Highly Migratory Shark Fisheries Research by the National Shark Research Consortium (NSRC), 2003-2004

Semi-Annual Performance Report
January 1, 2004 to June 30, 2004

Award Number: NA16FL2813

Submitted by: Robert Hueter
Principal Investigator

Submitted to: NOAA/NMFS
St. Petersburg FL

Submitted on
July 2004

Mote Marine Laboratory Technical Report No. 1002
SEMI-ANNUAL PERFORMANCE REPORT

Award Number: NA16FL2813

Project Title: Highly Migratory Shark Fisheries Research by the National Shark Research Consortium (NSRC), 2003-2004

Recipient: Mote Marine Laboratory, Sarasota, Florida

Award Period: July 1, 2003 to June 30, 2004

Period Covered by this Report: January 1, 2004 to June 30, 2004

Primary Project Tasks: The following primary tasks were scheduled for this six-month period:

Mote Marine Laboratory Component
  1a. Life history, migration, stock structure
  1b. Relative abundance
  1d. Bull shark freshwater use
  1e. Population genetics
  1f. EFH
  1g. Elemental analysis
  1h. Ecosystem modeling
  1i. Satellite tag workshop
  1j. Publications, conferences

Moss Landing Marine Laboratories Component
  2a. Life history data matrix
  2b. Age, growth, demography
  2c. Age validation
  2d. Reproduction
  2e. Feeding ecology
  2f. Stable isotope analysis
  2g. Habitat, nursery grounds
  2h. Population genetics
  2i. Surveys
  2j. Fisheries analysis
  2k. Taxonomy
Virginia Institute of Marine Science Component

3a. Relative abundance
3b. Age, growth, demography
3c. Habitat, migration
3d. Energetics, osmoregulation
3e. Genetics
3f. Publications, conferences

University of Florida Component

4a. FL survey
4b. Tagging, tracking
4c. Fatty acid analysis
4d. Bomb radiocarbon ageing
4e. Age-structured model
4f. Gestation
4g. ISAF
4h. Website, media
4i. Requests for data
4j. Publications, conferences

Summary of Results:

General Project Overview

Significant progress was made during this six-month period of the project. Important advances by the NSRC were made in both field and laboratory approaches to understanding the life history, abundance and environmental aspects of shark species involved in commercial and recreational fisheries. NSRC researchers and students continued to make important scientific contributions through publications and conference presentations during the six-month period.

Mote Marine Laboratory Report (R.E. Hueter, P/I)

MML’s Center for Shark Research (CSR) served as project coordinator. No major problems were encountered in the administration of the project. The following technical accomplishments were achieved during the reporting period:

Life history, migration, stock structure. Two surveys for large coastal sharks were undertaken during the period, one in spring (March) and one in summer (June) aboard the R/V Eugenie Clark. Each survey consisted of five days longline and drumline fishing between the north end of Anna Maria Island and the south end of Longboat Key on Florida’s Gulf Coast. The spring survey caught
51 sharks, comprising *Carcharhinus limbatus* (28), *C. brevipinna* (7), *C. plumbeus* (7), *C. leucas* (4), *G. cirratum* (2), *C. acronotus* (1), *Negaprion brevirostris* (1) and *Sphyrna mokorran* (1). Two satellite PAT tags were released on mature female *C. limbatus*. Both remained on the sharks for approximately 25 days and were located near Anclote Key when they detached. A full complement of depth and temperature data were returned via satellite for both tags. The summer survey caught 50 sharks, including *Carcharhinus limbatus* (7), *C. leucas* (7), *G. cirratum* (27), *C. acronotus* (6), *Negaprion brevirostris* (2) and *Sphyrna mokorran* (1). No PAT tags were deployed during this survey.

In the overall CSR shark-tagging program, 30 tag recaptures were reported during the period for six species comprising *C. limbatus* (13), *C. leucas* (7), *Negaprion brevirostris* (4), *S. tiburo* (3), *R. terraenovae* (2), and *C. brevipinna* (1). The longest time at liberty was from a bull shark tagged in the Florida Everglades (near Highland Beach) and recaptured after 1,099 days at large (3.5 nm from the tagging site). The longest distance traveled was from a young-of-the-year blacktip shark tagged off Yankeetown, Florida, that was recaptured in the Florida Keys (near Islamorada) after 171 days at large. This recapture represents a minimum distance traveled of 283 nautical miles.

Three trips (46 days total) were made to the remote Dry Tortugas shark study site by project staff during the reporting period. Field investigations into the natural reproductive cycle of the nurse shark, *Ginglymostoma cirratum*, continued in January and May with VR2 hydrophone monitor downloading and replacement of six-month batteries. In June, two researchers aboard the *S/V Eos* and a team of eight research staff aboard the charter vessel *Tiburon* documented 49 courtship events in nurse sharks. Twelve adult sharks were captured and tagged or retagged. Ten ultrasonic coded Vemco transmitters were attached and 29 Vemco VR2 tag monitors were downloaded, rebatteried and replaced. The team comprised staff from Mote Marine Laboratory (2), Albion College (3), National Geographic Remote Imaging (2), and Southern Illinois University (1). Results of our 187 hours of observation in the first six months of 2004 also included 5 CritterCam attachments to investigate the hypothesis of deepwater mating and courtship behavior. We captured 12 adults by dip net this year, six sharks new to us and six sharks previously tagged. Our dominant male, Notch (first seen in 1992), Thumb (1995) and females 9909 (1995) and 9908 (1997) all showed up around 30 June. With these data we have finally linked the occurrence of nurse shark reproductive events with the advent of both the full and new moons in June. The capture of female 9908 was the fifth encounter with this large female. This shark has shown the farthest excursions from the tagging site, having been recaptured, released and reported by a commercial fisherman 70 nm north of Tortugas in 2002. She returned to mate right on schedule in June 2004, demonstrating alternate year reproductive site fidelity after traveling for a long time and, for a nurse shark, a great distance.

