

Examination of Local Movement and Migratory Behavior of Sea Turtles During Spring and Summer Along the Atlantic Coast Off the Southeastern United States

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For

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During Spring and Summer Along the Atlantic Coast Off the Southeastern
United States**

by

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Executive Summary

Following a four summer (2000-2003) regional abundance and health survey of sea turtles in coastal waters of the Southeastern U.S., the SCDNR in-water sea turtle trawl project shifted focus in 2004-2005 to investigating the distributional patterns of juvenile loggerheads in these coastal waters, in order to better understand the significance of low tag-recapture rates observed during 2000-2003. The shift in focus to a single trawling area (the Charleston, SC, shipping entrance channel) greatly increased trawling replication at fixed stations, which enabled better understanding of residence patterns in areas of potentially concentrated abundance. Because this same location was trawled by two different efforts during 1990-1993, historical comparisons of catch rates at this locale were also possible.

Overall catch per unit effort (turtles per station) in 2005 was 0.3, a decrease of 63% with respect to catch rates at the same stations in 2004 (0.75 turtles per station). Although unlikely that trawl duration discrepancies exclusively accounted for inter-annual differences in catch rates, it is worth noting that trawling durations at the two most productive stations (D1, D3) were considerably shorter (22%) in 2005 than in 2004 due to encountering and avoiding obstructions. A strong seasonal component in turtle catch rates was observed in 2004 and 2005, with catch rates in May two to three times greater than in August. Abundance of two known prey items, blue crabs and horseshoe crabs, also exhibited the same seasonal abundance patterns in both years.

Recapture rates were comparable between 2004 (3.3%) and 2005 (4.25%), with recapture rates in the Charleston harbor entrance channel continuing to be greater than recapture rates for surrounding SC coastal waters. Four of six loggerheads recaptured since trawling in the harbor entrance channel began in May 2004 were originally collected at this location, with two others having been collected within 5 km of this location during 2000-2003 in-water efforts. Unlike 2004, recapture events were only observed during trawling in May 2005, and no within year recaptures were observed.

Although the entrance channel is a focal point for turtle abundance, loggerheads do not appear to spend much of their time in the vicinity of the channel. Most satellite-tagged loggerheads immediately departed the entrance channel after tag and release, and resided primarily on the shoals and patchy live bottom reef areas within 10-50 km of the coast. During summer and fall, several of these turtles briefly re-visited the entrance channel on occasion, particularly before departing for their respective over-wintering locations.

Complete over-wintering data (through March) was collected for four satellite-tagged loggerheads, while two others ceased detections in early-March. All of these turtles over-wintered on the middle to outer continental shelf off of SC and GA through late February. In late February and early March, two of these loggerheads appeared to have entered the Gulf Stream, at locations within 80km of each other; however, these turtles were only tracked until 8-9 March 2006. In mid-April 2006, transmissions ceased for a third loggerhead while it was still on the outer shelf off of Charleston, just prior to three other loggerheads returning to areas previously occupied during summer and fall 2005.

Introduction

Loggerhead sea turtles (*Caretta caretta*) inhabiting coastal waters along the southeastern United States represent the progeny of multiple rookeries (Bowen et al. 1993; Sears et al. 1995; TEWG 2000, Maier et al. 2004). Tagging studies of nesting female loggerheads suggest that most return to the same beaches in successive breeding seasons (Bjorndal et al. 1983) and it is widely accepted that most females return to their natal regions to nest. Although considerable effort has been expended to study adult females on nesting beaches, much less is known about the distributional patterns of juveniles and adult males in coastal water bodies.

Prior to May 2000, in-water studies targeting sea turtles were primarily conducted at shipping entrance channels (Kemmerer et al. 1983; Standora et al. 1993a,b; Dickerson et al. 1995; Keinath et al. 1995) or at opportunistic inshore collection locations (i.e., pound nets: Byles 1988; Epperly et al. 1995; Morreale and Standora 1993). The need to conduct, "...long-term, in-water indices of loggerhead abundance in coastal waters" (TEWG 1998) led to the development of a regional in-water survey of loggerheads during summers 2000-2003 (Maier et al. 2004). Coastal waters 1-15 km offshore between Winyah Bay, SC, to St. Augustine, FL, were thoroughly sampled in a nearly simultaneous manner using three research vessels annually. High catch rates were reported (Maier et al. 2004); however, very low recapture rates (<2%) were also reported, the cause of which was not readily evident.

Beginning in May 2004, in an effort to better understand the seasonal distributional patterns of juvenile loggerheads collected in coastal waters sampled during the 2000-2003 regional survey, the focus of the in-water survey was modified to intensively target one small trawling area to: (1) examine the effect of intensive trawling on recapture rates and (2) quickly obtain an adequate sample size of turtles to outfit with satellite transmitters. At the time that this research was initiated, satellite telemetry had only been attempted with four juvenile loggerheads in coastal waters south of Cape Hatteras (NMFS; USACOE; Whalenet); thus, detailed information on seasonal habitat utilization patterns of juveniles was virtually non-existent for this region.

In order to facilitate historical comparisons of catch-per-unit effort (VanDolah and Maier 1993; Dickerson et al. 1995), the shipping entrance channel of Charleston harbor was selected for this trawl survey. Logistical considerations, including close proximity to a turtle rehabilitation facility at the SC Aquarium in Charleston, also contributed to the decision to restrict trawling to the single location.

This annual report highlights the major findings for research activities primarily carried out during 2005. More detailed analyses will be included in the 2004-2006 Final Report and manuscripts which will be submitted for peer-review in 2007.

Methods

Study Area

Trawling in 2005 was conducted from the jetty ends out to 9km offshore in the Charleston, SC, shipping entrance channel (32°42'N, -79°48'W; Figure 1) for two weeks in May and August. Seven of 12 index stations first utilized in 1990-1991 (VanDolah and Maier 1993); gear loss due to bottom obstructions in 2004 resulted in permanent elimination of five stations (E1-E3; B2, D2) and shortening of two others (D1 and D3).

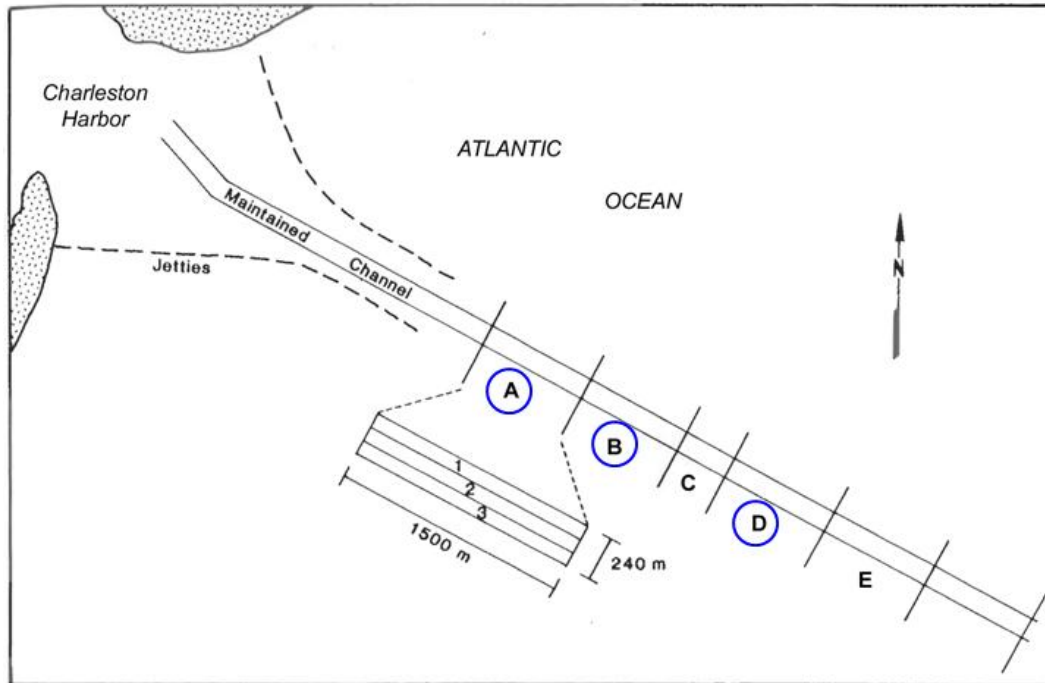


Figure 1. Index trawling blocks (from VanDolah and Maier, 1993) in the Charleston Harbor shipping entrance channel in 1990-1991 (all) and 2004-2005 (blue circles).

Capture and General Processing

Sampling was conducted aboard a double-rigged shrimp trawler (R/V *Lady Lisa*) measuring 75 feet in length and towing at speeds of 2.5-3.0 knots. Standardized NMFS nets routinely used in turtle surveys associated with channel dredging operations were used for this research: paired 60-foot (head-rope), 4-seam, 4-legged, 2-bridal; net body is of 4" bar and 8" stretch mesh; Top's sides of #36 twisted with the bottom of #84 braided nylon line; 60' corkline to cod end; cod end consists of 2" bar and 4" stretch mesh.

Nets were towed for 10-15 minutes (doors set on bottom to start of haul back), roughly one third of the 45 minutes allowed by NMFS Permit 1245. Nets were brought on-board using winches. Turtles were immediately removed from nets and examined for life-threatening injuries, before being visually/electronically scanned for existing tags. If not previously tagged in this study, a sequential project identification number was assigned to each turtle.

Blood samples were collected for all sea turtles >5kg body weight with a 21ga, 1.5 in. needle from the dorsal cervical sinus of loggerhead turtles as described by Owens and Ruiz (1980). Blood samples consisted of a maximum of 45 ml total volume and did not exceed the total recommended volume (10% of total blood volume) based upon total weight as described by Jacobson (1998), who estimated that total blood volume in reptiles was 5 to 8% of total body weight. Blood samples were used as follows:

- genetic stock identification - 5 ml (University of South Carolina)
- sex determination - 5 ml (University of Charleston)
- CBC/Blood chemistry -- 5 ml (Antech Diagnostics)
- Toxicological screening and immunological bioassay - 30ml (National Institute of Standards and Technology; Medical University of SC)

A suite of morphometric measurements were collected for all sea turtle species. Six straight-line measurements (cm) were made using tree calipers: minimum (CLmin) and notch-tip (CLnt) carapace length; carapace width (CW); head width (HW); and body depth (BD). Curved measurements of CLmin, CLnt and CW were recorded using a nylon tape measure. Additional curved measurements included plastron width (PW), and two tail length measurements (tip of plastron to tip of tail (PT) and tip of cloaca to tip of tail (CT)). All measurements represented standard measurements accepted by sea turtle researchers globally (Bolten, 1999). Body weight (kg) was measured using spring scales; turtles were placed in a nylon mesh harness and carefully raised off of the deck.

All sea turtles >5kg received two Inconel flipper tags and one Passive Integrated Transponder (PIT) tag (Biomark, Inc.). Triple tagging minimized the probability of complete tag loss. Inconel flipper tags were provided by the Cooperate Marine Turtle Tagging Program (CMTTP). Per instructions provided by the CMTTP, tags were cleaned to remove oil and residue prior to application. Inconel tag insertion sites, located between the first and second scales on the trailing edge of the front flippers, were swabbed with betadine prior to tag application. PIT tag insertion points, located in the right front shoulder near the base of the flipper, were swabbed with betadine prior to intramuscular injection of the sterile-packed PIT tag.

Prior to releasing turtles, a digital photograph of each turtle in a standard 'pose' (dorsal surface exposed, taken looking from anterior to posterior) was recorded. Additional photographs of unusual markings or injuries were also recorded.

By-catch

By-catch species were identified to the lowest possible taxon and a count or estimate of abundance noted. Sex and appropriate length (cm) measurements were included for all elasmobranchs, as well as finfish and invertebrate species of interest. Particular emphasis was placed on by-catch species that represented potential sea turtle prey items, such as blue crabs (*Callinectes sapidus*) and horseshoe crabs (*Limulus polyphemus*).

Satellite telemetry

ST-20 (Telonics, Inc) satellite transmitters were attached directly to the second vertebral scute on the turtle carapace using epoxy (Papi et al., 1997; Polovina et al., 2000; Griffin,

2002). Prior to attachment, barnacles and other organisms were removed with a paint scraper, the carapace sanded, washed with betadine and dried with acetone. A roll of 1.0 cm diameter “Sonic Weld” (Ed Greene & Company; Sparta, TN) was placed around the bottom edge of the transmitter to form a well, followed by application of “Fast Foil” epoxy (Power Fasteners Inc.; New Rochelle, NY) to the entire bottom surface of the transmitter within the well using a caulking gun. Turtles were released approximately two hours after initial collection in close proximity (<3 km) to where originally collected.

Satellite telemetry data consisted of (1) geographic position at each surfacing; (2) water temperature at each surfacing; and (3) four descriptive dive cycle metrics for each of four, six-hour collection periods per day: time(s) of last dive; number of dives per collection period; mean dive duration(s) per collection period; and percent of time submerged per collection period. Satellite telemetry data were automatically processed, distributed and received by the Argos system. Daily data e-mails were sent to project personnel; however, data were primarily managed using “STAT” (Satellite Tracking and Analysis Tool; Coyne and Godley, 2005). Data were downloaded from “STAT” monthly to a relational database (MS Access) on a local area network for analyses.

Results

Capture and Recapture

Forty-seven sea turtles, all loggerheads, were collected in 162 trawling events totaling 2,216 minutes (36.9 hrs) of trawling, representing an average catch rate of one loggerhead every 47.1 minutes. Total loggerhead catch in 2005 (n=47) was 61% lower than in 2004 (n=122), and it took 1.5 times as long, on average, to catch a loggerhead in 2005 than it did in 2004 (average time to catch loggerhead in 2004 = 19.1 minutes).

Greater catch rates were observed in May than in August in both 2004 and 2005. In May 2005, 36 loggerheads were collected in 70 trawling events totaling 991 minutes (average time to catch a loggerhead = 27.5 minutes); however, in August 2005, 11 loggerheads were caught in 92 trawling events totaling 1,225 minutes (average time to catch a loggerhead = 1.85 hours). August '05 CPUE was the lowest bi-weekly CPUE of five sampling blocks since this project began in 2004, and was comparable to CPUE's reported during 1991 (VanDolah and Maier, 1993); however, sampling effort was considerably greater (92 stations) in 2005 than in 1991 (24 stations; 12 during daylight).

