex, size class, and location were assessed using indices of dietary overlap and diversity as well as correspondence analyses.

Outcomes and Significance. Sandbar sharks fed on 57 families of prey. An ontogenetic shift was observed with juvenile sharks feeding primarily on crustaceans, primarily the mantis shrimp *Squilla empusa*, and larger juveniles feeding on teleosts, primarily the Atlantic croaker *Micropogonias undulatus*, elasmobranchs, primarily clearnose skates *Raja eglanteria*, and cephalopods.

The diverse prey base suggests sandbar sharks may have relatively weak impacts on populations of individual prey species. Conversely, sandbar shark populations are not likely to be strongly affected by fluctuations in prey populations. This work was published in a peer-reviewed journal (Ellis and Musick 2006).

3d2. Estimation of Consumption Rates of Juvenile Sandbar Sharks.

Objectives. This study aimed to estimate daily ration and seasonal prey consumption rates for six age classes of juvenile sandbar sharks in Chesapeake Bay using a bioenergetics model.

Methods. We used rates of anabolism, catabolism, and waste loss to build a bioenergetics model that predicted daily energy consumption (C_D , in joules per day, J/d):

$$C_D = RMR_D + SDA + G_D + F + U$$

where RMR_D = Routine metabolic rate, SDA = specific dynamic action, G_D = Growth, F = fecal losses, and U = excretions. Published diet composition data and populations estimates were used to predict the impact of sandbar sharks on populations of prey organisms in Chesapeake Bay.

Outcomes and Significance. Mean daily rations (% body mass/day) were estimated between 2.17 ± 0.03 (age-0) and 1.30 ± 0.02 (age-5). Using published VPA estimates of sandbar shark population size adjusted for a skewed age structure, the nursery population was estimated to be 11,627 \pm 2,483 sharks. This population of sandbar sharks was predicted to consume ~124,000 kg of prey over the summer.

The results suggest that the overall prey consumption by teleost predators in Chesapeake Bay is one to two orders of magnitude greater than that by juvenile sandbar sharks and the consumption of blue crabs by sandbar sharks was estimated to be 0.57% of the annual commercial harvest in the estuary. The results of this study were published in a peer-reviewed journal (Dowd et al. 2006b).

3d3. Standard and Routine Metabolic Rates of Juvenile Sandbar Sharks.

Objectives. The objective of this study was to measure standard and routine metabolic rates (SMR and RMR) for juvenile sandbar sharks.

Methods. Sandbar sharks between 57cm and 124 cm TL were captured by using hook and line from tidal lagoons near Wachapreague, Virginia and maintained in shoreside tanks (temperature 22–29°C, salinity 34–36‰). SMR measurements were taken from sharks maintained in flow-through, sealed box respirometers. RMR measurements were taken from sharks in an annular respirometer (1250 L; diameter 167 cm).

Outcomes and Significance. SMR was measured for 23 sharks and paired SMR and RMR measurements were recorded from 15 sharks. SMR and RMR increased with increasing body mass and heart rate decreased with increasing body mass and the mean SMR Q_{10} was 2.9. RMR's average 1.8 times SMR's and this ratio was not correlated with body mass.

Sandbar sharks were found to use a significant portion of their metabolic scope just to maintain daily functions, a limitation that may explain their relatively slow growth rates. The results of this study were published in a peer-reviewed journal (Dowd et al. 2006a).

3d4. Osmoregulatory Abilities of Juvenile Sandbar Sharks.

Objectives. This study's goal was to investigate the salinity tolerance of juvenile sandbar sharks.

Methods. Juvenile sharks were kept in three closed-system aquariums and the salinity decreased at predetermined weekly intervals (from 33, to 27, and to 20 ppt) by addition of fresh water. The sharks were weighed and blood samples taken once per salinity treatment. Hematocrit, hemoglobin, red blood cell count, total protein, plasma osmotic pressure, pH, pCO₂, pO₂, sodium, potassium, chlorine, urea, lactate, and glucose concentrations were measured from each sample.

Outcomes and Significance. After one week at 27 ppt, the following changes were observed: 5.7% decrease in hematocrit, 7.6% decrease in total protein concentration, 7% decrease in plasma osmotic pressure, 21.8% decrease in plasma urea concentration, 3.6% decrease in plasma sodium and average weight (kg) increased 2.3%. At 20 ppt, after the entire treatment, the following changes were observed: 8.7% decrease in hematocrit, 6.6% decrease in hemoglobin, 16% decrease in total protein concentration, 14% decrease in plasma osmotic pressure, 0.9% decrease in pH, 5.3% decrease in plasma sodium content and 49% decrease in plasma urea concentration. The sharks' average weight (kg) increased 7%. No significant changes in plasma glucose, potassium, chlorine and lactate were evident.

Previous VIMS research shows that sharks use areas in Chesapeake Bay with salinity down to about 20 ppt. These data, and evidence from other shark species, supports the hypothesis that sandbar sharks are coping with the osmotic stress by maintaining plasma ion levels in spite of an increase their extracellular water content.

3d5. Batoid Energetics.

Objectives. The objective was to measure Resting Metabolic Rate of seven individuals of the southern stingray and the bluntnose stingray to help in determining their energetic demands within the ecosystem.

Methods. Stingray metabolism was measured using an annular respirometer.

Outcomes and Significance. Due to funding restrictions, work on this project was not completed. Stingrays may have significant impacts on ecosystem energy budgets as some of the largest predators in the estuaries. Results from this study would be beneficial in constructing bioenergetics' models for these species.

3e. Genetic Studies.

3e1. Genetic Polyandry in Sandbar Sharks in the Western North Atlantic.

Objectives. The goal of this study was to investigate the occurrence and frequency of genetic polyandry in *Carcharhinus plumbeus* from the western North Atlantic

Methods. Twenty females and their litters were genotyped using five species-specific, polymorphic microsatellite loci. Two separate programs with different algorithms were used to estimate the number of sires represented in each litter.

Outcomes and Significance. Most litters had more than one sire (85%). The average number of sires per litter was 2.3. The number of sires was not correlated with realized female fecundity indicating no direct benefits to genetic polyandry.

Understanding mating system is important from both an evolutionary and management standpoint. These particular results will be used in an attempt to understand the relationship between effective size and census size. The results of this study have been published in a peer-reviewed journal (Portnoy et al. 2007)

3e2. Estimation of Effective Population Size and Reproductive Periodicity in Sandbar Shark Nurseries in Virginia.

Objectives. We estimated the effective number of breeders, for multiple years, utilizing the Delaware Bay and the lagoons of the Eastern Shore of Virginia as nursery grounds.

Methods. Over 600 individuals (YOY and one year olds) have been genotyped at 8 polymorphic microsatellite loci. Three different methods of estimation have been employed; the linkage disequilibrium method, a one sample method employing approximate Bayesian computation, and a variance method utilizing cohort data. These numbers will be examined in relation to census size estimates.

Outcomes and Significance. Final estimates are still being made. Preliminary estimates suggest that the effective number of breeders is on the order of several hundred per year per nursery ground.

Using molecular techniques and limited sampling to estimate the effective number of breeders may be a useful tool from a management perspective. This is especially true if the number is tightly coupled to census size, as it may be in species with low mortality and evenly distributed reproductive successes.

3e3. Global Phylogeography of Sandbar Sharks.

Objectives. This study aimed to understand the patterns of historical dispersal for *Carcharhinus plumbeus* as well as contemporary gene flow.

Methods. Samples collected from throughout the species range were genotyped at 8 polymorphic

microsatellite loci and the mitochondrial control region (1069bp) was sequenced.