Diver identifiable fin and body tags continued to be used to determine the identity, frequency and time period in which individual nurse sharks participate in mating. Since 1993, we have tagged or identified 224 nurse sharks, (92 adults and 132 juveniles) in the Dry Tortugas study population. Starting in 2000, we have been able to capture 51 large (219 – 275 cm TL) reproductively active sharks from our study population using large dip nets. Of the identified adults, 38 were subsequently sighted (visually recaptured or netted), at least once, and some more frequently. One individual has been seen 65 times over the course of 12 years and many juveniles have been physically recaptured multiple times. Two have been recaptured seven times. Observations from tagging and natural markings indicate that most adult males visit the study site faithfully every year, with two dominant
males and one male consistently observed since 1992. Results from our tagging studies continue to support our hypothesis that adult females visit the study area to mate in alternate years. Females that have actively mated in one year have never returned the following year, but some sharks regularly return after an absence of two or more years.

**Relative abundance.** A total of 40 inshore gillnet sets (20 in Pine Island Sound, Florida, and 20 in Yankeetown, Florida) were made during this reporting period as part of an ongoing study estimating shark relative abundance. These efforts resulted in the capture of 422 sharks comprising *Carcharhinus limbatis* (172), *Sphyrna tiburo* (168), *Rhizoprionodon terraenovae* (81), and *Sphyrna mokarran* (1). Of these captured sharks, 332 were tagged and released using CSR plastic dart tags. These tagged sharks comprised *C. limbatis* (141), *S. tiburo* (128), and *R. terraenovae* (63). Additionally, 125 bycatch specimens were captured which included 12 species of teleosts and 1 elasmobranch species. Offshore, relative abundance data were collected via the two week-long surveys conducted aboard the *R/V Clark* as described above.

**Bull shark freshwater use.** To examine the use of freshwater habitats by young bull sharks (*Carcharhinus leucas*), a series of 20 acoustic receivers (Vemco VR2) were deployed within the Caloosahatchee River, southwest Florida in 2003. These receivers recorded the presence of individuals fitted with transmitters when they swam within listening range of the receiver (c. 650 m). Six of the 18 bull sharks collected within the river in 2003 were observed to remain within the river for the entire winter season and are still using the river as of June 30, 2004. In addition to the one-year-old sharks being monitored in the river, another 12 neonate bull sharks will be fitted with transmitters in the river in July 2004. A further 8 transmitters will be deployed in the river during the month of August.

Examination of movement patterns and habitat use by bull sharks within the Caloosahatchee River has revealed correlations between salinity level and river usage. As reported previously, salinity levels within the river were very low (0.2 ppt) for up to four weeks at the beginning of this study (August 2003). When salinity levels increased in December 2003 (16.8 ppt) the sharks expanded their use of the river habitat. Data collected by the South Florida Water Management District was recently analyzed to display the continuous salinity patterns within the Caloosahatchee River from August 2003 to March 2004. These data show regular releases of freshwater from Lake Okeechobee affecting the salinity levels within the river. Examination of how far sharks were recorded up the river (based on river kilometers) revealed that sharks responded to declines in salinity by moving toward the mouth of the river. As salinity levels increased, sharks tended to move farther up river. Correlation values for six of the long-term (c. 8 months) residents within the river habitat revealed a relationship between salinity level and distance up the river ($r = 0.26 - 0.59$). From January to March overall salinity levels were higher within the river than earlier in the study, and during this latter period sharks were seen to regularly penetrate the entire monitored region (c. 28 kilometers from the river mouth). These preliminary results demonstrate our ability to monitor salinity changes and animal movements in response to those changes within the study site.

**Population genetics.** Fin clips were collected from 64 blacktip sharks within Florida waters. These samples came from various areas comprising: Yankeetown, 36; Pine Island Sound, 21; Ten
Thousand Islands, 4; and Everglades National Park, 3. An additional 41 fin clip samples were collected from juvenile and adult bull sharks along the Florida coast. These samples include collections from: Caloosahatchee River, 23; Indian River Lagoon (thanks to NSRC partner University of Florida), 10; and the Everglades, 8. All samples will be transferred for analysis to collaborating geneticists at Southern Illinois University at the end of the summer field season.

**Essential Fish Habitat.** Initial CSR studies on pollutant exposure in sandbar and blacktip sharks from U.S. east coast nurseries indicated that certain populations of these species (especially on the Atlantic coast) are exposed to potentially health-threatening levels of organochlorine pesticides and polychlorinated biphenyls. Because of this, our follow-up studies in 2003-2004 focused on determining whether physiological responses to such levels of pollutant exposure occur in these animals. As one part of this effort, we attempted to develop probes for measuring shark vitellogenin, a female-specific yolk protein precursor occurring in all nonmammalian vertebrates, which is often used as an indicator of pesticide exposure in male fish. We isolated proteins from blacktip shark yolk and developed polyclonal antisera against these proteins in rabbit. Unfortunately, despite considerable effort, we have had little success in using these antisera to detect vitellogenin experimentally induced in male bonnethead sharks in previous studies because antisera react non-specifically with several serum proteins, making them unreliable for accurately detecting vitellogenin in test animals. More recently, we have also screened antisera against swordfish yolk proteins to determine its potential usefulness for measuring elasmobranch vitellogenin. Interestingly, although this antiserum is not suitable for detecting yolk proteins from sandbar, blacktip, or bonnethead sharks, it is quite capable of specifically identifying vitellogenin in both yolk and serum from Atlantic stingrays. In addition, moderate reactions between the anti-swordfish probe and sandbar/blacktip/bonnethead yolk protein isolates has identified a candidate protein for isolation and antiserum production that may yet allow us to accomplish this objective.