Inter-annual water temperature differences at the time of sampling were noted. Sampling was generally conducted on the same dates in May 2004 and May 2005, but mean water temperatures were 1-2°C cooler in May 2005 than in May 2004 (Figure 2). Conversely, mean water temperatures in August 2005 were 2-3°C warmer than in August 2004 (Figure 2). Sampling in August 2005 was conducted two weeks earlier than in August 2004 and weather conditions were not as inclement in August 2005 as in August 2004.

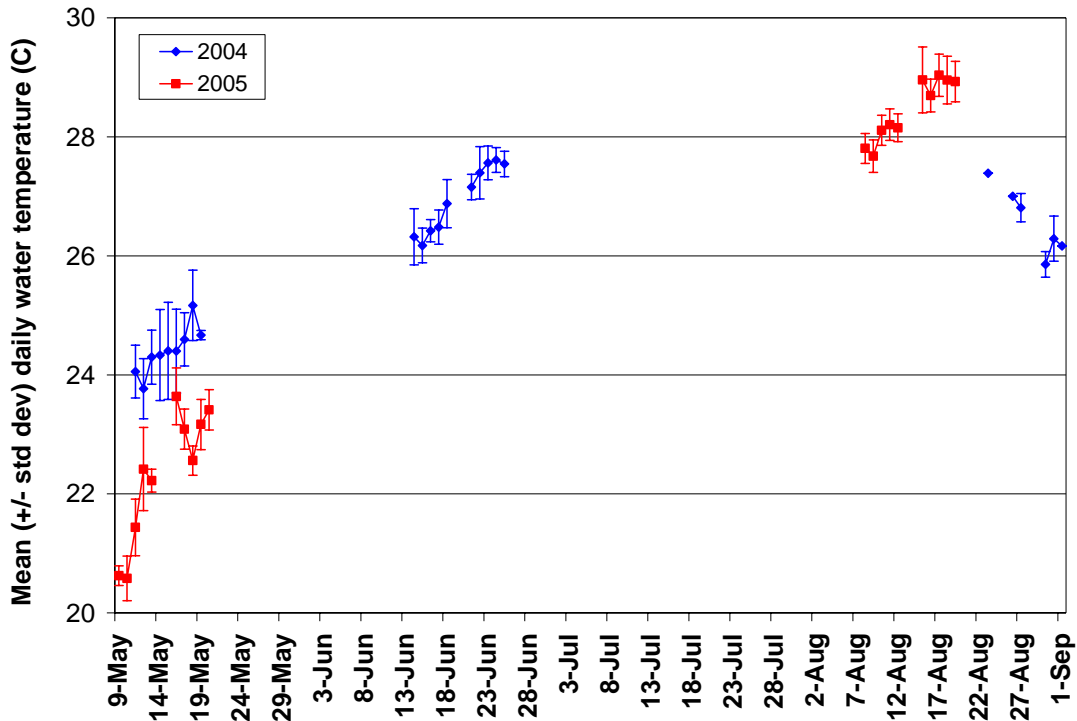


Figure 2. Mean (+/- std dev) daily surface water temperatures during sampling.

Loggerhead catches were highly variable among stations, with similar relative catches among stations in 2005 and 2004 (Table 1). Sixty to sixty-five percent of all loggerheads were collected in the “D” block in both years. Catch rates in the “B” and “D” sampling blocks also continued to be several times greater at #3 stations, the southern (green navigational buoy) side of the harbor entrance channel.

Highly variable catch rates within sampling stations noted in 2004 persisted in 2005 (Table 2). No turtle and single turtle catches at “A1” and “A2” were similar between 2005 and 2004; however, considerably fewer turtles (and no multiple turtle catches) were collected at “A3” in 2005 than in 2004. Zero turtle catches at “B” block and “D” block stations were considerably more common in 2005 than in 2004; consequently, turtle catches, particularly multiple turtle catches, were considerably lower in 2005 than in 2004 at these stations.

Two of 47 loggerheads (4.25%) collected in May 2005 represented recapture events, both of which were released in previous years. The first recaptured turtle (CC0329) was released in August 2004 with a satellite transmitter and monitored daily for 285 d. Following standard sampling procedures, this turtle was re-released with satellite transmitter intact and monitored for an additional 50 d until the transmitter expired. The second turtle (CC6045) was recaptured after 1,397 days at large and was initially tagged by SCDNR in July 2001. This turtle was recaptured within 5km of where previously collected and re-released with a satellite transmitter (which expired after 106 d).

Table 1. Summary of loggerhead catch and effort data with respect to trawl station.

2004

Station	N loggerheads	% Loggerheads Caught	N Trawl Events	Total Trawl Time	Mean Trawl Time
A1	3	3	21	305	14.5
A2	1	1	20	307	15.4
A3	4	3	19	296	15.6
B1	11	9	23	328	14.3
B3	23	19	23	343	14.9
D1	12	10	22	298	13.5
D3	66	55	22	302	13.7

Overall 120* 150* 2179

*12 additional sampling events (and two loggerheads caught) at B2, D2 and E1-E3

2005

Station	N loggerheads	% Loggerheads Caught	N Trawl Events	Total Trawl Time	Mean Trawl Time
A1	3	6	23	350	15.2
A2	1	2	23	340	14.8
A3	2	4	23	347	15.1
B1	3	6	23	342	14.9
B3	10	21	23	338	14.7
D1	5	11	23	244	10.6
D3	23	49	24	255	10.6

Overall 47 162 2216

Table 2. Percent of trawling events with zero, one, or multiple loggerheads by station.

2004

	A1 (n=21)	A2 (n=20)	A3 (n=19)	B1 (n=23)	B3 (n=23)	D1 (n=22)	D3 (n=22)
0 loggerheads	86%	95%	79%	57%	44%	63%	18%
1 loggerheads	14%	5%	16%	30%	26%	27%	32%
2 loggerheads			5%	13%	17%	5%	9%
3 loggerheads					13%	0%	5%
4 loggerheads						5%	5%
5 loggerheads							13%
6 loggerheads							0%
7 loggerheads							0%
8 loggerheads							13%
9 loggerheads							5%

2005

	A1 (n=21)	A2 (n=20)	A3 (n=19)	B1 (n=23)	B3 (n=23)	D1 (n=22)	D3 (n=22)
0 loggerheads	87%	96%	91%	87%	70%	83%	37%
1 loggerheads	13%	4%	9%	13%	22%	13%	42%
2 loggerheads					4%	4%	17%
3 loggerheads					4%		0%
4 loggerheads							0%
5 loggerheads							4%
6 loggerheads							
7 loggerheads							
8 loggerheads							
9 loggerheads							

Size Distribution

Size-frequency distributions were similar in 2004 and 2005 (Figure 3). Seventy-four percent of loggerheads in 2004, and 70% of loggerheads in 2005 were 60 to 79.9 cm SCLmin (Figure 5). Conversely, only two percent of loggerheads in 2004 were >90 cm SCLmin, compared to nine percent of loggerheads in 2005 being in this size class. Increased proportion of large loggerheads in 2005 was attributed to collection of four adult male loggerheads during May sampling. Similar to 2004 (Figure 4a), minimum carapace length could not be determined for one loggerhead (CC0385, Figure 4b) due to a pre-existing injury which removed ~15% of the posterior carapace.

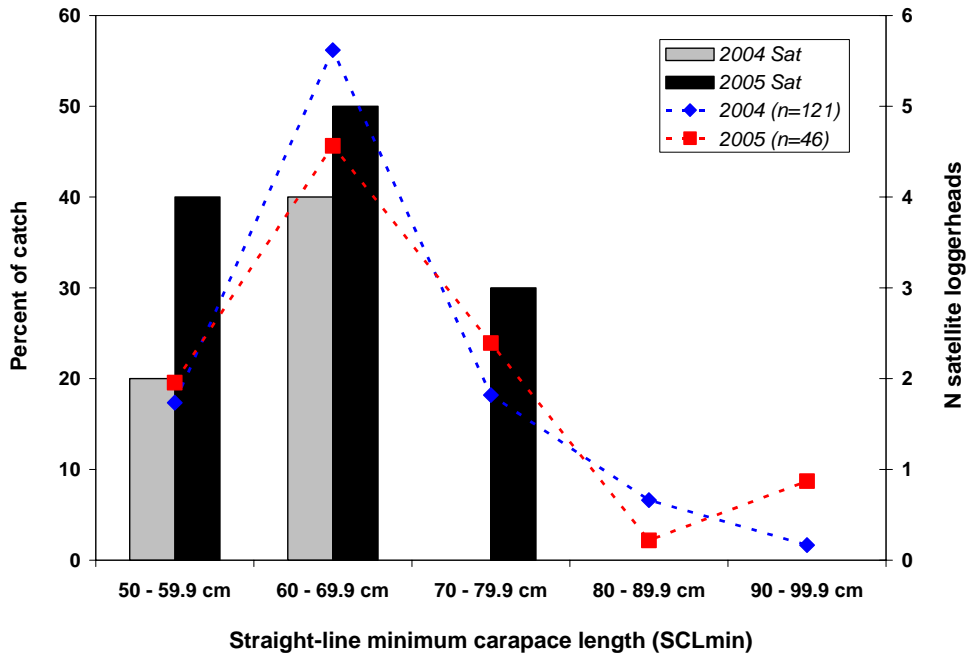


Figure 3. Size distribution of juvenile loggerheads collected in the Charleston Harbor shipping entrance channel, 2004-2005.



Figure 4. Pre-existing injuries precluding length measurement in 2004 (A) and 2005 (B).

Blood Analyses

Blood samples were collected for all 47 loggerheads and processed for distribution to respective collaborators. Three blood parameters (hematocrit, total protein, glucose) were measured at sea. No inter-annual or inter-seasonal differences in mean values for these parameters were noted for 2004 and 2005 (Table 3).

Table 3. Descriptive statistics for blood parameters measured at sea, 2004-2005.

year-mo	Hematocrit			Total Protein			Glucose		
	N	Mean	Stdev	N	Mean	Stdev	N	Mean	Stdev
2004-05	49	33	6	49	5.1	0.7	49	80	16
2004-06	54	34	5	55 ⁺	5.9	0.9	54	103	33
2004-08	16	33	4	16	5.3	0.8	16	84	15
2005-05	36	32	6	*	*	*	36	79	18
2005-08	11	32	5	11	3.5	0.8	11	95	21

⁺red top tube clotted; hematocrit and glucose not determined

*results under review

Blood samples for 13 loggerheads were analyzed (Complete Blood Profile) by Antech Diagnostic Laboratories. One sick turtle (CC0365) blood sample was analyzed by Antech; however, this turtle was successfully rehabilitated from the SC Aquarium and released with a satellite transmitter three months later. The remaining twelve Antech samples consisted of 11 loggerheads satellite tagged in 2005 and one satellite tagged loggerhead from 2004 that was recaptured in May 2005. Substantial inter-annual differences in Antech blood parameters between 2004 and 2005 were not noted (Table 4).

Testosterone radio-immunoassays (Dr. David Owens, Grice Marine Biology Lab) for sex determination and genetic analyses (Dr. Joseph Quattro, University of SC) are pending. Samples have not been analyzed due to problems encountered with a change in reagents (and therefore the need to evaluate methods) for the testosterone analyses, and problems sequencing the DNA samples. These data will be available by September 2006.

Other Collaborative Blood and Tissue Samples

Blood samples for toxicological analyses were collected for multiple researchers. Dr. Jennifer Keller (NIST) received a 10ml blood sample for all but one loggerhead (CC0370) as well as replicate toxicological samples for seven loggerheads. Dr. Margie Peden-Adams (MUSC) received a 10ml blood sample for all but two loggerheads (CC0343, CC0370). Mr. Rusty Day (NIST) received blood and keratin scrapings for all 12 loggerheads outfitted with satellite transmitters, one recaptured loggerhead (CC0329) and one of our large adult males (CC0350) collected in May. None of these results are available at this time; however, have been archived for future analyses.

Cloacal swab samples were collected for 30 of 47 loggerheads (Dr. Jan Gooch, NOAA). Gram negative bacteria *E. coli* – 2; *Proteus vulgaris* – 1; *Pseudomonas stutzeri* -1) were isolated from 4 of 21 (19%) cloacal swab samples collected in May 2005; however,

inappropriate methods for preserving these samples at sea (-20°C vs. -80°C) may have contributed to the low culture rate. Conversely, gram negative bacteria (*Shewanella algae/putrefaciens* – 1; *E. coli* – 1; *Pseudomonas spp.* & *Morganella morganii* -1; *Pseudomonas aeruginosa*, *Salmonella spp.* & Poly D – 1) were isolated from 4 of 9 (44%) cloacal swab samples collected in August 2005 using -80°C preservation. A complete summary of these findings are provided in Appendix 1.

Barnacle samples were collected for Dr. John Zardus (The Citadel) from six loggerheads in May (four juveniles outfitted with satellite transmitters, one large adult male, and one recaptured loggerhead, CC0329). Prior to being transferred to the custody of Dr. Zardus, these samples were examined by Mr. David Knott of the Southeastern Regional Taxonomic Center (SERTC) of the South Carolina Department of Natural Resources. Mr. Knott was able to identify several invertebrate species cohabitating with the barnacles, including tanaid crustaceans, which were preserved for the SERTC collection to facilitate development of a taxonomic guide for this diverse group of small crustaceans for which little local information on occurrence and distribution exists. Given the success of this collection method, growth removed from four loggerheads prior to attaching satellite transmitters in August 2005 were also saved and preserved for SERTC.

Table 4. Clinical blood values for ‘normal’ loggerheads collected from the Charleston harbor entrance channel (2004-2005) vs. the regional survey (CY2000-2003).