Outcomes and Significance. This project is underway with samples analyzed from all locations (South Africa, E. Australia, W. Australia, Taiwan and Hawaii) except the western South Atlantic and Eastern North Atlantic.

Understanding factors that lead to a species distribution may provide insight into important selective forces currently acting upon that species. In addition understanding contemporary gene flow is important in exploited species.

3e4. Genetic Polyandry in the Smooth Dogfish (*Mustelus canis*) and Spiny Dogfish (*Squalus acanthias*).

Objectives. Genetic polyandry is widespread in elasmobranchs though the rate varies. Continued investigation into polyandry in species with different reproductive modes is important for comparative purposes.

Methods. Females and litters are being genotyped using microsatellite loci to detect the number of sires per litter. In addition, stored sperm are being genotyped in *Mustelus canis* to compare the number of sires in a litter with the number of matings in which a female engaged.

Outcomes and Significance. Using *Mustelus canis* as a model, we hope to understand whether sperm storage permits females to exercise control over which matings result in fertilization. Currently the markers to be used in this project are still being optimized.

Much discussion has been generated in recent years about why female elasmobranchs would mate multiply. Sperm storage is a predicted outcome of the evolutionary tug-of-war that males and females engage in over mating rate. Understanding the function and mechanisms of sperm storage will further our understanding of observed mating behaviors.

3f. Publications and Conferences. During the past five years of this project, scientists and students in the VIMS Shark Research Program produced two books, four edited volumes and 35 scientific papers published or in press (see Appendix). We provided the longest time series of fisheries-independent catch data used by NMFS in stock assessments for the large coastal shark species complex, small coastal shark species complex, and species-specific stock assessments for sandbar sharks and dusky sharks in Atlantic waters. Data concerning salmon sharks were also provided to the NPFMC for assessment purposes. We also contributed to more than 30 IUCN SSG species assessments. VIMS biologists attended 23 national and international conferences and made 56 presentations on elasmobranch management and biology. In addition, we have trained 15 VIMS graduate students (6 Master's, 9 Doctoral) and we have served as adjunct faculty on the graduate committees of 3 students outside of VIMS.

**UNIVERSITY OF FLORIDA
FLORIDA PROGRAM FOR SHARK RESEARCH**

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4a. Fishery-independent Sampling of Commercially Exploited Sharks.

Objectives. The goal of this study was to continue fishery-independent surveys in the geographic region traditionally monitored by the Commercial Shark Fishery Observer Program (CSFOP) in order to provide year-round sampling and monitoring of sharks within the region. In addition, hook timers were used to identify how long individual sharks remain on the line after being caught on hooks.

Methods. Fishery-independent longline sampling was conducted aboard four leased commercial shark fishing vessels during the period. FPSR scientists collected data during 151 sets off the east and west coast of Florida. A total of 250 hook timers were used per set and soak times ranged from four to 12 hours.

Outcomes and Significance. A total of 3,344 sharks of 23 different species were caught during this time period. The most commonly caught species were the sandbar (*Carcharhinus plumbeus*) (776), nurse (*Ginglymostoma cirratum*) (585), Atlantic sharpnose (*Rhizoprionodon terraenovae*) (571) and blacktip (*Carcharhinus limbatus*) (403) sharks. Some of these sharks were tagged and released alive, others, primarily those that arrived at the boat dead, were necropsied and sampled to provide tissues and materials for biological studies by FPSR scientists and NSRC collaborators.

Results from this study fill in the seasonal gaps in biotic observation currently dictated by open fishing season-only data gathering and provide a temporal continuum for studying such critical biological processes as the reproductive cycle and age and growth. In addition, because fishery-independent sampling duplicated methods employed in the CSFOP, these two data sets can be combined to provide a more comprehensive view of shark populations in the region. This project will continue to contribute significantly to improved demographic analyses, stock assessments, and management decisions by NMFS. In addition, hook timer data has provided more detailed data pertaining to the relationship between soak time and capture depth on fishing mortality and catch per unit effort (CPUE) of individual shark species and shark species aggregates. Shark specimens collected during these surveys have also contributed to several ongoing shark life history studies, shark genetic studies, as well as a shark jaw morphometrics study.

4b. Studies of Age, Growth, and Reproduction of Commercially Important Sharks.

4b1. Analysis of Variability in Vertebral Morphology and Growth Ring Counts in Two Carcharhinid Sharks.

Objectives. This study was designed to elucidate possible sources of variation in vertebral age determination in commercially exploited sharks. Specifically, we analyzed the potential age variability introduced by region of the vertebral column that is sampled for growth ring enumeration.

Methods. Specimens of sandbar (*Carcharhinus plumbeus*) and blacktip (*C. limbatus*) sharks were collected through fishery-independent sampling. Vertebrae (n=10) from the cervical (above the branchial cavity) and thoracic (below the first dorsal fin) regions of the vertebral column were dissected and morphological measurements were taken. A subset (n=3) of vertebrae from each region was sectioned sagittally and growth bands were enumerated. Sources of variation in vertebral morphology were analyzed through ANCOVA tests. Variability in growth band counts was analyzed through measurements of approximant percent error and coefficient of variation.

Outcomes and Significance. Ten sandbar and ten blacktip sharks were used in this study. Our data indicate that vertebrae morphology varies significantly between the two regions of the vertebral column. However, inter-regional variability in growth band counts was not significantly higher than intra-regional variability.

These findings suggest that vertebrae from both regions of the vertebral column are suitable for age determination. However, as vertebral morphology varies between the regions, separate correlation models should be used for back calculation techniques.

4b2. Age and Growth of the Scalloped Hammerhead Shark, *Sphyrna lewini*, in the Northwest Atlantic Ocean and Gulf of Mexico.

Objectives. The objective of this collaborative study (with NMFS Panama City laboratory scientists) was to develop precise updated age and growth data for this highly exploited shark in the northwest Atlantic Ocean and Gulf of Mexico.

Methods. Scalloped hammerhead sharks were collected through fishery-dependent and fishery-independent longline fishing. Sharks were measured in the field and vertebrae were removed. Sagittal cut cross-sections were made using a low-speed diamond saw. Growth bands were enumerated and von Bertalanffy growth models were developed. Measurements of error were analyzed to ensure a precise model was developed.

Outcomes and Significance. Of the 311 original samples, only 4 were discarded as unreadable. Measurements of error were very low with an approximate percent error of 3.2%. Growth models were significantly different for males and females with male sharks having growth parameters of $L_{\infty} = 214.8$ cm fork length, $k = 0.13 \text{ year}^{-1}$, $t_0 = -1.62 \text{ year}$ and female growth parameters of $L_{\infty} = 233.1$ cm fork length, $k = 0.09 \text{ year}^{-1}$, $t_0 = -2.22 \text{ year}$. These data indicate that female scalloped hammerheads reach larger sizes but exhibit slower growth. The oldest sharks aged were 30.5 years for one male and one female shark.

This study represents the only suitable growth model for development of species-specific stock assessments for this species of shark in the northwest Atlantic Ocean and Gulf of Mexico. These data will allow for better assessment and management of this highly vulnerable shark species.

4b3. Age and Growth of the Great Hammerhead Shark, *Sphyrna mokarran*, in the Northwest Atlantic Ocean and Gulf of Mexico.

Objectives. The objective of this collaborative study (with NMFS/Panama City Laboratory) was to develop the first growth model for the poorly studied and vulnerable great hammerhead shark.

Methods. Great hammerhead sharks were collected through fishery-dependent and fishery-independent longline fishing. Sharks were measured in the field and vertebrae were removed. Sagittal cut cross-sections were made using a low-speed diamond saw. Growth bands were enumerated and von Bertalanffy growth models were developed. Measurements of error were analyzed to ensure a precise model was developed.