Due to the difficulties encountered in developing a protein assay for detecting vitellogenin in shark serum, we recently began to develop methods for measuring vitellogenin gene expression in the liver of these animals. Using degenerate oligonucleotide primers to vitellogenin, which were designed based on conserved amino acid sequence data from other fishes, we obtained putative fragments of the vitellogenin gene from bonnethead shark liver via reverse transcriptase and the polymerase chain reaction. These fragments will be sequenced to confirm identity following their ligation into a cloning vector and transformation in bacterial cells. If these fragments are confirmed to be vitellogenin, we will be able to use them to develop molecular probes for detecting the presence of vitellogenin mRNA in test animals. We will also be able to use sequence information on these fragments to predict amino acid sequence of the shark vitellogenin protein, thereby streamlining our efforts to develop a vitellogenin assay for shark serum.

Since our upcoming research will also focus on exposure levels of metal toxicants, we have begun to develop methods for detecting physiological alterations associated with these contaminants. In Spring of 2004, we began efforts to develop an assay for shark metallothionein, a protein that is induced by exposure to mercury, cadmium, copper, silver, and zinc in most fishes. Commercially available antisera against fish metallothionein were screened for their effectiveness to detect this protein in bonnethead sharks liver, gill, and testis using both immunoblotting and immunocytochemistry. The results of these assays indicated that one of the antisera would be useful
for detecting shark metallothionein via either immunoblot or enzyme-linked immunoassay procedures, but not immunocytochemistry. We intend to validate these results later in the year by examining metallothionein induction in primary cultures of shark hepatocytes exposed to various metal contaminants.

**Elemental analysis.** The analysis of elements in fish otoliths has been used to investigate the use of freshwater and estuarine habitats by marine organisms. In particular the ratio between strontium (Sr) and calcium (Ca) is a good indicator of changes in habitat use. High levels of Strontium indicate periods in fresh or brackish water. While this methodology has been used in teleosts, it has never been used in elasmobranchs. During the study period vertebrae from a number of bull sharks of various sizes were collected for trial using this method. Bull sharks were chosen because their nursery areas are often in freshwater. These vertebrae are currently being prepared for analysis in the second half of 2004.

**Ecosystem modeling.** Development of the ecological model of Charlotte Harbor, Florida using Ecopath with Ecosim continued during the period. This model includes 35 ecosystem components from primary producers to top-level predators, including sharks. During the study period development of the Ecospace model continued that allows for the examination of the influence of movement patterns and localized hydrographic phenomena on the functioning of the ecosystem. It also allows the exploration of policy development in terms of the impact of protected areas. The spatial model operates on a grid size of 1 km² and includes seven different habitat types based on depth, submerged aquatic vegetation and marine/freshwater influence. The second area of development has been the commencement of hydroacoustic surveys to validate the biomass estimates used in the initialization of the model. These surveys have been 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 indicates that during periods of hypoxia in the harbor the prey fish form smaller schools and inhabit shallower habitats.

**Satellite tag workshop.** As reported in our last semi-annual report, a workshop to address multiple issues concerning the use of satellite tag technology and its application to shark research was convened at Mote Marine Laboratory, Sarasota, Florida on 3-5 December 2003. A total of 22 participants attended from four countries and 18 institutions, including representatives from all the NSRC institutions. All invitees were requested to complete a comprehensive survey regarding their experience with satellite tag use and their recommendations for its application in shark research prior to the workshop. A post-workshop survey was also administered to gauge the success and usefulness of the meeting. The questions posed to the invitees and their responses were compiled in a Mote Marine Laboratory Technical Report (Sharks and Satellite Tagging: Achieving the Potential, MML Technical Report No. 962) authored by Drs. Heupel and Hueter. This report was distributed to all participants and to other interested parties. The report text is also available via the CSR web page (http://www.mote.org/sharks/tagging_workshop.pdf). This workshop is likely to be the first of many that will discuss this topic.

**Publications, conferences.** The Mote CSR hosted the IUCN/SSC Shark Specialist Group North and
Central America Region Red List Workshop in June. Over 50 delegates from 13 nations participated in the week-long workshop to determine the population status of sharks and rays inhabiting North and Central American waters. CSR scientists also participated in other scientific meetings including: the annual meeting of the American Elasmobranch Society / American Society of Ichthyologists and Herpetologists in Norman, Oklahoma; the annual meeting of the American Association for the Advancement of Science in Seattle; and the ASLO/TOS Ocean Research Conference in Honolulu. Eight presentations were made by CSR scientists at these and other meetings, and CSR staff produced 13 peer-reviewed publications in review, in press or published during the first half of 2004. Dr. Hueter also participated in one NMFS advisory panel meeting (Highly Migratory Species Advisory Panel, Silver Spring, Maryland, February).

Moss Landing Marine Laboratories Report (G. Cailliet, P/I)

**Life history database.** The life history matrix (LHM) was completed and is now available on the PSRC web site for the general public (http://psrc.mlml.calstate.edu). The LHM 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 eastern North Pacific Ocean (ENP) chondrichthians (including the sharks, rays and chimaeras). The ENP as defined here includes the area ranging from the eastern Bering Sea to the southern tip of the Pacific Baja California peninsula. The LHM includes 105 species reported to occur in the ENP. The missing information on life histories of individual species is being compiled into a gap analysis that will guide us toward future research projects to fill those gaps. A demonstration of the LHM was presented at the Western Groundfish Conference in February 2004 and at the American Elasmobranch Society meeting in May 2004. The LHM will be updated periodically as new information becomes available.

**Age, growth, and demographic studies.** Age determination and validation studies are underway to fill some of the gaps in the life history of poorly-studied species. Species currently under investigation include the sandpaper (*Bathyraja kincaidii*), roughtail (*Bathyraja trachura*), and California (*Raja inornata*) skates. To date, 188 sandpaper, 265 roughtail, and 289 California skates have been cleaned and thin-sectioned for age estimation. Age estimates have been calculated for the sandpaper skate with 50% of females maturing at 6.8 years and 50% maturity for males estimated at 7.3 years. The use of caudal thorns as an ageing structure is a new technique that, if successful, may provide a non-lethal ageing method for this elasmobranch group.