Blood Chemistry	2004					2005					2000-2003 All Boats				
	N	Mean	Min	Max	St Dev	N	Mean	Min	Max	St Dev	N	Mean	Min	Max	St Dev
Albu-AN	19	1.0	0.7	1.3	0.1	12	0.9	0.5	1.2	0.2	147	1.1	0.4	2.8	0.3
AST-AN	19	180.4	73.0	289.0	58.6	12	156.8	86.0	222.0	37.6	147	209.9	72	564	81.2
UrNi-AN	19	63.3	38.0	95.0	16.6	12	52.3	24.0	98.0	23.6	146	78.9	16	150	26.9
Calc-AN	19	7.5	6.1	8.4	0.6	12	6.7	5.1	9.4	1.0	147	7.8	1.6	11.7	1.5
Chlo-AN	19	118.8	110.0	133.0	5.4	12	117.3	112.0	121.0	2.7	147	117.5	92	141	7.4
CPK-AN	19	1319.6	286.0	4220.0	1123.6	12	1146.8	184.0	2535.0	729.4	147	1235.3	126	13830	1313.8
Glob-AN	19	2.4	0.9	4.0	0.9	12	2.4	1.7	3.2	0.5	147	3.2	1.4	5.1	0.9
Gluc-AN	19	97.3	75.0	147.0	19.4	12	80.1	47.0	126.0	20.0	147	106.8	7	202	33.2
Phos-AN	19	7.6	5.2	10.9	1.3	12	7.1	5.6	9.6	1.1	147	7.5	4.9	11.4	1.2
Pota-AN	19	4.6	4.0	5.7	0.5	12	4.4	3.7	5.4	0.6	147	4.9	3.2	19.9	1.5
Sodi-AN	19	158.2	150.0	171.0	5.1	12	156.1	150.0	163.0	3.6	147	156.9	137	186	6.0
ToPr-AN	19	3.4	1.9	5.0	0.9	12	3.3	2.3	4.4	0.6	147	4.3	1.8	6.6	1.0
Uric-AN	19	1.0	0.5	1.6	0.3	12	1.0	0.7	1.3	0.2	147	1.6	0.1	4	0.7

Complete Blood Count	2004					2005					2000-2003 All Boats				
	N	Mean	Min	Max	St Dev	N	Mean	Min	Max	St Dev	N	Mean	Min	Max	St Dev
Hema-AN	18	32.9	25.0	41.0	4.0	12	32.2	27.0	38.0	3.3	120	35.1	21	80	5.9
WBC-AN	19	8.6	5.0	13.0	1.9	12	8.4	5.0	13.0	2.5	153	11.1	4	25	4.0
Baso-AN	19	0.3	0.0	2.0	0.6	12	0.8	0.0	3.0	1.2	153	0.2	0	3	0.6
Eosi-AN	19	3.7	0.0	10.0	3.2	12	4.9	0.0	15.0	6.1	153	0.9	0	16	2.4
HePo-AN	19	23.4	7.0	54.0	11.0	12	41.8	0.0	82.0	27.6	153	35.5	7	86	18.1
Lymp-AN	19	70.0	31.0	90.0	15.6	12	49.6	12.0	90.0	25.0	153	61.7	13	93	19.4
Mono-AN	19	2.1	0.0	13.0	4.0	12	2.0	0.0	4.0	1.7	153	1.1	0	7	1.5
AzMo-AN	17	0.5	0.0	5.0	1.4	12	0.8	0.0	4.0	1.6	27	2.7	0	10	2.2
AbPo-AN	19	2000.0	660.0	4860.0	1018.5	12	3725.0	0.0	10660.0	2976.9	153	14.7	0	270	47.0
AbBa-AN	19	24.2	0.0	200.0	53.5	12	75.8	0.0	300.0	111.3	153	80.6	0	1260	212.0
AbEo-AN	19	273.7	0.0	990.0	258.2	12	327.5	0.0	900.0	372.2	153	3784.6	700	22880	2472.3
AbLy-AN	19	6044.7	2790.0	10920.0	1993.9	12	4010.0	1470.0	8100.0	2256.7	153	7146.5	1280	21000	4067.7
AbMo-AN	19	185.3	0.0	1100.0	363.4	12	183.3	0.0	480.0	162.0	153	123.2	0	840	174.8
AAMo-AN	17	51.2	0.0	550.0	141.9	12	95.0	0.0	480.0	178.3	27	221.5	0	700	170.4

Physical Condition of Turtles

Twenty-six percent of ($n=12$ of 47) loggerheads collected had pre-existing injuries for which human or shark interactions were suspected. In 2005, flipper damage was the most frequently observed injury, occurring in 8 of 12 loggerheads with injuries. Unlike in 2004 when minor to major flipper wounds were usually associated with damage to the carapace, only 2 of 8 loggerheads with flipper wounds also had carapace wounds. Six other loggerheads had damage to the carapace, most often to the M9-M11 scutes. Plastron wounds were observed in three loggerheads with carapace wounds, as well as one additional loggerhead (which also had a flipper injury).

While most injuries appeared to be very old and/or not too extensive, two turtles (CC0380, CC0385) collected in August had extensive injuries. Loggerhead CC0380 was observed with a chronic bite wound of 25cm gape width on the carapace (which included the right-side M8-M11 and C4-C6 scutes) as well as multiple linear plastron erosions. CC0385 (Figure 4b) was missing approximately 10-25% of the posterior carapace and plastron, as well as the tail/cloaca. This wound was probably only a few weeks old.

By-Catch

By-catch taxon consisted of 84 (generally identified to genus and species) listings totaling 4,278 individual items during 2005 trawling efforts. Overall bycatch (numbers of individuals) in 2005 was less than half of bycatch recorded for 2004, with 25% fewer taxon (84 vs. 103) recorded. Bycatch items were grouped into sixteen generic groupings for descriptive analyses (Figure 5). Jellyfish, observed in numbers more than double recorded in 2004, were the most dominant bycatch grouping in 2005, and were nearly 5 times more frequently observed than the next most abundant species grouping (Figure 5). Although jellyfish as a group increased dramatically, due to the surge in the collection of “box” jellies in 2005, cannonball jellyfish (*Stomolophus meleagris*) and “non-box stinging jellies” actually decreased several fold between 2004 and 2005 (Table 5).

Finfish were the next most abundant bycatch grouping in 2005, but were observed at levels which were more than five times lower than observed in 2004 (Figure 6). Four finfish species seen in total abundances of >200 individuals accounted for more than half of finfish catches in 2004; however, these species were collected with frequencies 5 to 51 times lower in 2005 (Table 5). Four finfish species seen in 2005 were not seen in 2004 and 16 finfish species were seen in 2005 but not in 2004; however, all of these species occurred with low frequency ($n=1$ to 26). Also of interest (but not included in Table 5 due to small sample size) was the collection of five Atlantic sturgeons (*Acipenser oxyrhincus*) in May 2005, not previously collected by this research study.

Sessile invertebrate catches in 2005 were observed in similar low abundance as finfish catches. Most sessile invertebrates collected were classified into one of six groupings, which were observed at levels two to 52 times lower in 2005 than in 2004 (Table 5).

Changes in elasmobranch abundances were mixed between years. Dasytid stingrays and smooth butterfly rays (*Gymnura micrura*) were seen in slightly lower abundances in 2005

than in 2004. Conversely, bullnose rays (*Myliobatis fremenvilli*) abundances were five times greater in 2005 than in 2004. Most elasmobranch species were seen in comparable numbers between years, or sample sizes were too small to discern trends.

Blue crabs (*Callinectes sapidus*) and horseshoe crabs (*Limulus polyphemus*), two important prey items of commercial interest, were seen with substantially reduced abundances in 2005 than in 2004. In both years, similar abundance trends between May and August were observed for both species (Figures 6, 7). Seasonal declines in both blue and horseshoe crab frequency and abundance paralleled turtle catch-per-unit effort.

Other invertebrate species of interest, as potential forage items for loggerheads, also declined precipitously between 2005 and 2004 (Table 5). Portunid and spider crabs (*Libinia* sp.) catches were 14 and 20 times lower in 2005, respectively. Fewer stone crabs (*Mennipe mercenaria*) and whelks (*Busycon* sp.) were collected in 2005, but overall abundances in both years were too small to discuss trends.

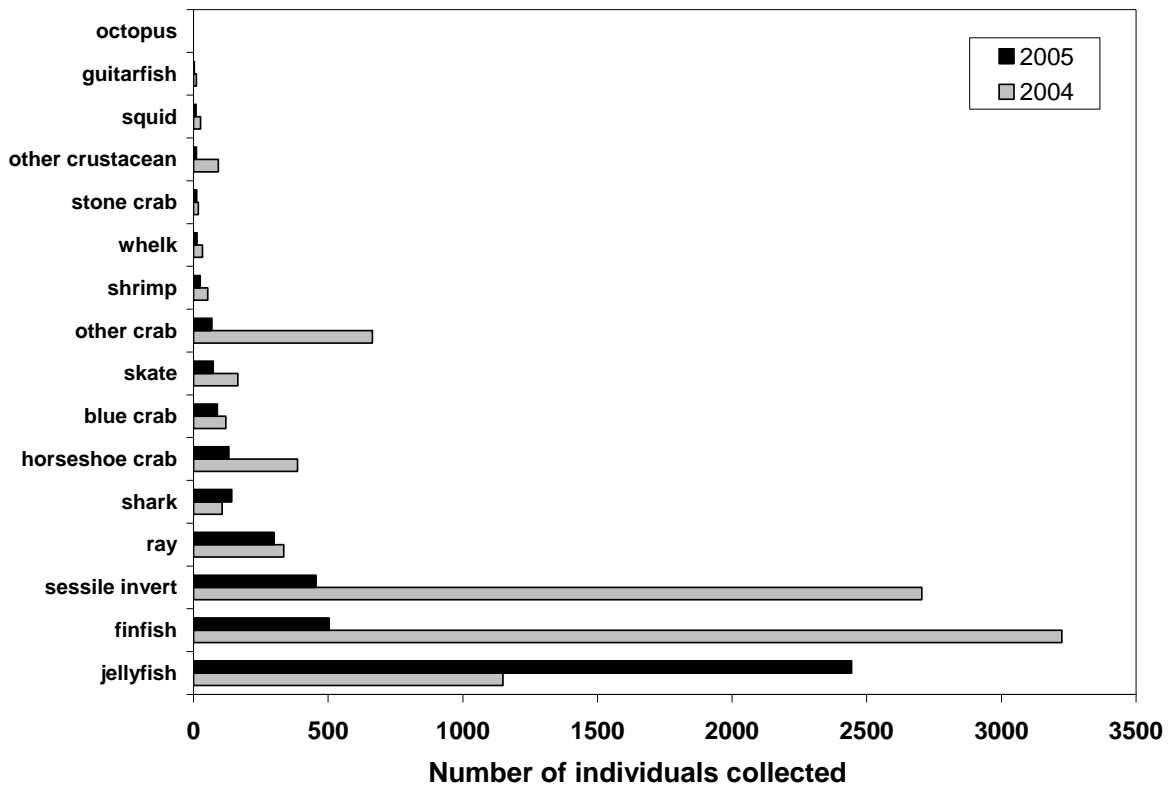


Figure 5. Relative abundance of by-catch (by groupings) collected in 2004-2005.

Table 5. Relative change between abundance of important by-catch groupings between years at index trawl stations in the Charleston harbor shipping entrance channel.

Category	Species or Grouping	2004	2005	Order of Change
Jellyfish	<i>Stomolophus meleagris</i>	336	105	-3.2
Jellyfish	Box Jellies and Sea Wasps	261	2320	8.9
Jellyfish	Lion's Mane and Sea Nettles	578	105	-5.5
Jellyfish	Moon Jellyfish	0	5	comparable
Finfish	<i>Peprilus triacanthus</i>	1040	56	-19
Finfish	<i>Larimus fasciatus</i>	387	77	-5
Finfish	<i>Anchoa</i> sp.	266	23	-12
Finfish	<i>Chaetodipterus faber</i>	205	4	-51
Sessile Inverts	Sea Porks & Other Tunicates	1827	133	-14
Sessile Inverts	Alcyonidum & Other Bryozoan	391	128	-3
Sessile Inverts	Sponges	112	31	-4
Sessile Inverts	Soft Corals (<i>Leptogoria</i>)	52	0	-52
Sessile Inverts	Sea Cucumbers	168	69	-2
Sessile Inverts	Urchins	92	13	-7
Rays	<i>Dasyatis</i> sp.	182	133	-1.4
Rays	<i>Gymnura micrura</i>	112	83	-1.3
Rays	<i>Myliobatis freminvillei</i>	9	46	5.1
Rays	<i>Rhinoptera bonasus</i>	31	37	comparable
Sharks	<i>Carcharhinus acronotus</i>	5	7	comparable
Sharks	<i>Carcharhinus isodon</i>	1	0	comparable
Sharks	<i>Carcharhinus plumbeus</i>	0	15	15
Sharks	<i>Ginglymostoma cirratum</i>	0	1	comparable
Sharks	<i>Mustelus canis</i>	2	13	6.5
Sharks	<i>Rhizoprionodon terranovae</i>	26	13	-2
Sharks	<i>Sphyrna lewini</i>	57	76	1.3
Sharks	<i>Sphyrna tiburo</i>	15	17	comparable
Other Inverts of Interest	Horseshoe crabs	386	131	-2.9
Other Inverts of Interest	<i>Callinectes sapidus</i>	120	88	-1.4
Other Inverts of Interest	Other Portunid Crabs	493	25	-19.7
Other Inverts of Interest	<i>Libinia</i> sp.	169	12	-14.1
Other Inverts of Interest	<i>Mennipe mercenaria</i>	18	12	comparable
Other Inverts of Interest	<i>Squilla</i> sp.	92	8	-11.5
Other Inverts of Interest	<i>Buscycon</i> sp.	33	13	-2.5

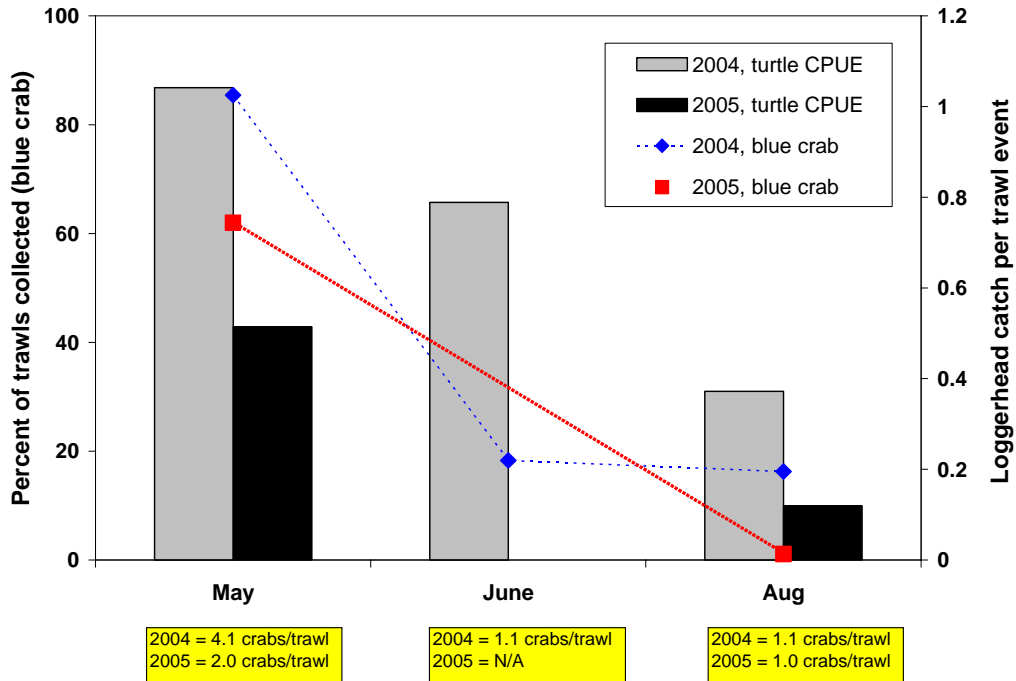


Figure 6. Seasonal declines in blue crabs and turtles, 2004-2005.