Outcomes and Significance. Our data indicate that great hammerhead sharks exhibit faster growth until the onset of maturity. Growth models were significantly different for males and females with male sharks having growth parameters of $L_{\infty} = 286.7$ cm fork length, $k = 0.12 \text{ year}^{-1}$, $t_0 = -2.51$ year and female growth parameters of $L_{\infty} = 304.6$ cm fork length, $k = 0.11 \text{ year}^{-1}$, $t_0 = -2.79$ year. These data indicate that female great hammerhead sharks reach larger sizes and have slightly slower growth rates. The oldest specimen aged was 48 years old.

This study represents the first report of age and growth data for this species in any part of its range. These data will be used in species-specific stock assessments to better manage this highly vulnerable shark. While growth rates were similar for great hammerhead and scalloped hammerhead sharks, the maximum age and size were much higher in great hammerheads.

4b4. Reproduction of the Sandbar Shark, *Carcharhinus plumbeus*, in the Northwest Atlantic Ocean and Gulf of Mexico.

Objectives. This study was conducted to elucidate reproductive parameters for the sandbar shark. The most complete previous study on the reproduction of this species was published in 1960.

Methods. Specimens for this study were collected through fishery-dependent and fishery-independent bottom longline fishing. Fork length and total length measurements of the sharks were taken in the field. Samples of the reproductive tract were dissected in the field and preserved in 10% formalin for later analysis. Morphological measurements and weights were taken of the gonads and oviducal glands, epididymis and seminal vesicles. Observations of uterine condition and contents were also made. Gonadosomatic indices were developed for mature males and females. Histological analysis was used to detect changes in the architecture of reproductive tissue, to confirm the timing of sperm production, and to examine the possibility of female sperm storage.

Outcomes and Significance. Our data show that the sandbar has a discrete reproductive cycle. Male sandbar sharks begin sperm production in late winter/spring and mating occurs in late May/June. Female sandbar sharks exhibit a three-year reproductive cycle with a gestation period of 12 months. Oocyte development occurs in late winter/spring, with ovulation occurring in June. No evidence of sperm storage in female reproductive tracts was observed.

This study is the most comprehensive study on sandbar sharks in this region. The sandbar shark is the most important species in the coastal shark fishery. The occurrence of a three-year reproductive cycle is in contrast with previous studies and has significant implications for fishery management. These data are available to NMFS stock assessment scientists and will be used in a collaborative study with scientists from NMFS Panama City laboratory, focused on determining the age of maturity for this species.

4b5. Reproductive Cycle of the Blacknose Shark, *Carcharhinus acronotus*, in the Gulf of Mexico.

Objectives. This study, which is a collaboration with NMFS scientists from the Panama City and Pascagoula laboratories, was designed to determine the reproductive cycle of the blacknose shark in the Gulf of Mexico. Previous studies have only been conducted on blacknose sharks found in the Atlantic Ocean.

Methods. Specimens for this study were obtained from fishery-dependent and fishery-independent bottom longline fishing. Fork length and total length measurements of the sharks were taken in the field. Samples of the reproductive tracts were dissected in the field and preserved in 10% formalin for later analysis. Morphological measurements and weights of the gonads and oviducal glands were taken. Histological analysis was used to confirm the timing of sperm production in the testes. Observations of uterine condition and contents were also made.

Outcomes and Significance. The data from this study indicate that the blacknose shark has a discrete annual reproductive cycle in the Gulf of Mexico. The gestation period is nine to 10 months. Sperm production and oocyte development appear to be synchronous and occur in the spring. The occurrence of simultaneous oocyte development and gestation suggest that female blacknose sharks reproduce annually.

The apparent annual reproductive cycle of blacknose sharks in the Gulf of Mexico contrasts with the biennial reproductive cycle exhibited in blacknose sharks in the northwestern Atlantic Ocean. These data are being used by NMFS scientists in species-specific stock assessment models.

4b6. Analysis of Shark Embryonic Fitness in the Sandbar Shark and the Atlantic Sharpnose Shark, *Rhizoprionodon terraenovae*.

Objectives. This study was designed to investigate the occurrence of “runts” in shark litters and analyze potential reasons for this occurrence.

Methods. Litters from sandbar (*Carcharhinus plumbeus*) and Atlantic sharpnose (*Rhizoprionodon terraenovae*) sharks were collected through fishery-dependent and fishery-independent bottom longline fishing. Litters were dissected in the field and placed on ice and frozen for later analysis. Embryonic lengths and weights were recorded and DNA samples were preserved for all embryos. Fulton condition values were determined for each embryo and comparisons with maternal size were analyzed. Fatty acid and lipid levels were analyzed in maternal and embryonic livers to see if nutrient partitioning was evident.

Outcomes and Significance. Sandbar shark runts are not genetically distinct and do not have depressed levels of lipids or fatty acids in their livers. These data suggest that the occurrence of runts is not genetic but may be related to nutrient partitioning of non-lipid metabolites. While low Fulton condition levels were not correlated with maternal size, the range of condition levels was smaller for larger mothers. This would suggest that larger mothers are better able to provide nourishment for all pups of a given litter than smaller mothers. We do not currently have enough sharpnose shark samples to begin analyses.

This is the first study to examine the occurrence of “runts” in shark litters. Better knowledge of embryonic fitness and nourishment may allow for more accurate determinations of neonate mortality rates for population modeling.

4b7. Paternity Analysis of the Sandbar Shark, *Carcharhinus plumbeus*, in the Northwest Atlantic Ocean and Gulf of Mexico.

Objectives. This study, in collaboration with scientists from Virginia Institute of Marine Science, was designed to determine the mating patterns of the commercially important sandbar shark (*Carcharhinus plumbeus*).

Methods. Embryonic and maternal DNA samples from 20 litters were collected during fishery-dependent and fishery-independent longline fishing, and stored frozen or in 95% ethanol. Molecular microsatellite markers were utilized to determine paternity patterns.

Outcomes and Significance. Our results show that the majority (85%) of female sandbar sharks mate with multiple males in a single reproductive season. Regression analysis did not demonstrate a significant relationship between female reproductive success and female body size or sire number and female body size. There was a high incidence of reproductive skew noted in litters, and two groups of males with highly different mean reproductive success were observed. Analysis using Bateman's principles suggest that there is less direct benefit for females that acquire multiple mates than for males who bias paternity within litters.

In light of past morphological and behavioral studies, these data suggest that patterns of polyandry in elasmobranchs may be determined by coercive mating, and that breeding behavior has likely evolved in the context of sexual conflict. This research adds to a small number of studies examining paternity patterns in elasmobranchs.

4b8. Population Genetics of the Blacknose Shark, *Carcharhinus acronotus*, in the Northwest Atlantic Ocean and Gulf of Mexico.

Objectives. Previous reproductive studies of the blacknose shark indicate regional variation in the reproductive cycle. The objective of this project was to determine if genetic variation in blacknose sharks was present between these two regions, which may account for variation in reproductive parameters.

Methods. DNA samples were taken from specimens caught at four separate sites: off shore of Louisiana, Pensacola (Florida), Fort Lauderdale (Florida), and Cape Canaveral (Florida). Mitochondrial DNA analysis was utilized to determine any genetic variability.

Outcomes and Significance. DNA from twenty specimens was collected from the four sites. Our preliminary results show very little variability in the population genetics among specimens sampled from the four sites.

Our preliminary findings suggest that any variation in the timing of reproduction may not be due to purely genetic variation. Variation in environmental parameters and prey availability may be factors influencing variability in reproductive timing.

4b9. Bomb Radiocarbon Method for Validating Age and Growth in the Sandbar Shark.