Demographic analyses of the diamond stingray (*Dasyatis dipterura*), based on vital rates recently calculated by PSRC personnel, are now completed and is being written up as Masters thesis. Reproductive rates, generation time, intrinsic rate of increase, and stable age distribution were estimated using modified life history tables, Leslie matrices and Monte Carlo simulation to incorporate uncertainty and variability in life history parameters. Elasticity analysis was completed to examine the effects of changes in survival, growth, and reproduction on specific life stages of the population. Annual population growth rates range from 1.05-1.01 and were indicative of relatively slow growth and low productivity. Net reproductive rates ranged from 8.5-1.2 per generation, which
varied between 14.4 and 16.7 years. Elasticity analysis followed the general pattern indicated by long-lived vertebrates: variation in juvenile survival had the greatest relative impact on population growth. Results of this study provide critical baseline formation necessary for the effective management of this species in the Mexican Pacific.

**Age validation studies.** Validation of the periodicity of growth band deposition in shark vertebrae and age estimates based on vertebral band counts are being investigated using bomb radiocarbon. To date, 14 samples from three shortfin mako sharks (*Isurus oxyrinchus*) have been tested. Preliminary results suggest that the vertebral bands are formed annually. In addition, we are analyzing white shark (*Carcharodon carcharias*) vertebrae for bomb radiocarbon, to validate the age estimation methodology, and stable isotopes (C-13, N-15), to assess trophic level and aid in interpretation of radiocarbon data. We are also conducting radiocarbon age validation studies on northwest Atlantic species, including the sandbar (*Carcharhinus plumbeus*) and tiger (*Galeocerdo cuvier*) sharks, using vertebrae from several institutions including the Florida Program for Shark Research at the University of Florida. With the help of the NMFS lab in Santa Cruz, we have purchased a micromilling machine and are routinely using it in the coring of vertebrae for age validation and stable isotope studies.

**Reproduction.** Investigations of the reproductive biology based on 304 California, 1,179 longnose (*Raja rhina*), 265 roughtail, and 296 sandpaper skates are in progress. The median size at 50% maturity for female California, longnose, and sandpaper skates were estimated at 56.5, 80.2, and 49.7 cm total length (TL), respectively. Males were calculated to reach 50% maturity at 50.2 cm TL for California, 69.6 cm TL for longnose, and 52.2 cm TL for sandpaper skates. Gravid females were only observed during December for the California and longnose skates, but for the sandpaper skates gravid females were found in June, October, and December. Analysis of roughtail skate reproductive data is currently underway. This investigation provides the first details on the reproductive biology of sandpaper, roughtail, and California skates. A total of 527 brown (*A. brunneus*), 98 longnose (*A. kampae*), and 348 filetail (*Parmaturus xaniurus*) catsharks has been examined. Depth distribution and variability between sexes and among size classes is being compared for these three species of catsharks. The size at 50% maturity for males and females of all three species is currently being analyzed and calculated.

**Feeding ecology.** Dietary analysis on the feeding ecology of 527 brown, 98 longnose, and 348 filetail catsharks, and 330 California, 1,131 longnose, and 296 sandpaper skates is currently ongoing. Preliminary dietary analysis of 287 California skates (*R. inornata*) has been completed. This species feeds mainly on benthic shrimp, crabs, and demersal teleosts.

**Stable isotope analyses.** The feasibility of stable isotope analysis is being tested to track the ontogenetic movement patterns of the blue shark (*Prionace glauca*). Analysis has been performed at the UC Davis Stable Isotope Facility. Vertebrae from eight blue sharks and muscle tissue from 20 specimens from the ENP were obtained from three locations (Southern Baja California, San Diego, and British Columbia) and were examined. Analysis of nitrogen isotopes within vertebrae for all animals in all locations showed that there was little change in nitrogen isotope level (14.87 %o ±
0.82), indicating that there may be little change in ontogenetic diet changes. Conversely, there were large variations found in carbon isotope values within vertebrae for all blue sharks that may indicate movement throughout life. Changes of 2 to 3 ‰ in carbon were evident in all of the individuals sampled and showed a cyclic nature that likely reflects patterns of onshore and offshore movement. Future work will include incorporation of the estimated ages of individuals to examine patterns of carbon isotope change within vertebrae as a function of time, and incorporation of muscle tissue samples to elucidate local productivity patterns and hence isotopic composition patterns spatially. Because there are presently no publications on stable isotope composition in shark vertebral centra, our work is unique. To our knowledge the only study of this nature was done by Sterling Peverell in Australia on the Sr/Ca ratios in the sawfish, Pristis microdon, suggesting annual growth zones and ontogenetic habitat changes.

**Habitat and nursery ground studies.** Preliminary habitat assessments of skates collected from fishery-independent surveys conducted by the NMFS Santa Cruz Laboratory (SCL) were completed during this period. Trawl and longline set locations were associated with a geological classification scheme available for the Monterey Bay National Marine Sanctuary. A geo-referenced map was developed that detailed elasmobranch abundance in relation to depth and bottom type. Longnose skate were most abundant in trawls at depths of 120-532 m and consistently associated with fine grain substrate. Starry skate (R. stellulata) and brown catsharks were primarily captured by long line over rocky substrate or rock-sand interfaces. Video transect footage taken in Fall 2002 during manned submersible dives in Ascension and Año Nuevo canyons (Monterey Bay, CA) were viewed and evaluated for the presence of elasmobranchs. Longnose skate and California skate were observed in association with muddy substrate at depths of 200-350 m. An aggregation of several hundred brown catsharks was observed in Ascension Canyon at 350 m in association with rock gullies and promontories. Bundles of brown catshark egg cases were observed in the same location. Solitary brown catsharks were also observed between 250-350 m on mixed rock and mud habitats or near rock walls. The habitat associations, distribution, and abundance of these and other elasmobranch species will continue to be evaluated from additional archived video available through the Monterey Bay Aquarium Research Institute (MBARI).