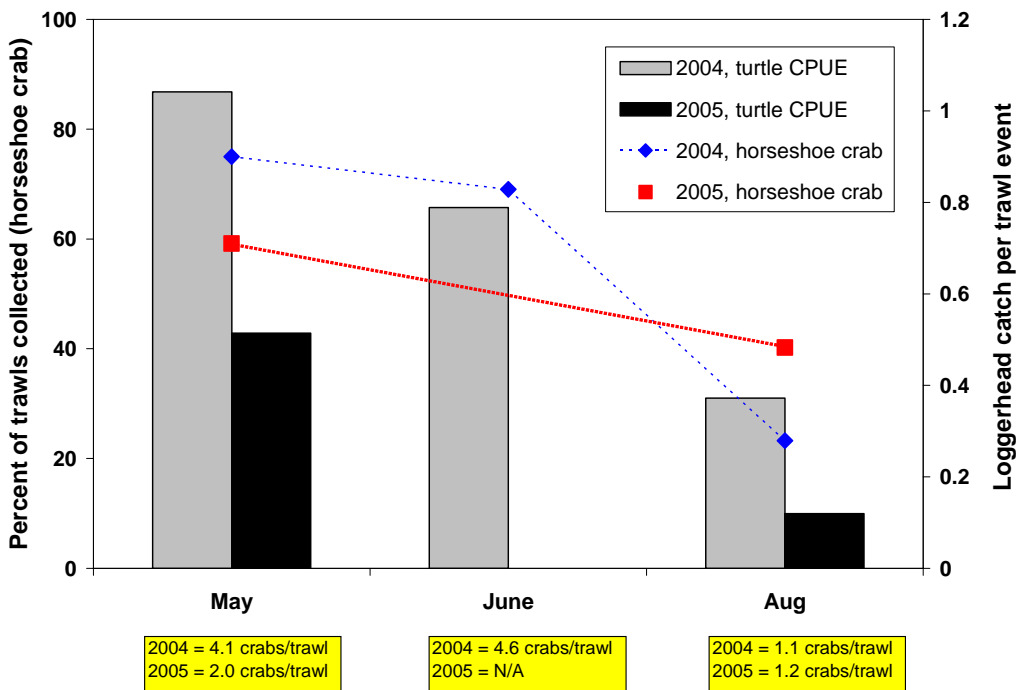


Figure 7. Seasonal declines in horseshoe crabs and turtles, 2004-2005.

Satellite Telemetry

Twelve juvenile loggerheads were tagged and released with satellite transmitters in 2005 in two groups. Six loggerheads (56.6 to 72.9 cm SCLmin; mean = 62.8 cm) were released in May and six loggerheads (59.8 to 73.4 cm SCLmin; mean = 66.3 cm) were released in August. During the spring and summer, with few exceptions, each of these loggerheads was detected daily; however, between mid-November and mid-March, periodic absence of detection for 1-2 days at a time was not uncommon for some loggerheads (Figure 8). Between 11 May 2005 and 30 May 2006, total daily detections (all transmitters combined) ranged from 5 to 152 per day for this group of loggerheads. Although detections were frequent and sometimes abundant, “good” detections (location classes 1, 2 and 3) were infrequently observed for many loggerheads (Table 6).

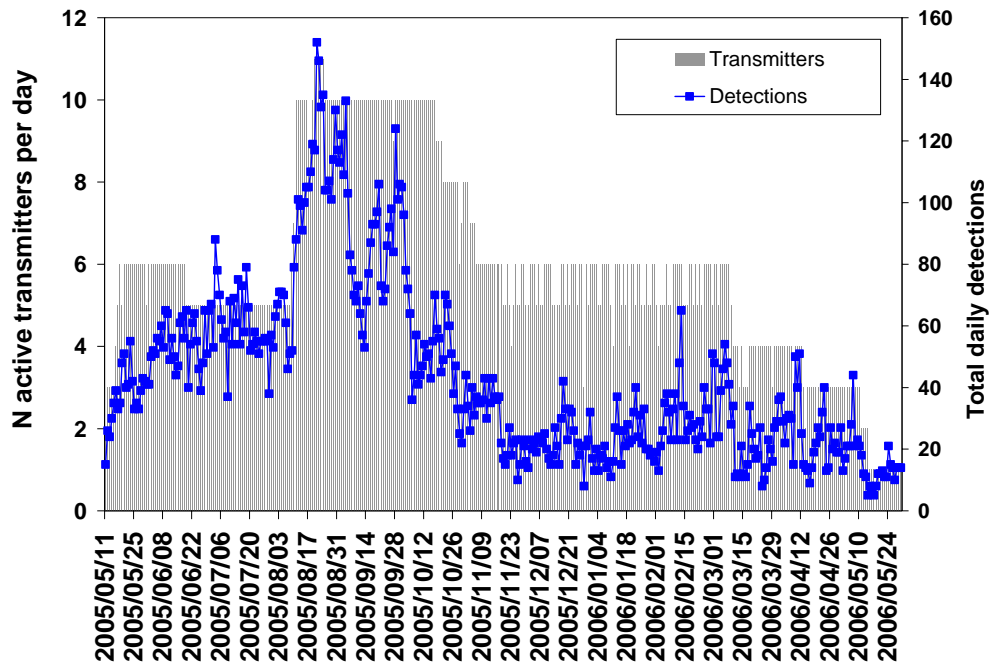


Figure 8. Daily data collection for satellite-tagged loggerheads released in 2005.

Table 6. Summary of location class distribution for loggerhead detection events. “DAL” stands for days at large.

Release	tag_id	SCLmin	N detect	DAL	"Good"					"Bad"					Sensor Only	% Sensor
					3	2	1	% Good	0	A	B	Z	% Bad			
May	57683	57.7	1732	156	12	47	64	7.1	38	195	544	27	46.4	805	46.5	
May	57684	56.6	219	33	2	10	19	14.2	20	35	61	7	56.2	65	29.7	
May	57685	72.9	2499	380	44	62	90	7.8	77	171	616	33	35.9	1406	56.3	
May	57686	68.5	1034	106	1	2		0.3	2	28	240	3	26.4	758	73.3	
May	57687	62.3	2362	301	78	189	171	18.5	112	231	643	48	43.8	890	37.7	
May	57688	59.3	1721	160	10	20	25	3.2	39	142	597	26	46.7	862	50.1	
August	58939	62	864	84	2	3	8	1.5	6	44	248	12	35.9	541	62.6	
August	58940	70	1172	211	34	49	74	13.4	107	124	322	39	50.5	423	36.1	
August	58941	68.3	1503	247	50	98	123	18.0	147	150	348	41	45.6	546	36.3	
August	58942	59.8	1529	278	101	119	136	23.3	127	151	399	51	47.6	445	29.1	
August	58943	64.5	1494	273	74	96	133	20.3	126	174	414	47	50.9	430	28.8	
August	58944	73.4	567	78	7	8	31	8.1	32	99	206	25	63.8	159	28.0	

Mean

11.3

45.8

42.9

Transmitters for four of six loggerheads released in May expired considerably earlier than expected, based on longevity for five loggerheads satellite-tagged in 2004. Mean transmitter life for these loggerheads (ID57683; ID57684; ID57686; ID57688) was 114 d (range = 33 to 160 d; Table 6). All four of these loggerheads remained in SC coastal waters for the entire monitoring period; however, distributional patterns were varied and somewhat different with respect to 2004.

ID57684, a 56.6 cm SCLmin “clean” (i.e., virtually no barnacle load with a mahogany-colored carapace) juvenile loggerhead, was caught at station “D1” on 17 May 2005. This turtle immediately departed the Charleston harbor shipping entrance channel and slowly traveled close to and parallel to the shoreline for two weeks (Figure 9), stopping briefly near several landmarks (Bulls Bay; Cape Romain; Winyah Bay). Approximately 20km north of Winyah Bay, SC, this turtle changed course and headed in a more easterly direction for approximately 75km. Residence at this general location was maintained for the next three weeks, prior to transmitter failure for unknown reasons on 18 June.

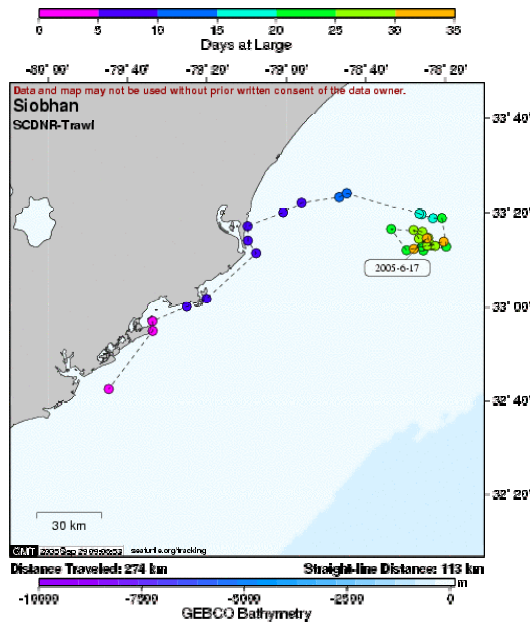


Figure 9. Short-term distributional pattern of a satellite tagged loggerhead (ID57684) from 17 May to 18 June 2005.

ID57683, a 57.7 cm SCLmin, “clean” (i.e., virtually no barnacle load with a mahogany-colored carapace) juvenile loggerhead, was caught at station “B3” on 18 May 2005. Similar to ID57684, this loggerhead also immediately departed the Charleston harbor shipping entrance channel and headed northeast parallel to shore and past the same landmarks; however, ID57683 made this journey further offshore than ID57684. During the first six weeks of monitoring, ID57683 traveled from Charleston, SC, to nearly 60km north of Winyah Bay, SC, before returning south (as far as Bulls Bay, SC) and then ultimately north to Winyah Bay, SC, again (Figure 10a). For the next four months, this turtle was generally resident offshore and within 20km of Winyah Bay, SC; however, on at least occasions, this turtle may have actually entered Winyah Bay, SC (Figure 10b).

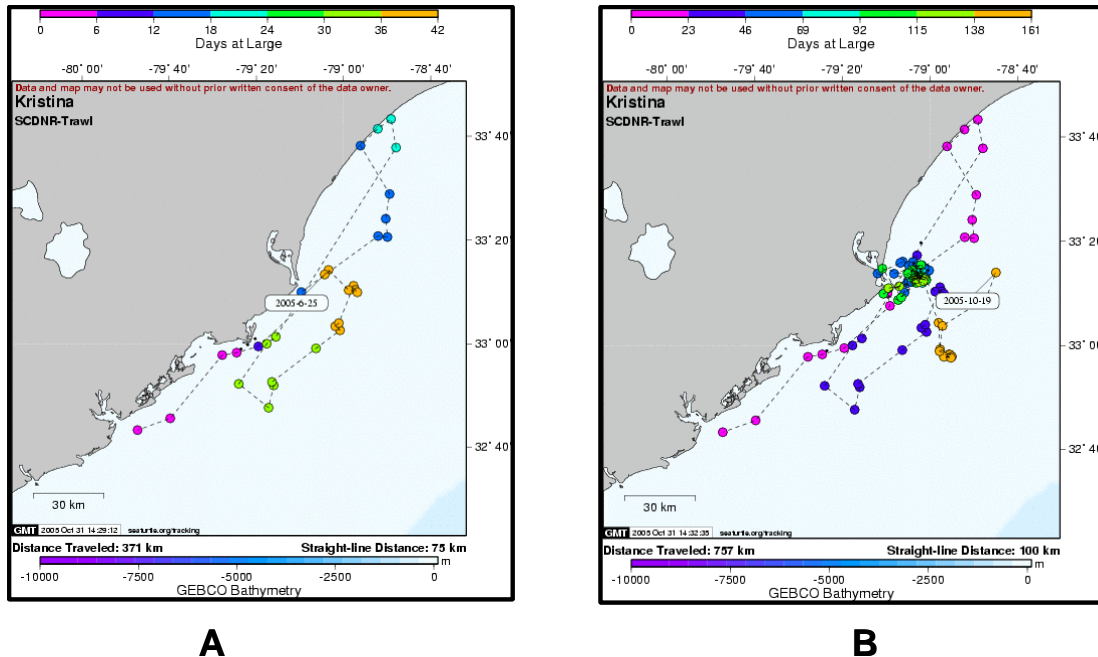


Figure 10. Seasonal distributional pattern of a satellite tagged loggerhead (ID57683) from 18 May to 20 October 2005. This loggerhead meandered back and forth parallel to the coastline between Charleston and Myrtle Beach, SC, for six weeks following tag and release (A), prior to establishing residence in the nearshore waters adjacent to Winyah Bay, SC, for the next four months (B).

ID57686, a 68.5 cm SCLmin juvenile loggerhead, was caught at station “D3” on 11 May 2005. This loggerhead was originally tagged and released near Charleston, SC, on 15 July 2001 during the fishery-dependent phase of the 2000-2003 regional in-water turtle survey (Maier et al., 2004). This turtle was subsequently detected near Charleston daily during 106 d of satellite telemetry monitoring; however, only three “good” location class detections were recorded. The first “good” location was in the entrance channel shortly after tag and release. The second “good” location was recorded in the Stono River Inlet on 31 May 2005 (20 d post-release). The third “good” location was recorded approximately 5km offshore of Sullivan’s Island, SC, on 7 July 2005 (58 d post-release). The transmitter for this turtle prematurely ceased on 24 August 2005.