Objectives. The goal of this study was to validate current age and growth estimation methodology and models for the over-exploited sandbar shark, *Carcharhinus plumbeus*, in the western north Atlantic Ocean.

Methods. FPSR scientists, in collaboration with scientists from other consortium members (Moss Landing Marine Laboratory) and NMFS personnel in Narragansett, RI, obtained archived vertebral samples from sandbar sharks alive during the 1950's and 1960's. This time period

coincides with prolific atmospheric testing of atomic bombs resulting in documented increases in radiocarbon levels in the world's oceans. Previous studies with long-lived teleosts were able to validate age estimates derived from hard part structures through comparisons of radiocarbon levels in coral reefs. This approach has been attempted with a few elasmobranch species resulting in successful age validation or correction.

Outcomes and Significance. Fifteen sandbar shark vertebrae were chosen from archival material. Age estimates using traditional vertebral growth ring counts were determined. Small core samples were taken from individual growth bands using a micromilling machine. Core samples were analyzed using an Accelerator Mass Spectrometer at Lawrence Livermore National Laboratory. Radiocarbon chronologies, established from corals in Florida and Bermuda, were used for temporal calibration of the shark vertebral growth zones. The results from this study were inconclusive. Radiocarbon data from sampled vertebrae did not conform to established trends of increasing levels.

These data suggest that this technique may not work for all species of fishes. Variation in vertebrae mineralization among species may account for variation in resultant radiocarbon levels.

4b10. Examination of Ovarian Tissue of the Atlantic Stingray, *Dasyatis sabina*.

Objectives. This project aimed to elucidate the physiological mechanism and reason for ovarian follicle enlargement during a period of reproductive inactivity in the Atlantic stingray.

Methods. Ovaries from mature female Atlantic stingrays were collected during the period in question (summer months) from Lake George, Florida. Ovaries were fixed in formalin and processed for standard histological analysis. Blood and uterine fluid were collected and processed for RIA analysis of estradiol concentration.

Outcomes and Significance. To date, only a few samples have been collected. This study was a thesis project for a veterinarian student who has since dropped out of school. We anticipate restarting this project in the coming year.

4b11. Investigations of the Role of Uterine Secretions in Gestation.

Objectives. To further our knowledge of the role of uterine secretions in embryonic nutrition and gestation, our goal was to identify the organic composition of uterine fluids and determine whether and how this composition changes over the course of gestation.

Methods. FPSR scientists collected uterine fluid and serum from pregnant bonnetheads in the Gulf of Mexico with the assistance of collaborating NSRC partners at Mote Marine Laboratory. We performed preliminary characterizations of the proteins found in the uterine fluid of pregnant females via 1-D gel electrophoresis and compared them with gel profiles of the serum.

Outcomes and Significance. To date, samples have been collected and analyzed for this project. However the student working on this project has since left the program and has not analyzed the results of the study. We anticipate restarting and finishing this project in the very near future.

4c. Movement and Migration Studies of Ecologically Important Elasmobranchs.

4c1. Movement and Migration of Bull Sharks, *Carcharhinus leucas*, in the Indian River Lagoon.

Objectives. The objective of this project was to gain new insights into the distribution, movements, and habitat use patterns of immature bull sharks (*Carcharhinus leucas*) in the Indian River Lagoon (IRL) nursery area along Florida's central Atlantic coast using a multifaceted approach.

Methods. The study included compiling and synthesizing all available fishery-independent data sets for bull sharks in this system, including published and unpublished information, as well as starting a new hook-and-line sampling effort to capture and tag bull sharks. In addition, between August 2003 and August 2005, five YOY and five juvenile bull sharks were tracked using active ultrasonic telemetry techniques for periods of two to 23 hours (mean = 13.4 hours). One other YOY shark had a transmitter surgically implanted, and was monitored by moored listening stations (Vemco VR2) over a period of 156 days in 2004.

Outcomes and Significance. Fishery-independent data sets yielded seasonal distribution and habitat information from approximately 385 individual bull sharks dating back to 1974. Bull sharks ranging in size from 66 – 249 cm total length (TL) were recorded from the system, although the lagoon is predominantly utilized by young-of-the-year (YOY) and juvenile sharks (<150 cm TL). They were present year-round, but were significantly less abundant during the winter months in the northern reaches of the lagoon. Bull sharks occurred over a broad range of habitats, including depths of 0.2 to 4.0 m, temperatures of 18 to 37°C, salinities of 1 to 42 ppt, dissolved oxygen concentrations of 3 to 8 mg L⁻¹, water clarity levels of 70 to 170 cm, and from seagrass, sand, and mud substrates. Although overall catch per unit effort was comparatively low in the system, higher than average catch rates occurred at power plant outfalls and near freshwater creeks.

Telemetry results showed that bull sharks have very small daily activity spaces ranging from 0.02 to 3.49 km² (mean = 1.40 km²), with YOY sharks having larger activity spaces than juveniles. High levels of site fidelity were exhibited at power plant outfalls and near freshwater creeks; areas that may expose these young sharks to high levels of wastewater pollutants. Activity space appeared to be influenced by salinity, and varied between dry and wet periods in the lagoon, with sharks spending over 75% of their time in salinities higher than 11 ppt, even when lower salinities were available within their activity space. YOY sharks appeared to select lower salinity environments than juveniles, creating a level of size/age segregation in the nursery.

This study provided evidence of the importance of the Indian River Lagoon as habitat for bull sharks. In particular this area functions as a nursery for YOY and juvenile bull sharks. Due to a relative lack of large predators in the IRL, young shark behaviors were hypothesized to be more influenced by prey distribution than by predator avoidance. However salinity appears to be an important factor in bull shark habitat selection and further studies are needed to determine the importance of both of these parameters for YOY and juvenile bull sharks.

4c2. Movement and Migration of Ray Species in the Indian River Lagoon.

Objectives. This study sought to gain insight into the movements and migration of rays in one of their primary Florida inshore habitats, the Indian River Lagoon (IRL) system. Rays, including the state protected spotted eagle ray (*Aetobatis narinari*), have been shown to occur within this area in high abundance and therefore likely represent an important component of the ecosystem. Despite their ecological significance, fundamental life history information is limited or lacking for many of these species.

Methods. In cooperation with Dynamac, Inc. at the Kennedy Space Center, we are currently maintaining a passive acoustic monitoring system that includes 32 moored receiving stations

(Vemco VR2 receivers) placed at key locations throughout the northern IRL. The system is currently set up for monitoring the movements of bluntnose stingrays (*Dasyatis say*), smooth butterfly rays (*Gymnura micrura*), spotted eagle rays (*Aetobatus narinari*), cownose rays (*Rhinoptera bonasus*) (FPSR initiatives), adult red drum (*Sciaenops ocellatus*), and green sea turtles (*Chelonia mydas*) (Dynamac initiatives).

Outcomes and Significance. This array of receivers is currently being used to track the movements of 30 bluntnose stingrays, 20 smooth butterfly rays, two spotted eagle rays and two cownose rays. We will continue this effort and will attach Vemco V13 or V9 coded transmitters to at least 26 more rays during the upcoming year.

Little is known about the movements of ray species within the IRL or the migration of these animals to and from the lagoonal system. Information on the environmental preferences, home range, habitat use, and seasonal migrations of ray populations is essential to adequately understand and protect these species and their critical habitats. Preliminary results from this study indicate that the bluntnose and smooth butterfly rays predominantly remain resident within the lagoon with evidence of only a small percentage (13%) leaving the array area. Around half of the total detections of bluntnose and smooth butterfly rays occurred at two receivers south of Haulover Canal indicating a strong preference for this locality within Mosquito Lagoon. These receivers occur near broad shallow grassy flats and rays may spend a significant portion of time within this habitat. Data analysis for this project continues and results will be used to examine the space use and residency of ray species within the Indian River Lagoon system.