A tracking study on the movement patterns of leopard sharks (Triakis semifasciata) in Elkhorn Slough was initiated during the spring 2003. The techniques include acoustic telemetry with a mixture of both active and passive tracking. To date, 15 female leopard sharks have been tagged with acoustic tags and nine of those tags are coded to work with the array of VR-1 receivers that have been placed around the slough. Three additional individuals have been tagged with passive tags. Preliminary analyses indicate that the movement patterns of these sharks appear to be highly influenced by the tides and suggest that they may utilize some areas more intensively than others. Initial results from both the active and passive portions of the study indicate that leopard sharks appear to spend most of their time in the Elkhorn Slough National Estuarine Research Reserve (ESNERR) marsh restoration site. This study will be continuing through 2004 to observe seasonal changes.

**Population genetic studies.** Genetic research has been initiated on all three thresher shark species; Alopias pelagicus, A. superciliosus, and A. vulpinus. A total of 204 samples has been obtained from
shark populations in the North and South Pacific, North Atlantic, and southwestern Indian Oceans. Total genomic DNA has been extracted from all tissue samples collected to date. A 1400 base-pair region of the mitochondrial control region is being amplified through PCR using primers developed for sharks by Dr. Ed Heist (Southern Illinois University). A 1200 base-pair region of the mitochondrial cytochrome \( b \) gene is also being amplified through PCR. So far, sequence data and chromatograms have been received for 114 of those samples. Through analysis of the sequence data, we intend to assess the levels of genetic diversity among populations of thresher sharks and determine if the potential for large-scale dispersal in thresher sharks is indeed translated into broad gene flow across their range.

**Shelf, slope and pelagic surveys.** In cooperation with NMFS Northwest Fisheries Science Center (NWFSC) and SCL, PSRC personnel will participate and assist in the annual shelf and slope groundfish surveys in Washington, Oregon, and California. In 2003 a total of 678 specimens representing nine species was collected for life history and taxonomic studies from the annual NWFSC slope survey. Chondrichthyan landings are continuing to be recorded from monthly NMFS SCL trawl and longline surveys in Monterey Bay. Measurements, wet weights, reproductive tracts, tissue samples, parasites, vertebrae for age and growth estimation, and/or stomachs for feeding analyses have been collected from 4,400 specimens to date. The primary elasmobranchs observed in NMFS SCL groundfish surveys are the big (*Raja binoculata*), California, and longnose skates.

**Chondrichthyan fishery database.** A regional fisheries database, incorporating all chondrichthyan species landed in waters of the western continental United States, was expanded during this period. Focus has been directed at acquiring and entering commercial and recreational chondrichthyan fisheries landings and regulations from Alaska, California, Oregon, and Washington from 1980 to present. Details were obtained from the Pacific States Marine Fisheries Commission via the RecFin and PacFIN databases, and through state fisheries agencies. Relevant peer-reviewed publications, technical reports, and management/regulatory plans have been acquired and incorporated into the database. State-specific information will be provided for public access through the PSRC website and available later in 2004.

**Taxonomic studies.** A review and revision of the skate genus *Bathyraja* (Family: Arhynchobatidae) are being undertaken for each of the species within this family from the ENP. Colleagues at the NWFSC, with assistance from PSRC personnel, are continuing to collect and save skates during their annual slope cruises. In addition, specimens from museum collections at the California Academy of Sciences, Los Angeles County Museum Natural History, Smithsonian Natural History Museum, Scripps Institute of Oceanography, and the University of Washington are being examined. PSRC personnel are continuing to consult and collaborate on this project with leading experts at the Shark Research Center, Cape Town, South Africa and at Texas A & M University.

**Publications and conferences.** Members of the PSRC attended and presented results from our research at two conferences: Western Groundfish Conference and American Elasmobranch Society (AES) meetings; one of the program’s graduate students, Tonatiuh Trejo, won the best student poster award at the AES meetings. Drs. Gregor Cailliet and David Ebert, along with three graduate
students, also participated in the IUCN Shark Specialist Group Workshop at Mote Marine Lab in Sarasota, Florida to assess the status of Central and North American elasmobranchs. The PSRC web site now has a monthly “Featured Elasmobranch” that highlights a different species each month. The new “Feature” was started in February 2004 to help educate the public on the diversity of elasmobranchs by focusing on a different species each month. We anticipate that this feature will attract the general public, especially educators, to visit the web site on a regular basis to find out more about our ongoing research efforts. PSRC staff produced a total of eight papers that were published, in press, or in review during this report period.

Virginia Institute of Marine Science Report (J. Musick, P/I)

**Relative abundance.** During the report period, January 1-June 30, 25 longline sets were made in the Chesapeake Bay and Virginia coastal waters that fished 2,540 standard hooks and 360 non-standard hooks (directed toward juvenile sandbar sharks). The catch included a total of 286 sharks, of which 154 were tagged: 164 sandbar sharks (*Carcharhinus plumbeus*), 6 Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*), 88 smooth dogfish (*Mustelus canis*), 16 dusky sharks (*Carcharhinus obscurus*), four blacktip sharks (*Carcharhinus limbatus*), three spiny dogfish (*Squalus acanthias*), three common thresher sharks (*Alopias vulpinus*), one spinner shark (*Carcharhinus brevipinna*) and one white shark (*Carcharodon carcharias*). The sharks captured were either sampled for age and growth and DNA studies, tagged with VIMS juvenile dart tags, or tagged with NMFS M-type dart tags. Over this time period all standard stations have been sampled and several ancillary stations have been sampled. Sampling will continue through October of this year.

**Age, growth, and demography.** VIMS scientists continue to study the age and growth of barndoor skate by using vertebral ring analysis in cooperation with scientists at the NMFS Northeast Fishery Science Center (NEFSC). During this period 100 vertebrae were selected and processed to begin the validation of annual ring deposition. The results of genetic studies, conducted in collaboration with Joe Quattro at the University of South Carolina, are showing a fairly large well mixed population that extends from US waters to the Scotian Shelf. In addition, over 100 stomach samples were processed in a continuing effort to study the life history of the barndoor skate. The results of these new life history parameters have also been incorporated into newly developed demographic models. NMFS survey data is also being used to validate model results.