ID57688, a 59.3 cm SCLmin juvenile loggerhead, was caught at station “B3” on 12 May 2005. This turtle departed the Charleston harbor entrance channel soon after tag-and-release and headed northeast to Bull Island, SC, where it remained for up to one week (Figure 11a). During the next two weeks, this turtle returned to and resided in the nearshore coastal waters within 15 km of Charleston. From late-June through August, this turtle remained resident in coastal waters offshore of Charleston (Folly Beach/Kiawah Island); however, this turtle was located nearly twice as far offshore as observed during May-June (Figure 11b). In early September, this turtle moved inshore again, even returning to the harbor entrance channel. No good detections were reported after 21 September 2005; however, this turtle was detected daily for the next 26 d prior to premature transmitter failure on 17 October 2005.

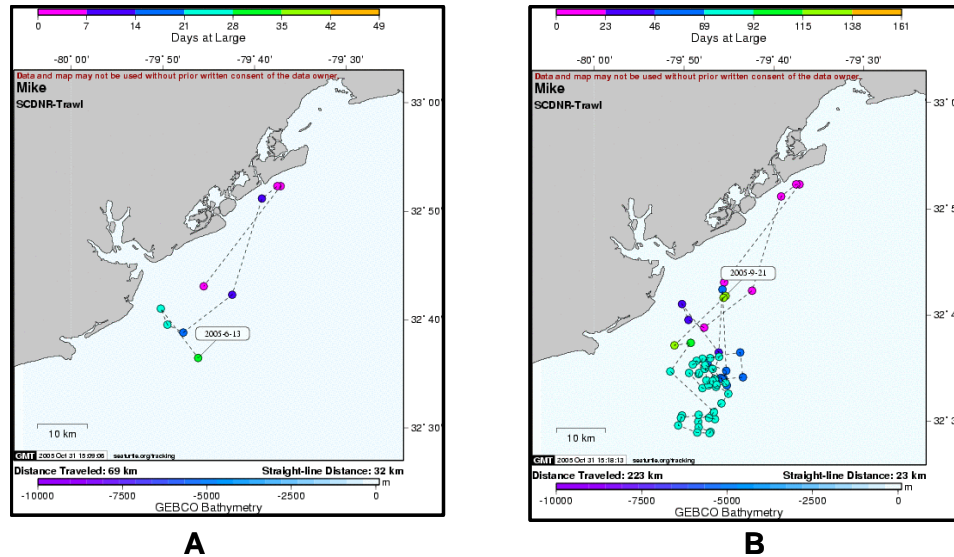


Figure 11. Seasonal distributional patterns of satellite tagged loggerhead (ID57688) from 12 May to 17 October 2005. This loggerhead remained offshore and to the south of Charleston for all but one week of monitoring, spending approximately half of its time within 15 km of shore (A) and half of its time in waters as far as 40 km offshore (B).

Over-wintering distributions were able to be documented for two of six loggerheads released in May 2005.

ID56787, a 62.3 cm SCLmin juvenile loggerhead, was caught and released at station “B3” in the morning on 12 May 2005. “Good” detections comprised less than 10% of total monthly detections (125 to 372 detections per month) through November 2005; however, between December 2005 and March 2006, “good” detections comprised 39-61% of monthly detections (104 to 372 detections per month). Between mid-May and mid-November 2005, good detections for this turtle were clustered 20-40 km off the coast between the Isle of Palms, SC, and Bull Island, SC (Figure 12a). Between December 2005 and mid-February 2006, this turtle traveled steadily northeast, 80-100km offshore, until entering the Gulf Stream at a location ~100km south and 50km east of Cape Fear, NC (Figure 12b). Detections continued daily until 8 March 2006, at which point the turtle was located approximately 1200 km off the coast of Virginia.

ID56785, a 72.9 cm SCLmin juvenile loggerhead, was caught and released at station “A3” in the morning on 16 May 2005. This turtle generally remained within 20km offshore of Kiawah Island, SC, and the Isle of Palms, SC, through mid-November 2005. Between mid-November 2005 and mid-March 2006, this turtle was approximately 70km offshore of Kiawah and Folly Islands (Figure 13). During April this turtle was briefly located in nearshore and estuarine waters near Edisto Island, before ultimately returning to the nearshore waters off where it was tagged and released a year earlier. The last good detection for this turtle on 5/15/06 placed the animal in the near-shore waters off of Capers Island, SC; however, this turtle was tracked until 5/30/06.

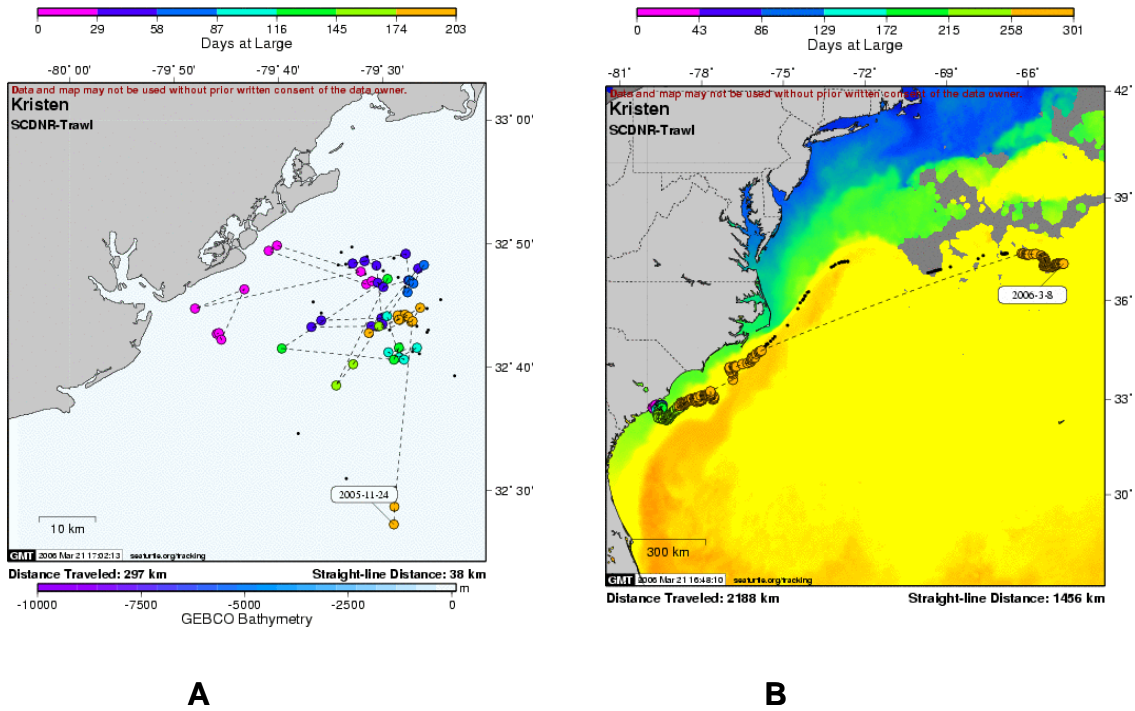


Figure 12. Seasonal distributional pattern of satellite tagged loggerhead (ID57687) from 12 May 2005 to 8 March 2006. Following localized distribution (A) and overwintering on the outer continental shelf, this turtle entered the Gulf Stream in late Feb 2006 (B).

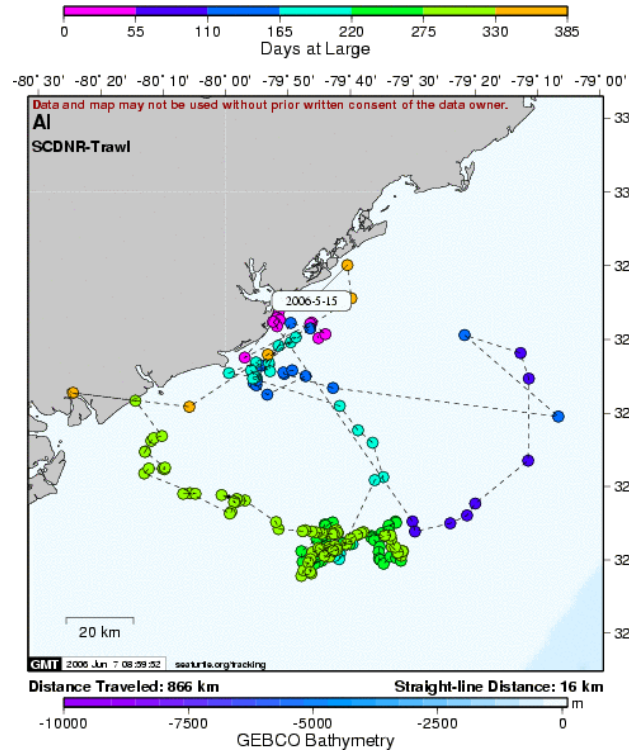


Figure 13. Seasonal distributional patterns of satellite tagged loggerhead (ID57685) between 16 May 2005 and 30 May 2006.

Transmitters for two of six loggerheads released in August expired considerably earlier than expected, based on transmitter longevity for five loggerheads satellite-tagged in 2004. Transmitter lives for these two loggerheads (ID58939; ID58944) were 84 d and 79 d, respectively, comparable to transmitter life for ID52600 which was also only monitored from August to November in 2004.

ID58939, a 62.0 cm SCLmin juvenile loggerhead, was caught and released at station “D3” in the morning on 11 August 2005. Although this turtle was detected daily, “good” detection events for this turtle were rare, and only totaled 13. Of these 13 “good” detections, all were located south of the Charleston harbor entrance channel, within 10 km offshore of Folly and Morris Islands (Figure 14). On several occasions, this turtle came very close to shore, and may be the same turtle as one sighted at the Folly Beach Fishing Pier in late August/early September, which appeared to be satellite tagged; however, this could not be confirmed (M. Arendt, personal observation).

ID58944, a 73.4 cm SCLmin juvenile loggerhead, was caught at station “B3” on 18 May 2005 and immediately sent to the SC Aquarium for rehabilitation due to lethargy and an overall emaciated appearance (Figure 15a). Renamed “Jetty” once at the aquarium, the turtle's condition and health improved rapidly (Figure 15b), which was attributed to treating his/her debilitated condition in the relatively early stages of debilitation process. To explore the possibility that debilitated condition may be associated with overwintering residence, “Jetty” was released with a satellite transmitter from Seabrook Island, SC, on 19 August 2005.

Immediately upon release, “Jetty” initiated a unique rapid and northeasterly movement which was sustained for three weeks (Figure 16a). Unlike turtles ID57683 and ID57684, which appeared to pause upon encountering landmarks such as Bulls Bay, Cape Romain and Winyah Bay, “Jetty” continued past these topographical features until reaching the coastal waters offshore of Topsail Island, NC. As such, “Jetty” was the first loggerhead in this project to travel, during the summer, substantially north of the northern sampling limit, as trawled during the 2000-2003 regional survey. “Jetty” remained highly resident offshore from New River Inlet, NC, for the two months until premature transmitter failure on 5 November 2005 (Figure 16b). “Jetty” was generally detected daily, but with sporadic occurrence of “good” detections, similar to ‘resident’ turtles in SC waters.

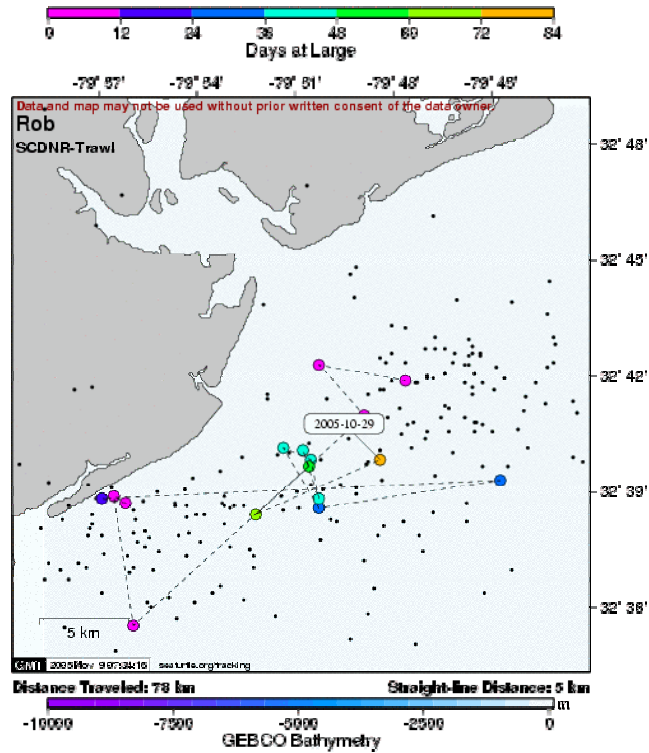


Figure 14. Summer/fall distributional pattern of a satellite tagged loggerhead (ID58939) from 11 August to 2 November 2005. This loggerhead was detected daily, although “good” detections were infrequent. All “good” detections were located in coastal waters adjacent to and within 10 km of Folly and Morris Islands.



A



B

Figure 15. “Jetty” was lethargic and emaciated (A) when collected on 18 May 2005. Collection in the early stages of debilitated turtle syndrome (DBS) contributed to a rapid rehabilitation at the SC Aquarium, allowing this turtle to be released approximately three months later (B). Photos courtesy of Kelly Thorvalson, South Carolina Aquarium.