4d. Elasmobranch Nursery and Pupping Areas within the Eastern Gulf of Mexico.

Objectives. The Cedar Key area of the Gulf of Mexico consists of several small islands situated just offshore between the Suwannee River mouth to the northwest and the Waccasassa River mouth to the southeast. The incidental capture of numerous elasmobranch species within this area off the western Florida coast by scientists working for the University of Florida and the Florida Fish and Wildlife Conservation Commission indicates this region may support a diverse and abundant assemblage of sharks and rays. The objective of this study is to examine the role of this area as habitat for elasmobranchs as part of the GULFSPAN nursery study.

Methods. In cooperation with the NMFS Southeast Fishery Science Center we initiated a monitoring study in the Cedar Key area in the Gulf of Mexico in May of 2007. Using standardized gill-netting gear, areas from the Waccasassa River mouth to the Suwannee River mouth are sampled on a monthly basis between May and October using stratified random sampling techniques. This study will continue at least through the year of 2008.

Outcomes and Significance. To date, 24 two-hour gill net sets have been completed within this region capturing 269 elasmobranchs of nine species. The most commonly caught species were the bonnethead, *Sphyrna tiburo* (97), the blacktip shark (77), and the Atlantic sharpnose shark (74). In addition 288 teleosts of 23 species have been captured. Sharks captured alive were measured, tagged, and released.

It has been suggested that bull sharks, blacktip sharks, Atlantic sharpnose sharks, bonnetheads, smooth butterfly rays, cownose rays, southern stingrays, bluntnose stingrays, and Atlantic stingrays all commonly occur within this region. Little is known about the life history and ecology of many of these species, particularly the ray populations. In addition, neonate and juvenile bull sharks appear to be abundant in the Waccasassa River mouth and the southern portion of Waccasassa Bay indicating this area may function as a nursery ground for bull sharks and possibly other elasmobranch species. This study will allow us to determine if this area

functions as a nursery and Essential Fish Habitat for several elasmobranch species. The use of gear consistent with that used to study elasmobranch nursery areas in the western Gulf of Mexico and along the southern Gulf coast of Florida will allow us to compare the productivity of this area with nearby areas. To date this area of the Gulf of Mexico is largely unstudied and represents a significant gap in our knowledge of elasmobranch species within the Gulf of Mexico.

4e. Fatty Acid Analysis for Elasmobranch Diet Reconstruction.

Objectives. This study was designed to evaluate the usefulness of fatty acid profiles in elasmobranch diet reconstruction.

Methods. Atlantic stingrays, *Dasyatis sabina*, were collected from Lake Jessup and transported to Mote Marine Laboratory and held in two 284-liter holding tanks with five specimens per tank. Following a week-long acclimation and fast, stingrays were fed to satiation three days a week on a tank specific diet of either penaeid shrimp (*Penaeus aztecus*) or fish (giant danio, *Devario aequipinnatus*). These two diets were chosen due to their differences in fatty acid profile. After six weeks of feeding, stingrays were euthanized via anesthesia without revival and muscle and liver samples were dissected. Ray tissues and food samples of the two experimental diets were stored at -80°C until analysis. Fatty acid profiles were analyzed using a modified Folch method. Data were analyzed using classification and regression tree analysis and principle component analysis.

Outcomes and Significance. Stingray muscle had much lower lipid content than liver and the two tissues were significantly different in fatty acid composition. However, there was no significant difference in fatty acid composition between fish-fed and shrimp-fed stingray liver or muscle samples. Both fish-fed and shrimp-fed stingray tissues were significantly different than corresponding diet samples in fatty acid composition. Statistical analysis did not group stingray muscle tissues with the corresponding diets.

These data suggest that fatty acid analysis may not be suitable for elasmobranch diet reconstruction. The slow metabolic turnover rate of dietary lipids in elasmobranch physiology may preclude fatty acid analysis from being a viable technique for diet reconstruction in these fishes.

4f. Life History and Morphology of Commercially Exploited Skate and Ray Species.

4f1. Age, Growth, and Reproduction of the Rondel Skate, *Raja texana*.

Objectives. The objectives of this research component were to analyze the age, growth and maturity of the roundel skate (*Raja texana*). In order to gain insight into this species' life history and assess the population status and susceptibility to exploitation, biological data such as: growth rates, mortality and productivity were needed.

Methods. Roundel skates were collected in the Gulf of Mexico through a collaborative effort with the National Marine Fisheries Service (NMFS) and Raffield Fisheries Inc. Collections were made during trawl surveys and during commercial butterfish trawl catches through the months of January, February, March, April, June and October 2005. All skates were snap frozen on board and then transferred to the University of Florida where they were measured and sampled. Researchers collected 251 roundel skates over the sampling period. Of those, 231 specimens ranging in size from 277 to 630 mm total length (TL) had readable bands on their vertebrae. A

block of ten vertebrae were extracted from each skate to be cleaned, washed and stored in 70% ethanol and three of those vertebrae were randomly selected to be mounted and cut.

Outcomes and Significance. FPSR scientists determined that the oldest age estimates obtained for the roundel skate were 8 years for males and 9 for females, which corresponded to total lengths of 495 mm and 630 mm, respectively. Age bias plots and coefficient of variation suggests that our ageing method represents a non-biased and precise approach to age assessment. Marginal increment and edge analyses suggest that growth bands are formed annually with a distinct trend of increasing growth at the beginning of March. Back calculations suggested a birth size of 113–118 mm TL. Growth was assessed using three models: the von Bertalanffy (VBGM), the von Bertalanffy with size at birth (VB with L_0), and the Francis model. Male and female growth was significantly different. The VBGM and Francis model predicted the same growth for males ($k = 0.228$) and females ($k = 0.228$), however the size at birth was underestimated using these models due to the paucity of 0–1 year olds. Maturity occurred at about 444 mm TL and 5.0 years for males, while females matured at about 534 mm TL and 5.8 years.

This species is found in the near shore waters throughout the Gulf of Mexico and is one of many elasmobranchs taken in large numbers as bycatch/byproduct in trawl and shrimp fisheries in that area. The information collected in this study suggests that the roundel skate's life history characteristics make it extremely susceptible to overexploitation. Data from this study suggest that the implementation of a fisheries management plan to protect this species from further population decline would be a prudent measure.

4f2. Steroid Hormone Analyses and the Reproductive Cycle of Two Exploited Skate Species.

Objectives. The objective of this study was to test a new and potentially innovative means to assess reproductive maturity and seasonal cycles through the use of steroid hormone analyses. This information will not only provide a better understanding of the control and periodicity of reproductive events, but also help determine the usefulness of this technique for use in other species.

Methods. As part of an ongoing collaborative project, FPSR scientists and scientists from the University of New Hampshire have been examining the life history of two exploited skate species from the Gulf of Maine. Plasma samples were obtained from mature smooth skates (*Malacoraja senta*) and thorny skates (*Amblyraja radiata*) of both sexes captured during all months of the year, (May 2001 though May 2003). Blood was collected immediately after the capture event by a cardiac puncture, centrifuged and stored in a cooler on board. All skates were dissected and processed at the University of New Hampshire and the Estradiol (E_2), Testosterone (T), and Progesterone (P_4) concentrations were determined using radioimmunoassay (RIA). Steroid hormone concentrations were averaged, compared to morphological reproductive parameters, and plotted to examine any monthly trends.