Age and growth studies of squaloid sharks are ongoing at VIMS. Due to vulnerability to fishing pressure, this age and growth data will be crucial in the proper management of these long-lived species. VIMS scientists have continued collaboration with Dr. Castro and John Galbraith (NMFS, Northeast Fisheries Science Center) in collecting samples of *Centrophorus granulosus* for age and growth and demographic analysis. Dr. Castro is in the process of revising his book, Sharks of North America, and in return for our assistance in collecting hard to find specimens for his book, he has agreed to provide us with fin spines to be used in our age and growth studies. Two deep-water sampling trips were conducted with John Galbraith in February and June of 2004, along the mid-Atlantic shelf and Bear Seamount, respectively. Another deep-water longline trip with Dr. Castro is planned in the Gulf of Mexico in September of 2004.
During the upcoming period, VIMS scientists will partner with the MAR-ECO project (www.mar-eco.no), a joint collaboration between U.S. scientists and scientists from 15 European countries, to study the faunal composition of the mid-Atlantic Ridge from the Azores Islands to Iceland. During this time, numerous samples will be obtained for age and growth analysis of several species of squaloid sharks (*Centrophorus squamosus*, *Centroscymnus coelolepis*, *C. owstoni*, *Centroseelachus crepidater*, *Etmopterus princeps*, *Deania calcea*, *Centroscyllium fabricii*). Samples will also be taken for studies of reproductive biology in these squaloid sharks. VIMS scientists will work with Dr. William Hamlett (Indiana University School of Medicine) on these reproductive studies. Specifically, oviducal glands from all species will be examined for evidence of sperm storage; also samples will be collected from reproductively active females to look for evidence of histotrophy in these species.

When VIMS scientists compared growth rates for sandbar sharks before and after stock collapse from overfishing between 1980 and 1992, they found little growth compensation and no change in the age at maturity. Now in 2003 the sandbar shark population is still less than 50% of that in 1980 and compensation has had more time to become apparent (growth may be affected over more year classes). VIMS scientists continue to examine sandbar shark growth rates (based on vertebral analysis) collected in 2001-2003 for comparison to published historical rates and those derived from archived vertebrae. VIMS scientists are continuing to work in cooperation with scientists from the University of Hawaii to collect sandbar shark vertebrae from the Hawaiian population which is purported to grow faster, mature younger, and reach a smaller maximum size. Sampling trips were conducted in September 2002, May 2003, September 2003, and June 2004. These trips have yielded 97 samples for age and growth studies, stomach content analysis, and genetic analysis. 51 sandbar sharks were tagged, injected with OTC, and released for age validation considerations over the time span. In addition 8 neonates collected from at term females were returned to the laboratory alive for captive OTC validation experiments. Preliminary results portray much slower growth rates than previously reported by Wass (1973).

**Habitat and migration studies.** Sampling directed at catching juvenile sandbar sharks within the Eastern Shore of Virginia nursery area commenced during the months of May and June. During this period, 50 longline sets were made that fished 2,500 monofilament ‘puppy’ hooks. 20 stations were fished every four weeks starting the first week of May and the physical parameters of temperature, salinity, tidal flow, and distance to ocean inlets were determined for each longline set. The catch included 601 sharks of four species: 258 sandbar sharks, 351 smooth dogfish, five Atlantic sharpnose sharks and one spinner shark. The catch also included several other species of elasmobranchs including: 34 clearnose skates (*Raja eglanteria*), eight southern stingrays (*Dasyatis americana*), six bluntnose stingrays (*Dasyatis say*), three smooth butterfly rays (*Gymnura micrura*), one spiny butterfly ray (*Gymnura altavela*), one cownose ray (*Rhinoptera bonasus*), and one little skate (*Leucoraja erinacea*). During this period 216 juvenile sandbar sharks were tagged with VIMS juvenile shark tags. Seven tag recaptures were reported during the period, five from this period, one from the summer of 2002, and one from the summer of 1998. Data on station location, physical attributes of each station, catch data, and time of year will be used to delineate this nursery spatially and temporally.

On June 2, 2004 15 acoustic receiver stations were re-deployed in Millstone Creek in
Wachapreague Inlet in the same positions as last year and one additional receiver was added to the array. Additionally on this date, two YSI's were deployed at each end of the array and programmed to take water temperature, salinity, and dissolved oxygen measurements every 15 minutes throughout the summer of 2004. During the previous summer 27 sharks were implanted with acoustic transmitters, six of these animals were detected by the receivers in the array during the beginning of June 2004. Additionally two of the 27 sharks were captured and killed by a commercial fisherman approximately 5 miles south and 3 miles offshore from the acoustic array and the transmitters were returned. These two transmitters and three new transmitters were implanted in juvenile sandbar sharks during the beginning of June. An additional 30 sharks will be implanted with transmitters during the months of July and August. The activity of these sharks will be studied as well as the effect of physical parameters on the movements of these sharks.

**Shark energetic and osmoregulatory studies.** Plans to study the salinity tolerance and metabolic rates of sandbar sharks at different salinities will commence in the upcoming period. Juvenile sandbar sharks present in the Chesapeake Bay nursery area are able to utilize areas within this estuary that have salinities down to approximately 20 ppt. We hypothesize this represents a physiological barrier against predation by larger sharks that are unable to utilize these low salinity waters. In order to test this hypothesis sharks will be captured by hook and line from the barrier island channels and lagoons off the Eastern Shore of Virginia and maintained at the VIMS Field Station at Wachapreague during July and August of 2004.

**Genetics.** VIMS scientists continue to work on estimating the effective population size of sandbar sharks utilizing east coast nurseries and the number of females that pup in these nurseries using modern genetic techniques. In addition comparisons can be made between recently collected samples and museum samples from the late 1970’s to determine if there has been any significant loss of genetic variation during the last thirty years of exploitation. Work is also being done to compare animals found in the Chesapeake Bay nurseries with those found in the Delaware Bay to examine philopatry and gene flow as well as to detect reproductive periodicity by finding kin groups. In order to accomplish these goals, over 700 fin clips have taken from juvenile and adult female sandbar sharks in the Eastern Shore and Lower Bay nurseries. Microsatellite markers have been designed and currently being optimized to ensure efficient accurate genetic screening. These markers will be used to examine the possibility of multiple paternity in sandbar sharks utilizing the Bay in the near future. In addition the sequencing of the control region will begin in the coming months enabling us to estimate the female effective population size and define the nature of gene flow in the population.