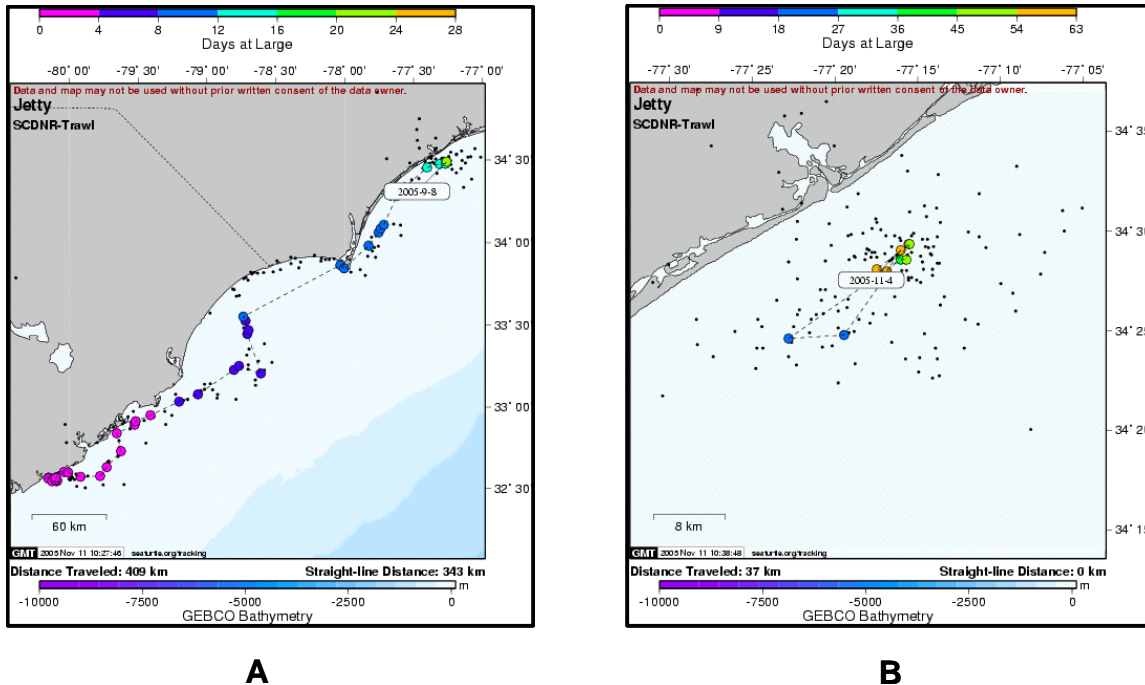


Figure 16. Summer/fall distributional pattern of satellite tagged loggerhead (ID58944) from 19 August to 5 November 2005. Following rehabilitation and release, “Jetty” swam in a northeasterly direction for three weeks until reaching Topsail Island, NC (A). For the next two months, “Jetty” was generally detected daily, with all “good” detections located within 10 km of shore in the vicinity of New River Inlet, NC.

Over-wintering distributions were able to be documented for four of six loggerheads satellite tagged in August 2005.

ID58940, a 70.0cm SCLmin juvenile loggerhead, was collected at station “A3” in the morning of 11 August 2005. Between mid-August and the end of November, this turtle resided 10-50km offshore of the Isle of Palms to Cape Romain (Figure 17). In early December 2005, this turtle moved to the outer continental shelf, approximately 80km offshore. Between mid-December 2005 and the end of February 2006, this turtle completed a “loop” on the middle to outer continental shelf offshore of the Grand Strand, SC, area. In early March 2006, this turtle moved further offshore, over the Florida-Hatteras Slope, perhaps as a result of entry into the Gulf Stream.

ID58941, a 68.3 cm SCLmin juvenile loggerhead, was collected at station “A1” in the afternoon of 9 August 2005. “Good” detections, though rare, were located between Folly Island and the Isle of Palms and within 20km of shore through November 2005 (Figure 18). In December 2005, this turtle was regularly located in an area approximately 40km offshore between the Isle of Palms and Bull’s Bay, SC. Between January and mid-April 2006, this turtle traversed a large area on the middle continental shelf (centered on Winyah Bay, SC) within 50-80km of shore.

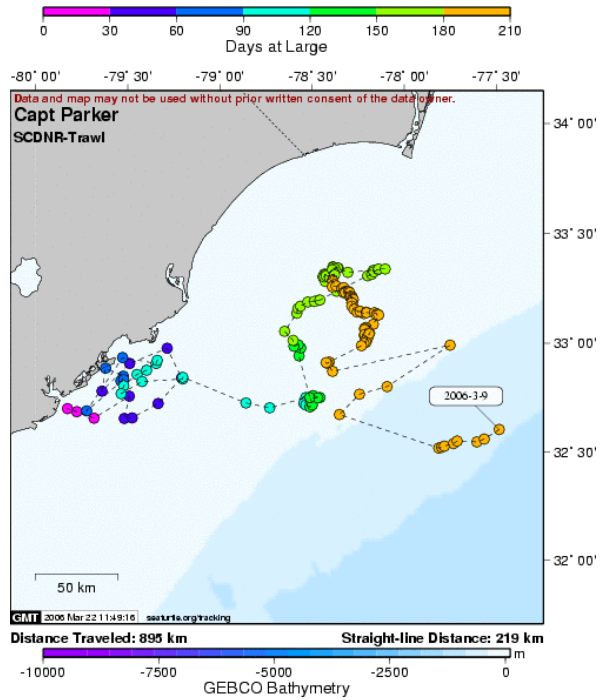


Figure 17. Seasonal distributional patterns of satellite tagged loggerhead (ID58940) from 11 August 2005 to 9 March 2006.

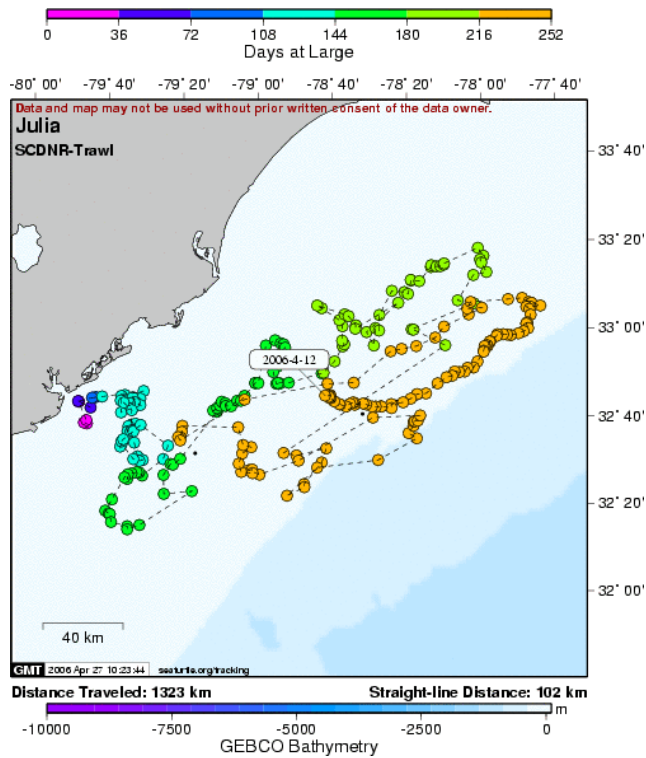


Figure 18. Seasonal distributional patterns of satellite tagged loggerhead (ID58941) from 9 August 2005 to 12 April 2006.

ID58942, a 59.8 cm SCLmin juvenile loggerhead, was collected at station “D1” in the morning of 10 August 2005. Prior to November 2005, “good” detections were rare for this turtle; however, all good detections were located within 25km of the coast between the Isle of Palms and Folly Island or in the vicinity of the Charleston harbor shipping entrance channel (Figure 19). During December 2005, this turtle traveled a linear distance of approximately 240km to the southwest, on a course that more or less paralleled the coast. Upon reaching a location approximately 30km east of Sapelo Island, GA, this turtle headed due east. During January and February 2006, this turtle remained more or less off the coast of and within 100km of Sapelo Island. In early March 2006, this turtle began moving to the north, then turned northeast upon reaching a point approximately due east and 30km offshore of the Savannah River inlet. By mid-April 2006, this turtle had returned to the areas off of Charleston previously occupied in summer/fall 2005.

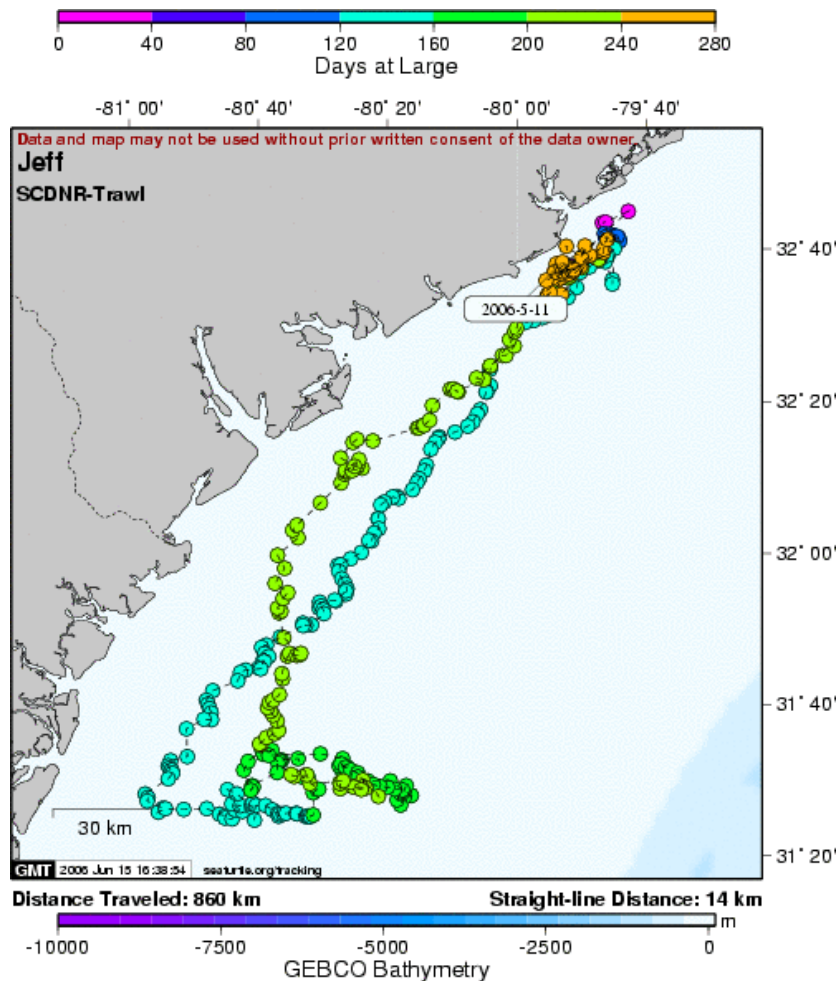


Figure 19. Seasonal distributional patterns for loggerhead ID58942 between 10 August 2005 and 14 May 2006. This turtle over-wintered on the middle to outer continental shelf off of northern GA, similar to loggerhead 49122 during Dec-March 2005.

ID58943, a 64.5 cm SCLmin juvenile loggerhead, was collected at station “D3” in the morning of 11 August 2005. Between mid-August and early December 2005, this turtle remained localized in an area approximately 25km southeast of Folly and Kiawah Islands, returning to the channel (based on “good” detections) only once (Figure 20). During December 2005, this turtle traveled as far south as an area approximately 120 km offshore of Ossabaw Island, GA; however, by the end of December this turtle had returned north to an area approximately 80km offshore of Fripp Island, SC. Between January and early March 2006, this turtle trekked steadily to the northeast, more or less parallel to the coast, until reaching a point east (and within 50km) of Winyah Bay, SC. During the last three weeks of March, this turtle made a rapid and directed movement from Winyah Bay towards Charleston; however, this turtle was not detected in the vicinity of the Charleston shipping entrance channel until early May.

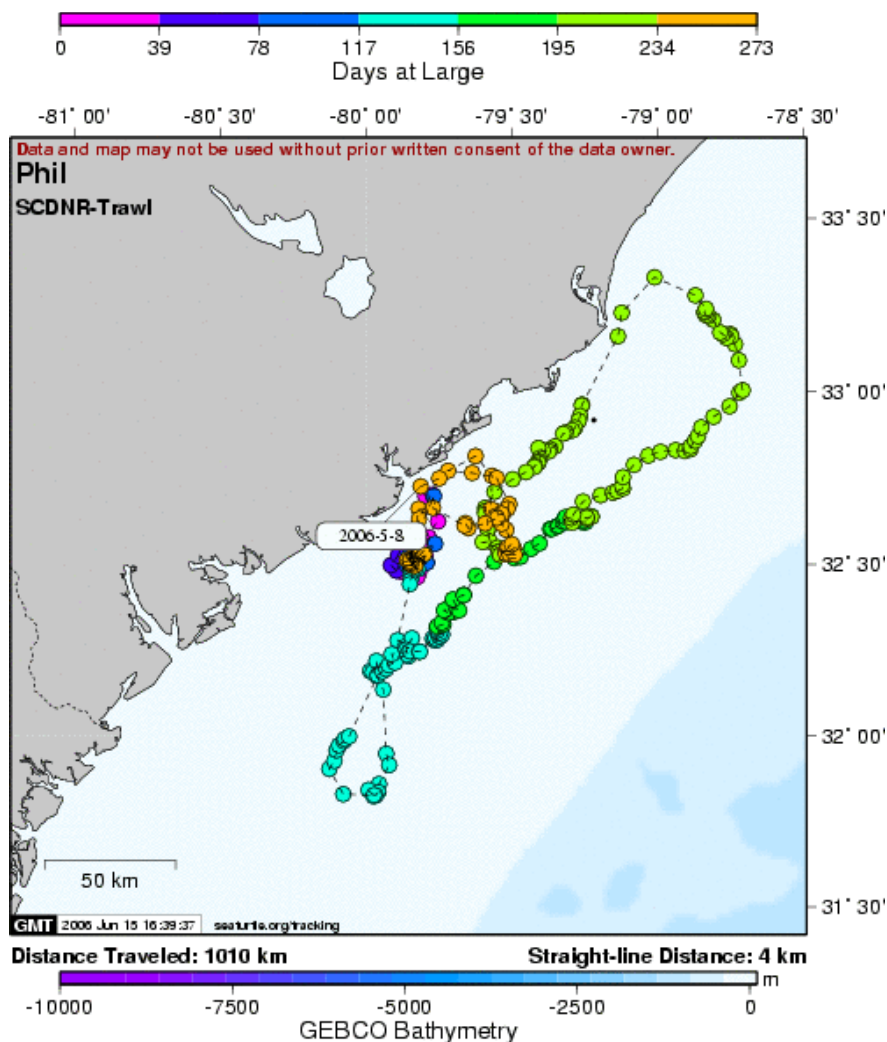


Figure 20. Seasonal distributional patterns for loggerhead ID58943 between 11 August 2005 and 10 May 2006. This turtle over-wintered primarily on the middle continental shelf off of SC, but traveled extensively between December 2005 and March 2006.