Outcomes and Significance. Concentrations of T, E_2 and P_4 in female smooth and thorny skates remained relatively constant throughout the year and between months. Estradiol concentrations did not correlate to any gross reproductive morphology measurements. Male smooth and thorny skates showed high monthly concentrations of testosterone, but did not show differences in levels between any months. There was a weak correlation between testosterone levels, testes weight and percentage of stage VI spermatocysts in the thorny skate, but not in the smooth skate. The maintenance of these high hormone concentrations throughout the year suggests that both of these skate species are capable of continuous reproduction.

Recent declines in biomass levels of both the smooth skate and the thorny skate, and the paucity of specific biological life history information concerning them, have prompted the

National Marine Fisheries Service (NMFS) to prohibit commercial harvests of both species in the U.S. portion of the western north Atlantic. Thus, obtaining life history information for these skate species is not only timely, but it has become imperative. Based on morphological parameters, each species exhibited an annual reproductive pattern. Future studies will explore hormone concentrations during a single egg-laying cycle in order to decipher the exact role that each hormone actually plays during particular reproductive events. This new technique has proven to be an effective way to obtain information on the reproductive maturity and seasonal cycles of these skate species in a non-lethal manner and may be applicable to other elasmobranch species.

4f3. Development of a Novel Technique to Age the Smooth Skate (*Malacoraja senta*).

Objectives. The objective of this study was to develop an alternative, and novel, aging method for the smooth skate, *Malacoraja senta*. Obtaining accurate biological data, such as age at size data and growth rates is necessary to understand how populations may respond to exploitation.

Methods. FPSR scientists collaborated with scientists from the University of New England to collect smooth skates off the coast of New Hampshire and Massachusetts. Skates were maintained alive onboard and transferred to the University of New Hampshire where they were euthanized and morphological measurements were taken. Vertebrae from 306 skates ranging in size from 230 mm to 680 mm TL were used to compare gross and histological methods. A block of ten vertebrae were extracted from each skate to be cleaned, washed and then frozen. Three of these vertebrae were randomly selected to either be sent out for histology or mounted for gross sectioning.

Outcomes and Significance. Although the histological method is more time consuming and costly than gross sectioning, the greater accuracy of ages provided by this method justify the increased cost of the technique. Using the counts from the gross sectioning method led to undercounting and thus underestimation of the true age of the skate. Additionally, the precision of the counts using this method may be less than the precision of counts using the histological method due to the difficulty associated with identifying the band pairs. At this time the size and age at maturity for the smooth skate is unknown due to the fact that there is an unexplained lack of congruency between male and female increment growth.

There is no direct fishery for this species, although it is frequently taken as bycatch. This indirect fishing pressure makes smooth skates, as well as other elasmobranchs, highly susceptible to overexploitation. It was evident that the gross sectioning method did not elucidate the band pairs as clearly as the histological method which can be used in future studies to age vertebrae.

4f4. Sexual Dimorphism in Skate Tooth Morphology.

Objectives. The objective was to examine differences in the tooth morphology of male and female specimens of the roundel skate (*Raja texana*) and the spreadfin skate (*Dipturus olseni*).

Methods. Specimens were collected during the months of January through November 2005 from the Gulf of Mexico. Variations in tooth morphology were compared between male and female roundel skates, as well as among mature and immature specimens of this species at different sizes. To account for intra-jaw tooth variations, lower jaw teeth were extracted using a dental pick at both a medial and lateral location. Teeth from both locations were photographed under magnification, and morphology software was used to denote landmarks and curves within each tooth. Jaws were removed from 109 (69 female: 40 male) roundel skates. Spreadfin skate teeth are currently being photographed in order to run similar comparisons, but due to a limited number of samples, only the differences between female and male tooth morphology will be examined.

Outcomes and Significance. Significant sexual dimorphism was observed in roundel skate teeth. Both medial and lateral teeth in immature and mature male and female roundel skates were significantly different in shape. However, the differences in shape were much more prominent in mature animals than immature animals for both male and female specimens. The more pointed shapes of mature male medial and lateral teeth of the roundel skate were significantly different than the stouter tooth shapes of mature females of this species.

Previous studies regarding skate dental dimorphism suggest that the differences in tooth morphology may be due to diverse prey preferences and habitat. However, more recent hypotheses suggest that the development of sharper, pointed teeth in male skates and rays enhances their ability to grasp females during mating events. Understanding this phenomenon will aid in determining the reason for the morphological differences. Results from this study indicate a significant difference between the tooth shape of male and female roundel skates and reinforce the mating success hypothesis.

4f5. Age and Growth of the Smooth Butterfly Ray (*Gymnura micrura*).

Objectives. The objective of this study was to examine the age, growth, and reproductive biology of the smooth butterfly ray, a common but little studied species in Florida inshore waters.

Methods. During the summer of 2007 we initiated a study of the age, growth, and reproductive biology of *G. micrura*. Vertebral and reproductive samples will continue to be collected from smooth butterfly rays captured both within the Indian River Lagoon and the Cedar Key region of the Gulf of Mexico. These samples will be used to calculate growth curves, determine ages and sizes at maturity, and determine fecundity levels for *G. micrura* within these regions. The ability to collect samples from both the Atlantic and Gulf coasts will allow us to determine if there are differences in the age, or reproductive parameters of smooth butterfly rays found within each of these regions.

Outcomes and Significance. To date, 12 smooth butterfly rays have been sampled and catalogued. These rays were primarily captured in the Gulf of Mexico. During the upcoming year additional samples will be collected. The smooth butterfly ray is found in shallow coastal waters throughout the state of Florida. It appears to be a common resident in two of the University of Florida's targeted sampling localities on the Atlantic coast and in the Gulf of Mexico. Despite the relatively high abundance of this animal within shallow coastal waters of the southeastern United States, little is known about its life history, movements, and migration. This study will provide the first age, growth and reproductive data on this species.

4g. Age-Structured Simulation Model for the Dusky Shark (*Carcharhinus obscurus*).

Objectives. The objective of this study was to conduct a demographic analysis of the dusky shark population and fishery that occurs in the northwest Atlantic Ocean.

Methods. Scientists at FPSR coded one age-structured and one spatially structured model for use in the dusky shark stock assessment. The age-structured model runs in a Bayesian framework and allows for multiple gear types, commercial and survey catch rates. Results from this model will indicate changes in total and mature biomass between 1972 and 2006 and 1972 and 2025. The spatial model runs in Excel and allows the user to open and close specified areas to fishing and allows for movement between areas.

Outcomes and Significance. Selectivity curves and catch rates were calculated for four data sets: 1. Virginia Institute of Marine Science (VIMS) 2. Commercial Shark Fishery Observer Program (CSFOP) 3. Pelagic Longline Observer Program (PLOP) and 4. Large Pelagic Survey (LPS). A Generalized Linear Model (GLM), executed in SAS, was used to standardize these catch rates. Models were fitted using a SAS GENMOD procedure (SAS Institute Inc., 1999) and forward stepwise approach, testing each factor one at a time. Biological parameters were collected from current literature and the total number of dusky sharks caught by region and gear type was calculated from the following sources: National Marine Fisheries Service (NMFS) South East Fisheries Science Center (SEFSC) quota monitoring system (QMS), the Northeast and Southeast regional general canvass landings, Marine Recreational Fisheries Statistic Survey (MRFSS), NMFS Headboat Survey (HBOAT) and Texas Parks and Wildlife Recreational Fishing Survey (TXPWD). All of this information has been included in the age-structured model and preliminary results show the dusky shark biomass in 2006 to be around 24% of the biomass in 1972. Fifteen sensitivity analyses have been identified and will be implemented in the upcoming months. Movement rates and biological parameters were added to the spatially structured model and areas which are closed to fishing have been identified. This model is still in the early stages and will likely be completed during the upcoming year.