In connection with the research being conducted to examine the growth of deep sea sharks, VIMS researchers are also examining genetic questions associated with deep sea sharks. Currently there may be confusion about the taxonomy of this group of sharks at the family level. Previously *Etmopterus* was included in Dalatiidae and *Centrophorus* was in Squalidae. Currently those genera are in the families Etmopteridae and Centrophoridae (respectively). It is our aim to clarify some of the taxonomic issues through genetic analysis. Tissue samples were collected in conjunction with age and growth samples, during the previously mentioned NMFS NEFSC cruise.

**Publications and conferences.** During this period two VIMS scientists attended the American
Elasmobranch Society meeting in Norman, Oklahoma and presented papers on VIMS shark research. Additionally three VIMS scientists participated in the IUCN Shark Specialist Group North and Central America Region Red List Workshop held at Mote Marine Laboratory. During this period three papers were submitted for publication: 1) Dowd et al. Standard and Routine Metabolic Rates of Juvenile Sandbar Sharks (*Carcharhinus plumbeus* Nardo), Including the Effects of Body Mass and Acute Temperature Changes, 2) Ellis and Musick. Ontogenetic changes in the diet of the sandbar shark, *Carcharhinus plumbeus*, in Chesapeake Bay, Virginia (USA) and adjacent waters, and 3) Musick and Ellis. Reproductive Evolution of Chondrichthians (in press).

**University of Florida Report (G. Burgess, P/I)**

**Fishery independent surveys.** Fishery independent longline sampling was conducted aboard leased commercial shark fishing boats in the Spring of 2004, after the winter commercial shark fishing season had closed. FPSR scientists were at sea 14 days in April, May, and June in the southeastern Gulf of Mexico and in the Atlantic off the coasts of the Florida peninsula. A total of 406 sharks of twelve different species were caught on these expeditions. The dominant species in the catch were Rhizoprionodon terraenovae (84), *Carcharhinus plumbeus* (83), *Carcharhinus acronotus* (76) and *Ginglymostoma cirratum* (61). Some of these sharks were tagged and released alive, others, primarily those that arrived at the boat dead or moribund, were necropsied and completely “worked up” to provide tissues and materials for biological studies by FPRS scientists and NSRC collaborators.

Data and specimens derived from our fishery independent surveys and from our separately funded Commercial Shark Fishery Observer Program have contributed to our ongoing biological studies. We have been examining age and growth characteristics in hammerhead sharks (*Sphyrna lewini* and *S. mokarran*) in cooperation with NMFS scientists. During this reporting period, we have processed the majority of hammerhead vertebrae samples, and anticipate completion of the scalloped hammerhead age and growth project during the next reporting period. We are continuing the collection and processing of tiger shark vertebrae for a life history study on this species. During the last reporting period we initiated a study to investigate differences in vertebral characteristics (size, shape, and growth band deposition) in different regions of the vertebral column in four species of sharks important in the commercial fishery. All vertebral samples for this project have been processed and we will be completing this study during the next reporting period. We have continued to collect vertebral samples for collaborators in NMFS and other NSRC institutions.

Specimens taken during our surveys have contributed to an ongoing study on shark jaw morphometrics. In collaboration with scientists at the University of South Florida, we are gathering morphometric data from carcharhinid, sphyrid, and lamnid jaws and teeth. The project goal is to provide a means of accurately discerning the approximate size and species of a shark by statistically analyzing measurements from a bite impression. This study includes data on more than one hundred and fifty specimens of twenty different species, including tiger (*G. cuvier*), bull (*C. leucas*), and white sharks (*Carcharodon carcharias*).
Tagging and tracking bull sharks. During the reporting period a total of 21 days were spent in the Indian River Lagoon sampling with longlines and rod and reel, carrying out tracking activities, and maintaining the automated tracking system. A total of 12 neonate and juvenile bull sharks were captured ranging in size from 66-112 cm total length. Three juvenile bull sharks were tracked manually, yielding a total of 46 hours of short-term movement and habitat use data. The array of 19 passive listening stations was deployed in April in conjunction with the Dynamac Corporation at the Kennedy Space Center, allowing us to detect movements of sharks along 75 km of intracoastal waters. As of the end of the reporting period, the movements and site fidelity of one neonate bull shark have been monitored by the array for a period of 25 days. Fin clip samples were collected from six bull sharks for DNA analysis being conducted by NSRC collaborators at Mote Marine Laboratory. Tagging and tracking activities will continue through the next reporting period.

Fatty acid analysis and diet. During the last reporting period we initiated an experiment dealing with the effect of diet on fatty acid composition of stingrays. This study serves as a validation model for other studies dealing with commercially important sharks. The experimental phase of this project was complete during the last reporting period. During this reporting period the biochemical analysis of the experimental tissues was completed at a collaborating lab (Dr. Graham Worthy, University of Central Florida). We have begun to analyze these data and this project will be completed during the next reporting period. This study was conducted with the cooperation of collaborating scientists from MML’s Center for Shark Research.

Bomb radiocarbon age validation. The FPSR, in collaboration with other NSRC members (Pacific Shark Research Center) and NMFS scientists in Narragansett, RI, is attempting to validate the age and growth of the sandbar shark (Carcharhinus plumbeus), the single most important species in the southeastern Large Coastal Shark management group. This study will involve the use of radioactive carbon markers in shark vertebrae as a tool for validating and refining current age and growth parameters. “Practice” vertebrae have been provided from our fishery independent sampling program. Archival vertebrae have been gathered from specimens stored at the Florida Museum of Natural History and at the NMFS Narragansett lab and shipped to Moss Landing Marine Lab. A subcontractual agreement has been developed with our consortium collaborators at the Pacific Center for Shark Research (PCSR) to perform sample coring and demineralization.