Seasonal water temperature exposure patterns were remarkably similar for satellite tagged loggerheads released in 2004 and 2005 (Figure 21), despite a slightly northern ‘shift’ in continental shelf over-wintering locations between December 2005 and March 2006. In 2004 and again in 2005, loggerheads began experiencing mean daily water temperatures of 20°C in early November, with movement away from the Charleston area generally occurring one to two months later. Typically, during January and March 2005 and 2006, loggerheads remained in waters 16-18°C; however, extended exposure to water temperatures <15°C was not uncommon. By mid- to late-April 2005 and 2006, when water temperature had reached 20°C, loggerheads had moved back to areas on the inner continental shelf that they had occupied the previous summer/fall at mean daily water temperatures approaching 30°C.

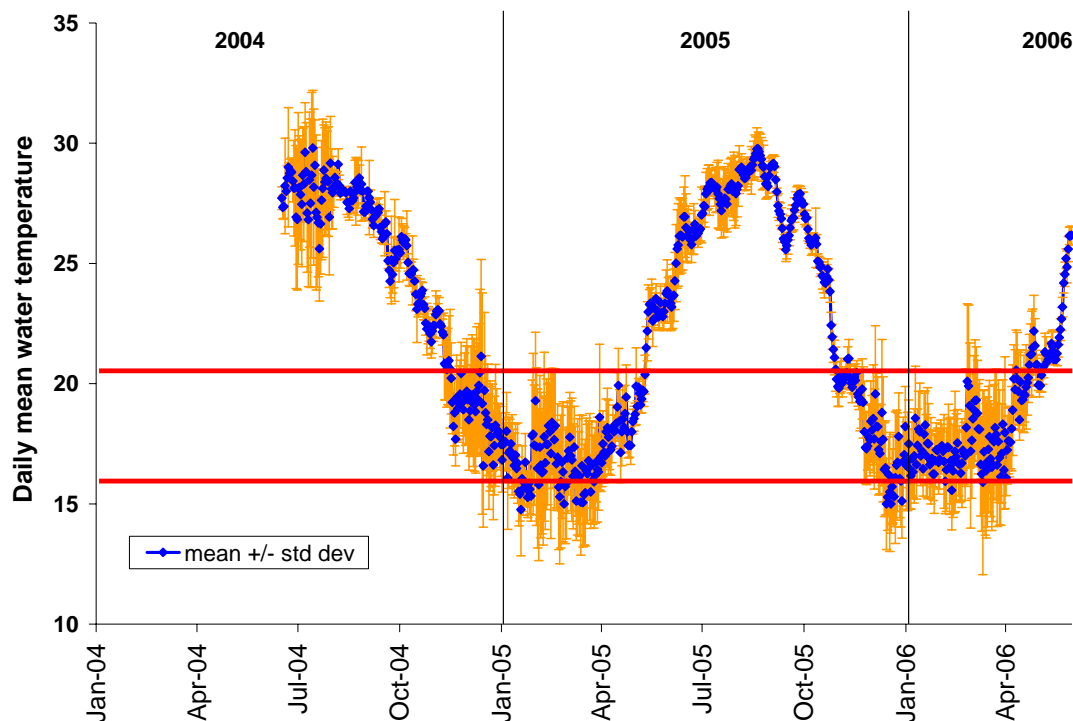


Figure 21. Seasonal water temperatures experienced by satellite-tagged loggerheads.

Seasonal submergence patterns were also remarkably similar for satellite-tagged loggerheads released in 2004 and 2005 (Figure 22). Between June and November 2004, and again between May and November 2005, mean time spent at the surface was less than 10% of each month. December 2004 and 2005 represented transitional periods, with an average of 16-23% of the month spent at the surface, respectively. With the exception of January 2006, loggerheads spent an average of 27-36% of their time at the surface between January and April in 2005 and 2006.

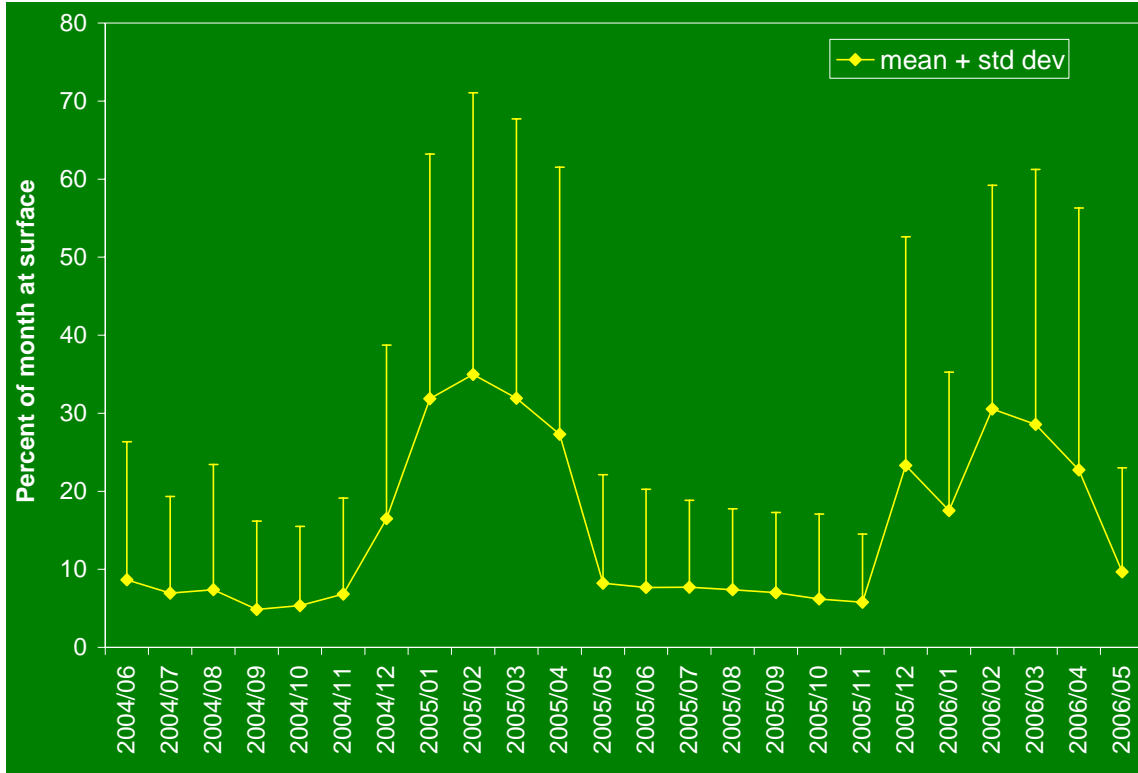


Figure 22. Seasonal submergence patterns among satellite-tagged loggerheads

Discussion

Seasonal decline in loggerhead abundance in the Charleston harbor shipping entrance channel between May and August was possibly a reflection of prey availability as opposed to seasonal changes in water temperature, given that this pattern persisted in both 2004 and 2005 independent of water temperature at the time of sampling. Decline in loggerhead abundance in the Charleston harbor shipping entrance channel between May and August is contrary to findings from two trawling studies conducted in this channel during the early 1990’s (VanDolah and Maier, 1993; Dickerson et al., 1995). VanDolah and Maier (1993) reported peak catch rates (turtles/trawl) in July 1991, which was attributed to maximum annual water temperature. Between May and July 1992, Dickerson et al. (1995) reported stable catch rates (0.17 to 0.22 turtles per station), with no appreciable monthly differences as reported by VanDolah and Maier (1993).

Loggerhead catch rates in both years were highly variable among and within sampling stations, including highly productive stations such as “D3” and “B3”; thus, illustrating the importance of determining catch-per-unit-effort rates based on rigorous sampling. Although the data collected by VanDolah and Maier (1993) and Dickerson et al. (1995) enable historical comparisons of catch-unit-effort for the current investigation, two study design issues warrant caution when comparing 2004-2005 results with these earlier studies. First, depending on year and month, each of the seven primary sampling stations was sampled 5 to 13 times during each two week sampling block, all during daylight. Although the stations in this study were essentially the same as those sampled by

VanDolah and Maier (1993), VanDolah and Maier (1993) only sampled each station once per month during the day; thus, the catch rates of VanDolah and Maier (1993) were based on minimal sampling replication at stations with highly variable catch rates. Second, although Dickerson et al. (1995) conducted comparable sampling effort (7-10 times per month, presumably during daylight only) as the current study, Dickerson et al. (1995) sampled fewer ($n=3$) and considerably longer (3km vs. 1.5 km) stations. Furthermore, to avoid unspecified “edge effects”, only the middle of the channel was surveyed by Dickerson et al. (1995). In the current study, middle channel stations (i.e., “B2”, “D2” and “E2”) were not surveyed due to bottom obstructions which impeded trawling. Although both edges of the channel were sampled, catch rates were substantially greater on the southern side of the channel, supporting the supposition of Dickerson et al. (1995) that edge effects may affect catch rates, perhaps due to differential habitats along the edge of the channel were vertical profiles persist due to channel dredging.

Overall, loggerhead catch rates in the Charleston Harbor shipping entrance channel were about one-third lower in 2005 than in 2004; however, catch rates in 2005 were comparable to catch rates observed during trawling efforts during 2000-2003. Catch rates at this sampling location remain high with respect to levels reported during the early 1990’s. During May, June and August 1991, VanDolah and Maier (1993) only collected nine loggerheads in 48 trawling events (0.19 loggerheads/event) in the Charleston Harbor shipping entrance channel, with half of these trawling events conducted at night when slightly greater catch rates than during the day were observed. Similarly, Dickerson et al. (1995) only caught 11 loggerheads during 56 trawling events (0.2 loggerheads/event) in the Charleston Harbor shipping entrance channel during May and June 1992.

Low recapture rates ($n=4$ of 162; 2%) in the current research study are consistent with high catch rates, particularly given the relatively high re-capture rates for turtles collected at this location in the early 1990’s (VanDolah and Maier 1993; Dickerson et al., 1995). Three loggerheads tagged and released in 2004 were recaptured during sampling efforts in 2004, and a fourth loggerhead tagged in 2004 was recaptured during sampling in 2005. Two loggerheads tagged prior to the current research study (both of which were tagged by the SCDNR in 2001 during the previous in-water research study) have also been recaptured during sampling in the shipping entrance channel. Conversely, 13% ($n=7$ of 53 loggerheads) and 9% ($n=4$ of 45 loggerheads) tagged by VanDolah and Maier (1993) and Dickerson et al. (1995), respectively, were recaptured during those studies.

Loggerhead length-frequency distributions in 2004 and 2005 differed from distributions reported for the Charleston harbor shipping entrance channel by VanDolah and Maier (1993) and Dickerson et al. (1995); however, it is unknown what affect, if any, sample size may have contributed to these differences in length-frequency distributions. Loggerheads >80 cm SCLmin represented 17% and 16% of turtles caught in VanDolah and Maier (1993) and the current investigation, respectively; however, loggerheads >80 cm SCLmin only represented 8% of the catch of Dickerson et al. (1995). Loggerheads <60 cm SCLmin accounted for 16% of the catch observed in the current investigation, 30% of the catch of VanDolah and Maier (1993) and 44% of the catch of Dickerson et al. (1995). Only two of 79 combined individual loggerheads collected by VanDolah and

Maier (1993) and Dickerson et al. (1995) were <50 cm SCLmin and none of the 162 individual loggerheads collected during the current investigation were <50 cm SCLmin. Loggerheads <50 cm SCLmin were also rarely observed during the 2000-2003 regional survey (Maier et al., 2004), perhaps because most turtles <50 cm SCLmin have not entered the benthic foraging stage of their life cycle, and are generally located elsewhere (i.e., eastern north Atlantic Ocean; Bjorndal et al., 1994).

Satellite telemetry data collected to date suggest that juvenile loggerheads collected in coastal waters off of Charleston, SC, remain fairly localized within these waters through most of the year (late April to late November), as opposed to undertaking long-distance migrations during these months, as reported for adult female loggerheads collected on nearby SC (SCDNR) and GA (NMFS, GADNR) nesting beaches. During these months, loggerheads may remain highly localized for extended periods, particularly at offshore locations where patchy live-bottom reefs are common, but movement among multiple locations within 10-20 km of the coastline is also common. Given these observations, low recapture rates during the 2000-2003 regional survey are to be expected. Although loggerheads have distinctly different over-wintering areas from December – March than the areas which they occupied between April and November, satellite telemetry data collected for these loggerheads suggests that there is a strong affinity to return to the same waters each spring after over-wintering. These data support the assertion of Day (2003) that loggerheads may exhibit strong site-fidelity to foraging areas, based on mercury contamination values for loggerheads located near the inlets of major industrial harbors vs. further offshore. Satellite data transmission for three loggerheads which either entered or appeared to enter the Gulf Stream and a fourth loggerhead that traveled to northern Florida was not of sufficient duration to determine if these turtles eventually returned to the coastal waters off of Charleston.

In addition to bioaccumulation of ingested contaminants, strong site fidelity and affinity to areas with heavy commercial and recreational vessel traffic may also pose health risks. Twenty-three percent of loggerheads collected in 2004 and 26% of loggerheads collected in 2005 exhibited injuries associated with boat strikes, entanglement in fishing gears, and/or shark bite wounds. During the 2000-2003 regional survey, only 5-13% of loggerheads collected exhibited such wounds (Maier et al., 2004), with highest propensity for such injuries among loggerheads collected near shipping channels. Frequency of observation of physical trauma to free-swimming loggerheads illustrates the dangers that sea turtles experience in the wild, but also underscores their hardiness with respect to conservation efforts to restore sea turtle populations to historical levels of abundance. Frequent observation of physical trauma to free-swimming sea turtles also suggests that such injuries, with respect to stranded turtles found on beaches, may not necessarily have occurred post-mortem.

Continued sampling in the Charleston harbor shipping entrance channel in May 2006 will enhance interpretation of May 2004-2005 results. Climatology and inter-annual variability present unique challenges when attempting to study the ecology and distributional patterns of marine species; thus, multi-year studies are desirable. Similarly, the diversity of seasonal distributional patterns exhibited by 18 loggerheads tracked via

satellite telemetry to date, particularly with respect to over-wintering strategies, illustrates the need to continue this type of research so that conclusions will be based on greater sample sizes. Although additional work is needed, the results from data collection efforts that began in 2004 have already shed considerable light on several poorly understood aspects of the life history of loggerhead sea turtles in coastal waters of the South Atlantic Bight. Some previously accepted notions of this life history, such as strong site affinity patterns, are corroborated by this work, while others, such as the conventionally accepted belief that exothermic sea turtles must over-winter in waters warmer than 20°C in order to survive, are clearly not universally applicable.