The current Federal Fishery Management Plan for Atlantic sharks manages 39 species, which are separated into three management categories: large coastal (ridgeback and non-ridgeback), small coastal and pelagic sharks. In 1999, 14 of these species were moved to the prohibited species list, including the dusky shark. Commercial and recreational fishermen are no longer allowed to catch or land this species. Despite this change in management, dusky shark populations continue to remain low due to intrinsic biological characteristics and high hook mortality. Data from this study will provide valuable input to future management and conservation measures for this population.

4h. Maintenance and Growth of the International Shark Attack File (ISAF) and Public Education about Shark Attack.

Objectives. The International Shark Attack File, internationally recognized as the definitive source of scientifically accurate information on shark attack, is a compilation of all known shark attacks. Requests for information and phone queries from the public, media and scientific communities were continuously answered throughout the five-year period. This aspect of the program serves to further the understanding of shark-related issues in the public and scientific community.

Methods. More than 4,000 individual investigations are currently housed in the ISAF, covering the period from the mid-1500's to present. Many of the data in the ISAF originate from the voluntary submissions of numerous cooperating scientists who serve worldwide as regional observers. Data submitted to the ISAF is screened, coded and computerized. Hard copy documentation, including original interviews and notes, press clippings, photographs, audio/video tapes, and medical/autopsy reports, is permanently archived. Biological researchers and research physicians study investigations housed in the ISAF.

Outcomes and Significance. In the last five years, a total of 850 new case files were entered into the ISAF database. In addition, 380 case files that already exist in the database were updated with new information. A project aimed at filling in missing parameter entries within the database for latitude and longitude, water temperature, salinity, and lunar phase and tidal cycle also occurred during this period in order to standardize and complete the database. Staff completed an ongoing project of identifying latitude and longitude points for attacks that occurred

in the USA. A University of Florida "University Scholars" student has been working on identifying the tidal range and lunar cycle for all attacks in the USA, and another student enrolled in the Geographic Information Systems (GIS) Applications Specialist program at Sir Sandford Fleming College in Lindsay, Ontario, is applying GIS technology to attack data. The database was also updated to allow more information concerning species identification, surfboard type/size/color, and other country case files to be included. Standardization of several database parameters including distance from deepwater, provoked vs. unprovoked spearfishing attacks, victim activity, and multiple victims was completed. In addition, progress was made on the creation of a new database system that uses a graphical user interface and runs through the Sequel server. This new database will allow us to maintain photos, questionnaires, newspaper articles and medical files electronically. This included hiring a computer technician to aid in the development and implementation of the new database. This project will be completed during the next year. Collaboration with colleagues working on the Australian Shark Attack File (ASAF) has resulted in more effective sharing of information between these two databases. Staffs from both Files are currently working on integrating the information from the ASAF into the ISAF, so that both files will operate in the same database. This will also be done sequentially with the Brazilian Shark Attack File, South African Shark Attack File and Californian Shark Attack File over the course of the next few years. Trends in conditions surrounding 4,290 shark attack cases have been analyzed and reported.

The goal of the database consolidation and analysis is an update of Baldrige's 1974 classic analysis of shark attack (*Shark attack: a program of data reduction and analysis*). The ISAF web pages, where a yearly summary of shark attacks and preliminary analyses of trends in attacks are posted, continue to be the most heavily visited of all pages on the FLMNH web. Since shark attack, shark fishery management, and shark conservation have increasingly become interrelated topics, responses to queries serve to further a broad understanding of shark-related issues in the public at large.

4i. Web- and Media-Based Public Education Focusing on Shark Biology, Ecology, Management, and Conservation.

Objectives. The objective of web- and media-based public education is to provide a scientifically accurate and readily accessible source of information and data on shark biology, ecology, management, and conservation to the general public and the media.

Methods. Throughout the five-year reporting period, all sections of the highly utilized shark section of the FLMNH's Ichthyology web site were continually monitored, revised, expanded, and updated.

Outcomes and Significance. The shark section of the FLMNH's Ichthyology web site [<http://www.flmnh.ufl.edu/fish/Sharks/sharks.htm>] provides a rich source of information on sharks and is the most highly utilized shark site on the World Wide Web. The home page and web site for the NSRC was designed and posted online at the beginning of the reporting period. Throughout the five year reporting period, NSRC member institution web pages have been continually updated with current research, education initiatives, personnel and in-depth featured projects. The IUCN Shark Specialist Group (SSG) has also been continually updated and expanded. These updates include news stories for each IUCN SSG region as well as features on the activities of this group.

Information and resources related to elasmobranchs on the web site have been expanded and greatly improved since the inception of the consortium. New elasmobranch species accounts have been added to the "Biological Profiles" section. To date, we have a total of 210 fish biological

profiles with 118 elasmobranch profiles now available online. Also, the image gallery has been expanded, now totaling over 5,000 images, with new submissions from existing photographers as well as submissions from new photographers.

Updates have also been continuously made to the International Shark Attack File (ISAF) web pages including new statistics and data collected and compiled each year during the reporting period. These statistics include annual updates on the number and location of attacks throughout the world as well as data on other aspects of shark attacks. The media relies upon the ISAF web site to provide accurate information and data on shark attacks in their reporting of this phenomenon. New and updated web pages have also been developed in the “Relative Risk” section of the ISAF web site. These pages compare shark attacks to common risks such as lightning strikes, tornadoes, drowning, bicycle accidents, boating accidents, hunting accidents, alligator attacks, collapsing sand holes, and animal bites. These pages provide valuable information regarding shark attacks and the relative risk of shark attacks compared to other activities to the media as well as the general public.

A “Sawfish Recovery Team” web page was also developed and posted online. Information is available on the listing history of the smalltooth sawfish (*Pristis pectinata*), key listing documents, sawfish in the news, sawfish references, sawfish recovery team members, sawfish brochure and marine sign, and sawfish in peril PowerPoint presentation as well as links to sawfish species profiles.

The education web page has been expanded to include “Most Commonly Asked Shark Questions”, “Most Commonly Asked Sawfish Questions” and “Most Commonly Asked Ray and Skate Questions”. Interactive shark-related puzzles and games for young web visitors were posted. An interactive shark identification key, “Field Key To Sharks Encountered in the US Atlantic Bottom Longline Shark Fishery And By Recreational Fishers”, was also developed and posted online. Another new interactive web site feature is the “Chondrichthyes Bibliography” search engine, offering access to a library of chondrichthyes resources. Other selected additions on the education web page include topics such as “Careers In Ichthyology”, “...About The Anatomy Of Rays And Skates”, “Skates Of The Western Gulf Of Maine”, “Stingray Reproduction References”, “Sawfish In The News”, “Sawfish References”, “Sawfish Links”, “Shark Games!” and “Megalodon Exhibit Opening”.

Ongoing maintenance activities continue to include every section of the shark web site including organizational information, news stories, image gallery, education, conferences and meetings, staff, outside links, and references. A “Shark Site Contents” page has been added to the web site, providing a site map of all shark-related web pages hosted by the Florida Museum of Natural History. This page provides easier navigation through the large amount of shark materials on our web site for visitors.

The FLMNH Ichthyology Division web pages, including the FPSR web pages, received 26,693,327 visits and 13,300,913 unique visitors (as compiled each month) during the five-year reporting period. These visit and unique visitor data provide insight into the dramatic increase in the use and importance of our web site to the general public, educators, and the media as seen below:

<u>Year</u>	<u>Visits</u>	<u>Unique Visitors</u>
2002 (Oct-Dec)	389,889	156,539
2003	3,281,513	1,614,450
2004	5,158,246	2,340,591
2005	6,369,911	3,244,662
2006	8,144,707	4,119,276
2007 (Jan-June)	3,349,061	1,825,395

Viewers drawn to the site by fascination with shark attacks find complementary explanatory material dealing with shark biology and ecology, conservation, and fishery management. The

result is that interested viewers come away with a more balanced view of elasmobranchs and measures needed to ensure their management and survival. We have found that providing a combination of web-based educational materials plus direct interplay with the media results in a very positive approach to educating the public about elasmobranch issues. It is also readily apparent by perusing the media, that our web site is frequently referenced as a major source of data pertaining to shark attacks throughout the world as well as other shark-related information including basic biological information, conservation, and ongoing research activities and results.