During this reporting period, test swipes were taken from all of the samples and analyzed at Lawrence Livermore National Laboratory (LLNL) by Accelerator Mass Spectrometry to determine any background contamination. Contamination of some vertebrae supplied by NMFS/Narragansett was confirmed. The problem was corrected and new samples supplied. All vertebrae were cored and demineralized at PCSR and sent to LLNL for radiocarbon analysis, which was completed in late June. We await the results of the analysis as of this writing. We expect to complete this project in the next reporting period.

Dusky shark population modeling. During this time period several data sets and parameters for the dusky shark were identified and the standardization of the Commercial Shark Fishery Observer Program (CSFOP) data set was initiated. Several statistical computer programs and computer languages were identified, purchased and/or downloaded onto computers within the Florida Program
for Shark Research. Communication with advisors outside of the FRSR was continued and plans have been made for site visits to NMFS Panama City and Mote Marine Laboratory to take a look at various age-structured models.

**Reproduction and gestation.** We have continued our studies of the maternal-embryonic relationship in the bonnethead shark. During this reporting period, we collected uterine fluid and serum samples from pregnant bonnetheads in the Gulf of Mexico with the assistance of collaborating NSRC partners at Mote Marine Laboratory. Our sample size was increased to include individual females from early and late pregnancy stages. We have 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. These analyses are ongoing, and part of a larger project to determine mechanisms of uterine fluid production and utilization and hormonal control of uterine development in the bonnethead.

We have also continued to collect samples of reproductive tissues of the sandbar shark and blacknose shark for reproductive studies planned by our lab and to meet the needs of collaborating NMFS scientists. Histological preparation and analysis of tissues has been delayed due to the continuing lack of an appropriate exhaust hood in our laboratory. We expect to have this problem remedied in the next reporting period.

**International Shark Attack File.** A total of 46 new case files were entered into the ISAF database during this time period. In addition 70 case files that already exist in the database were updated with new information. A project initiated during this time period aimed at filling in missing parameter entries within the database for, latitude and longitude, water temperature, salinity, lunar phase and tidal cycle was completed for Florida, Hawaii and California. The remainder of this project will be completed during the next time period. The database was 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 during this time period. In addition requests for information and phone queries from the public, media and scientific communities were continuously dealt with. The ISAF web pages were updated through the year 2003 and continue to be the most heavily visited of all pages on the entire FLMNH web-site.

**Public education.** The FLMNH Ichthyology Division web pages, including the FPSR web pages, received 138,280,100 “hits”, 2,816,649 visits, and 1,210,306 unique visitors during the reporting period. Materials relating to elasmobranchs on the site have been expanded and improved. New shark species accounts have been added to the “Biological Profiles” section, with an emphasis on those species occurring in the Pacific Ocean. Also, the image gallery has been greatly expanded during the reporting period with the addition of over 1,000 new images. The “In The News” fish and shark pages are updated daily with the latest news releases on research and conservation. The IUCN Shark Specialist Group and AES web sites have been continually upgraded and expanded. Pages for each member institution of the NSRC have been updated with current research and education initiatives and personnel. The “Featured Projects” web page, which features in-depth information on
research initiatives from each NSRC member institution, has also been expanded. A web section on whale shark research and conservation is a new addition off the whale shark web page, detailing FPSR scientist Dr. Rachel Graham’s activities in Belize. The “Education” web page now includes information on shark fishing methods as well as stingray reproduction references. Over the next reporting period, the web site will continually be expanded with the addition of new information in each appropriate section of the web site.

*Project Shark Awareness* 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. The FPSR will be hosting teacher-training workshops for Florida Sea Grant extension agents and 4-H leaders in the next reporting period in response to the high demand for this program. The teaching kits will be revised and significantly improved for these workshops. Funding has also been granted from another agency in support of future teacher-training workshops for secondary school educators within the state of Florida. An interactive shark identification key is currently undergoing final revisions and will be posted during the next reporting period in support of Project Shark Awareness.

**Requests for information.** The staff of the FPSR, especially those involved with the International Shark Attack File, have been busy responding to requests for information during the reporting period. We have handled 361 individual correspondences (email, phone, or postal mail) with various organizations, news agencies, scientists, and lay individuals asking for information and analysis on matters related to sharks and rays, especially shark attacks and related issues, shark ecology and biology, and shark conservation and fisheries. These requests have come from all over the world. FPSR staff members have also responded to numerous requests for interviews and for information on sharks from the general public, especially students. We have provided tissues, specimens, and information to collaborating scientists in three NMFS labs (Panama City, Miami, and Narragansett) and to collaborators in two NSRC programs (Center for Shark Research, MML; and Shark Research Program, VIMS). Two NMFS departments, the Protected Resource Division and the Highly Migratory Species Division, requested data and information, and these requests were addressed. We also provided technical support and tissue samples to support ongoing studies on various aspects of shark biology by scientists in South Africa, Hawaii, New York, and Florida.

**Publications, presentations and conferences.** During the reporting period, FPSR personnel are authors on two published scientific papers, and authors or co-authors on three papers accepted for publication and in press. Numerous manuscripts are in or nearing submission. Project personnel gave one poster and three oral presentations on elasmobranchs at the annual meeting of the American Elasmobranch Society. Two presentations were made at other professional conferences. We attended and were actively involved in seven meetings, conferences, or workshops relating to elasmobranch issues, including meetings of the IUCN Shark Specialist Group and the NMFS Sawfish Recovery Team. FPRS staff made six presentations to educational groups, with emphasis on shark fisheries, conservation, and education. We were especially active in support of *Project Shark Awareness* and with the Center for Ocean Sciences Education Excellence (COSEE) program. We presented a workshop to NMFS commercial shark fishery observers to train them in taking biological samples while at sea.
Respectfully submitted,

Robert E. Hueter, Ph.D.
Project Coordinator & Director, Center for Shark Research, Mote Marine Laboratory
August 6, 2004