4j. Classroom-Based Elasmobranch Education Initiatives.

4j1. Project Shark Awareness.

Objectives. The objective of classroom-based elasmobranch education initiatives is to educate communities in the state of Florida as well as other southeastern states about the myths and realities of elasmobranch behavior, biology, conservation, and research.

Methods. An innovative classroom-based education initiative, *Project Shark Awareness*, was developed during the reporting period with supplemental funding provided by other sources. This ongoing program seeks to educate communities in the state of Florida as well as other southeastern states about the myths and realities of shark behavior, biology, and conservation. This initiative consists of a two-day workshop including multimedia presentations, lectures, and a shark dissection and is augmented by teaching support materials. A teaching kit consisting of a PowerPoint presentation, teaching guide, handouts, laboratory and classroom activities, and evaluation materials is available to each participant. In addition, shark jaws, shark skin, shark embryos, shark vertebrae, stingray spines, and skate jaws are among the items in the hands-on component of the teaching kit, providing exciting hands-on learning opportunities for students. A password-protected companion web page within the FPSR education web site is available for workshop participants. This web page includes all teaching materials as well as any new or updated files that can easily be downloaded for use in teaching activities.

Outcomes and Significance. Over the reporting period, 120 educators attended seven *Project Shark Awareness* educator workshops held at the Florida Museum of Natural History. *Project Shark Awareness* curriculum has the greatest impact when presented in school systems by trained and qualified teachers, thereby providing a multiplier effect that will continue to grow year after year and reach thousands of students. The teaching materials continue to be improved and expanded with feedback from workshop participants. These workshops have been overwhelmingly popular with educators with high levels of interest for our program to continue.

Project Shark Awareness seeks to provide youth with increased educational opportunities through curriculum enrichment. The program seeks to help address society's greatest educational challenges and to provide quality learning experiences while at the same time educating youth about the myths and realities of elasmobranch biology, behavior, conservation, and research. *Project Shark Awareness* also enhances the value and effectiveness of other educational opportunities that teachers may take advantage of to educate their students concerning sharks. Living sharks and rays are displayed at Epcot's Living Seas, Sea World of Florida, the Florida Aquarium, and to a lesser extent at other public venues throughout the state of Florida. *Project Shark Awareness* is also used by teachers in conjunction with classroom excursions to public aquaria to further enhance learning and add excitement to the study of sharks and rays.

4j2. Sawfish In Peril.

Objectives. The objective of classroom-based elasmobranch education initiatives is to educate communities in the state of Florida as well as other southeastern states about the myths and realities of elasmobranch behavior, biology, conservation, and research.

Methods. *Sawfish In Peril*, the most recent addition to our education initiatives, is currently being designed and will be implemented in a manner similar to *Project Shark Awareness*. Very little is known about sawfishes, which historically were quite abundant in the tropical regions of the world. Today, the U.S. distribution of the smalltooth sawfish (*Pristis pectinata*) is restricted primarily to the waters off the coast of southwest Florida. Although it is difficult to estimate the exact numbers of sawfish remaining in U.S. waters, it is likely that the population has declined at least 95% over the past century. As a result, the smalltooth sawfish has recently been listed for protection under the U.S. Endangered Species Act. The largetooth sawfish (*P. perotteti*), once found in low numbers ranging from Texas to Florida, has probably been extirpated from U.S. waters. *Sawfish In Peril* educator workshops will result in a better informed public concerning the plight and protection of sawfishes native to U.S. coastal waters, as well as the fate of sawfishes in general, which as a group are globally threatened. *Sawfish In Peril* workshops will include multimedia presentations and lectures as well as a companion teaching kit and web page. The availability of this one-day workshop is dependent upon funding from external sources which are still being actively pursued.

Outcomes and Significance. The *Sawfish In Peril* education initiative has resulted in the production of a total of 150 smalltooth sawfish signs and 30,000 smalltooth sawfish brochures which are currently being utilized for education and outreach activities throughout the state of Florida as well as other southeastern states. Brochures have been distributed throughout the state of Florida with the assistance of Florida Sea Grant extension and the Florida Fish and Wildlife Conservation Commission (FFWCC) as well as numerous formal and informal education facilities. The FFWCC, in coordination with county personnel, has also been instrumental in marina/boat launch sign posting in coastal areas throughout the state where smalltooth sawfish are known to occur. These materials are available on the sawfish section of the FPSR web site and will be utilized in future *Sawfish In Peril* workshops, as they are currently included in *Project Shark Awareness* workshops.

Sawfish In Peril strives to assist the Florida Fish and Wildlife Conservation Commission to achieve its goals of restoring the richness and biodiversity of Florida's native nongame wildlife. This program will continue to increase the public's knowledge and awareness of sawfishes residing in our state's waterways. Floridians will develop a better appreciation of nongame wildlife and approaches for management and conservation of endangered species. We believe that *Sawfish In Peril* curriculum will have the greatest impact if presented in school systems by trained and qualified teachers, thereby providing a multiplier effect that will continue to grow year after year. As these young students become adults and become involved in decision- and policy-making, their knowledge and attitudes concerning sawfishes and other endangered wildlife will lead to an increased awareness and understanding of issues relating to conservation, fisheries, and human interactions. Success will be seen in the short term as people begin to learn about sawfishes and conservation measures to protect these unique fish. This is particularly important due to the endangered status of the smalltooth sawfish which reside locally off the coast of Florida. Hopefully, future generations of Floridians will view sawfishes less as modern-day sea villains to be feared and more as spectacular products of evolution to be revered, respected, and cherished as part of the ocean's biodiversity.

4k. Responses to Requests for Data, Analysis, and Technical Information.

Objectives. It is part of the mission of the FPSR to actively participate in disseminating information to the public via responses to requests for data, analysis and technical information about all aspects of shark biology as well as to disseminate information about shark attacks.

Methods. FPSR staff scientists respond to inquires from the public via phone and e-mail as well as responding to technical data needs whenever requested.

Outcomes and Significance. During this period, FPSR personnel continued to work with the Florida Fish and Wildlife Conservation Commission, NMFS/HMS, NSRC collaborators, and other scientists and NGO's to provide data, analysis, and other technical information as needed to advance the basic understanding, fishery management, and conservation of sharks. FPSR scientists responded to thousands of requests from the general public and media for information during this reporting period.

The dissemination of scientifically accurate information to the public as well as federal and state management agencies is critical for the conservation and management of elasmobranch species.

4l. Publications, Materials, and Conferences.

During the past five years over 40 peer-reviewed publications and 15 reports have been published by FPSR scientists and collaborators (see Appendix). FPSR scientists have presented data on over 75 occasions at multiple national and international meetings and hosted or participated in over 40 workshops.

APPENDIX

PUBLICATIONS OF THE NATIONAL SHARK RESEARCH CONSORTIUM, 2002-2007

(Institutional staff members' names in **bold**)

I. MOTE MARINE LABORATORY – CENTER FOR SHARK RESEARCH

Publications

2002

Castro, J.I. 2002. On the origins of the Spanish word 'tiburón' and the English word 'shark'. *Environmental Biology of Fishes* 65: 249-253.

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2003

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