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PATTERNS OF ANTIBIOTIC RESISTANCE IN BACTERIA ISOLATED FROM MARINE TURTLES

An internship report submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

ENVIRONMENTAL STUDIES

by

Lana Piñera-Pasquino

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The internship report of Lana Piñera-Pasquino
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Abstract:

Patterns of Antibiotic Resistance in Bacteria Isolated from Marine Turtles, by Lana

Piñera-Pasquino: Sea turtles face many natural and human-induced threats to their survival. This has prompted several sea turtle rehabilitation facilities to open in order to treat and release these animals. Treatment of these rehabilitated sea turtles has led to the discovery that some of their bacterial infections do not adequately respond to antibiotic treatment (Tom Sheridan, 2006, personal communication). This has led to questions as to where the sea turtles are acquiring these antibiotic-resistant bacteria. Widespread use of antibiotics in humans, domesticated animals, aquaculture and agriculture has led to their increased presence in the environment, and has created the selective pressure necessary for some bacteria to develop antibiotic resistance (Levy, 2001). Many studies have been done to determine the effects of antibiotic release on terrestrial ecosystems (Haapapuro, *et al.*, 1997; Sayah, *et al.*, 2005). However, very little research has been done on its effects in aquatic ecosystems (Depaola, *et al.*, 1995; Goni-Urriza, *et al.*, 2000; Kolpin, *et al.*, 2002), and even less has been done to determine its effects on marine ecosystems (Kelly, *et al.*, 2006). To determine the possible effects of antibiotic release in the environment on sea turtles, an internship was conducted at the South Carolina Aquarium's Sea Turtle Rescue Program. During this internship, sick and injured sea turtles were rehabilitated and released back into the wild. In addition, the occurrence of antibiotic-resistant bacteria found in wild Loggerhead sea turtles (*Caretta caretta*) was analyzed using the Kirby-Bauer method and a tube-dilution method with a 96-well suspension plate, and a preset panel of antibiotics designed by Dade Behring specifically for the National Oceanic and Atmospheric Administration (NOAA) (Bauer, *et al.*, 1966; NCCLS, 2003).

Twenty-one gram negative bacterial strains were isolated from *C. caretta* cloacal samples and analyzed for their resistance to specific antibiotics and also for the minimal inhibitory concentration (MIC) of each antibiotic. Resistance to multiple antibiotics was detected in all of the isolates, with the most common resistances being to lincomycin, clindamycin, erythromycin, penicillin, triple sulfa, cephalexin, and cephalothin. Determining possible patterns of antibiotic resistance in microbes from marine animals is vital in order to establish the significance of antibiotic release into marine environments.

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Chapter One: The South Carolina Aquarium Sea Turtle Rescue Program

Introduction: Natural threats for *Caretta caretta* include erosion of nesting beaches, nest depredation, nest loss due to erosion or inundation, and shark depredation. Human-induced threats to *C. caretta* populations include beach armoring, artificial beachfront lighting, recreational beach equipment (which act as obstacles for nesting females and hatchlings), poaching, destruction of resting and foraging grounds through dredging, longline fisheries, trawl fisheries, boat collisions, pollution, and incidental ingestion of trash (National Marine Fisheries Service and U.S. Fish and Wildlife Service, 1999). Because *C. caretta*, along with many other sea turtle species, face so many threats to their survival, the formation of sea turtle rehabilitation facilities has become more and more prevalent throughout the United States. One such facility is located at the South Carolina Aquarium, where one portion of the internship was performed. At the South Carolina Aquarium Sea Turtle Rescue Program, work was completed to rehabilitate sick and injured sea turtles with the goal of returning them to the wild. In addition to rehabilitating the sea turtles, their specific ailments were documented in order to identify any particular trends that may provide insight into any new threats to the *C. caretta* populations.

Methods: Sea Turtle Rehabilitation: The South Carolina Aquarium Sea Turtle Rescue Program accepts injured or sick sea turtles which have been found along the southeastern coastline. Once an injured sea turtle was delivered to the South Carolina Aquarium, its health was first determined through visual assessment and blood extraction. Measurements were taken, and included the turtle's weight, its straight and curved carapace width and length, and the concavity of its plastron. The amount of

barnacles and other marine organisms which were growing on the turtle's carapace, plastron, head and flippers was also observed. Once the measurements and blood had been taken, the turtle was placed in a tank of shallow freshwater. The freshwater allowed the turtle to re-hydrate itself, while killing the majority of the marine organisms which were attached to the turtle's body. Daily, the freshwater tank was drained, which allowed access to the turtle. While the turtle was out of water, the loose barnacles and other marine growths were removed, and any wounds were sprayed and gently brushed with a disinfectant. The disinfectant remained on the turtle's skin for approximately ten minutes, after which the turtle was sprayed clean with freshwater. This procedure was continued until about 90% of the marine organisms were removed, and the turtle was strong enough to be placed in a full tank of freshwater. The percentage of seawater in the tank was gradually increased during each water change until the tank contained 100% seawater.

Medical rounds, which were completed under the supervision of the South Carolina Aquarium's veterinarian, were performed weekly on all of the sea turtles in the facility. Once a week, each turtle was removed from its tank in order for its health to be assessed. Each turtle was weighed and measured to quantify its growth and weight gain. The amount of food and medication being administered to each individual turtle was occasionally modified as the turtle gained weight. When necessary, each turtle was also debrided with a brush to remove any loose, flaking skin, or any remaining barnacles. Blood was extracted from each turtle and analyzed every week until the turtle's health stabilized. Blood was extracted from the dorsal cervical sinus and analyzed to determine the turtle's packed cell volume (PCV), total protein (TP), and glucose levels.

Sea Turtle Maintenance: In addition to performing the medical rounds each week, routine husbandry tasks were performed daily. These tasks included cutting and weighing out the food being fed to each turtle, feeding each turtle and observing their eating behavior, administering sub-cutaneous and oral medications and vitamins, and recording the eating activities of each individual turtle, including how much they ate, how actively they ate, and what medications they were given with their food. Standard cleaning tasks necessary to maintain a sanitary facility were also performed on a regular basis and included mopping the floor, cleaning counter tops, cleaning containers used to hold turtles during medical inspections, and disinfecting any instruments used to clean the turtles or turtle tanks.

Release of Sea Turtles: A turtle was considered healthy enough for release when it had regained a healthy appetite, had increased both its weight and strength, and had a PCV in the high twenties. Rehabilitated sea turtles were released from the same area in which they were found whenever possible. However, due to complications with the tides, predators, water temperature, or the stress which is placed on the sea turtle during transportation to the release site, that was not always possible. In those cases, the sea turtles were released from areas which were approved by U.S. Fish and Wildlife and the National Oceanic and Atmospheric Administration (NOAA). In every case, the primary concern when releasing a rehabilitated sea turtle was its welfare.

Results: During the time frame of this internship, a total of ten *C. caretta* were admitted to the South Carolina Aquarium Sea Turtle Rescue Program. Five *C. caretta* died before or during treatment, four were successfully rehabilitated and released, and one currently remains at the facility and is scheduled to be released this summer. All ten

C. caretta were diagnosed with having Debilitated Turtle Syndrome (DTS). A sea turtle is determined to have DTS when it is emaciated and has a significant amount of barnacle and epibiotic coverage on its exterior. DTS can occur as the result of a sea turtle becoming weakened by a variety of reasons, from becoming cold-stunned to illness due to exposure to pollutants or ingestion of trash. One study found DTS to occur more frequently in sub adult, female sea turtles, with *C. caretta* being the most commonly afflicted species in the southeastern United States (Norton, *et al.*, 2004). Detailed information about DTS is still sparse; thus research needs to continue on the subject to gain greater insight into its possible causative factors.

Chapter 2: Laboratory Work

Introduction: Over the last couple of decades, the study of the environmental impacts of chemical pollution has focused primarily on what are considered “priority pollutants” (*i.e.* potent toxic or carcinogenic chemicals). Little attention has been paid to the effects that “less potent” pharmaceuticals may have on the environment and its inhabiting wildlife, and even smaller attention has been paid to its effects on marine life (Daughton and Ternes, 1999). Antibiotics have been used extensively in both human and animal life since their introduction into medicine in the 1940’s and 1950’s (Virella, 1997). Antibiotics and antibiotic-resistant bacteria are released in varying amounts into the environment due to the increased and sometimes haphazard use of antibiotics in the medical, veterinary, aquacultural, and agricultural fields (Goni-Urriza, *et al.*, 2000). The extensive use of antibiotics in both humans and animals has led to the development of antibiotic resistance in some bacterial strains. Some of the proposed sources through which antibiotics are being introduced to marine creatures are animal agriculture and the

improperly treated wastes of humans and animals (Chee-Sanford, *et al.*, 2001; Daughton and Ternes, 1999). Because of the serious implications of antibiotic release in the environment, research was conducted at the South Carolina Aquarium Sea Turtle Rescue Program. In addition to caring for the sick and injured sea turtles contained within the facility, cloacal samples were obtained from several of the turtles upon entry to the South Carolina Aquarium. Cloacal samples were also obtained from the sea turtles which were captured by a South Carolina Department of Natural Resources (SCDNR) research team for an unrelated study. The resistance of the sea turtles' microbiota to antibiotics was studied, utilizing laboratory equipment from both NOAA and the Clemson Veterinary Diagnostic Center (CVDC). This was accomplished by culturing cloacal samples extracted from these rescued sea turtles, isolating and identifying dominant gram negative bacterial strains, and testing them for resistance to antibiotics. Studying the extent to which antibiotic resistance is present in marine animals has far-reaching implications for both marine animal and human health. It is important to determine if the occurrence of antibiotic resistance found in marine animals represents a particular pattern. If a pattern can be established, then sources of the factors leading to the development of antibiotic resistance in marine animals may be able to be determined. The results of this study may serve to guide future research on this topic. Further research could be conducted to locate the origins of antibiotic release into marine environments. This may lead to greater care in the use of antibiotics, and stricter regulations on the release of antibiotics into the surrounding environment.

Methods: Cloacal samples came from two sources. One source was the sea turtles in the South Carolina Aquarium Sea Turtle Rescue Program. The second source

came from sea turtles caught by an SCDNR research team doing an unrelated sea turtle study. The sea turtles which were caught by SCDNR were retained briefly for measurements and sampling, and subsequently released. In all cases, samples were taken from *C. caretta* populations located off the southeastern coast of the United States.

Cloacal samples were acquired by inserting a sterile culturette swab into the cloaca of a sea turtle, and preserving it in a sterile media tube. The samples were stored in different manners, depending upon the circumstances in which they were taken. Samples taken at the South Carolina Aquarium were refrigerated until they could be properly stored in the laboratory at NOAA. In most cases, the samples were refrigerated for less than one hour before storage at NOAA. However, due to the unpredictable nature in which the sea turtles were admitted to the South Carolina Aquarium, samples were sometimes refrigerated for a day before proper storage. In one case, a sample was refrigerated for five days before storage at NOAA. The samples obtained by the individuals from SCDNR were stored in another manner, as the sampling boat (the *Lady Lisa*) remained out to sea for a week before returning to land. To preserve these samples until they could be stored at NOAA, the culturette tubes were either placed in a -80° C freezer, or frozen in liquid nitrogen and stored in a Dewar flask (Mark Mitchell, 2005, personal communication). The samples were stored on the *Lady Lisa* in this manner for seven to ten days.

Once the samples arrived at NOAA, the tips of the culturette swabs were cut off using sterilized scissors and dropped into 2 ml storage vials, containing 1 ml of 80% bacto tryptic soy broth (TSB) with 20% glycerol (Dade Behring, California). After the tip was placed in the vial, the vial was vortexed for approximately ten to fifteen seconds

to reduce clumping of the bacteria, labeled, and placed in the -80° C freezer located on the NOAA facilities. The samples which were stored in liquid nitrogen were the only exception to this procedure, as they were directly placed in the -80° C freezer. It was necessary to store the samples at NOAA, as budgetary issues required the cloacal samples to be shipped in bulk to CVDC for analysis. The samples were wrapped in bubble wrap, and placed on dry ice for transport to CVDC. At CVDC, the dominant gram negative bacterial strains were isolated and identified, and an antibiotic resistance analysis (ARA) was performed using the Kirby-Bauer method (Bauer, *et al.*, 1966). The ARA results, along with the isolated and identified bacteria, were returned to NOAA. An ARA was also performed in the laboratory at NOAA, and the minimal inhibitory concentration (MIC) of the isolates was determined using a tube-dilution method, involving a 96-well suspension plate, and a preset panel of antibiotics (Table 7). Because NOAA was awaiting the shipment of more ARA suspension plates, the isolates were stored again until the arrival of these plates. A small amount of the isolate was transferred to the 2 ml vials containing 1 ml of TSB and glycerol using a sterilized loop. The vial was then vortexed, and frozen in the -80° C freezer.

When the plates arrived, the isolates were removed from the freezer for transfer onto tryptic soy agar (TSA) plates (Dade Behring, California). The vials containing the isolates were placed on ice until they could be transferred to the TSA plates to minimize thawing. Ice flakes from the frozen isolates were streaked onto the plates using a sterilized loop. The inoculated TSA plates were placed in a 37° C incubator for 21 hours before being removed in order to transfer the bacterial colonies to the ARA suspension plates (Dade Behring, California). The isolates were prepared for ARA using the

following method. A small amount of the isolate was removed from the TSA plate using a disposable, sterile wooden rod, and placed into sterile, nutrient-free, inoculum water. After the inoculum water was inoculated, it was vortexed, and its optical density (OD) was measured using a Dade Behring MicroScan Turbidity Meter (Dade Behring, California) (Figure 1). The inoculum water was inoculated with the isolate until it reached an OD between 0.08 and 0.10. Once the inoculum water reached the proper OD, 0.1 ml of the broth was transferred to a tube containing 25 ml of a cation-adjusted Mueller-Hinton broth (CAMHB) using a Gilson Micropipette (Dade Behring, California). The inoculated CAMHB tube was gently shaken back and forth several times before being poured into a disposable, plastic inoculator-D set (Dade Behring, California) (Figures 2 and 3). The inoculator-D set, which is custom made by Dade Behring to accompany the Dade Behring MicroScan Renok Pipette (Figure 4), is used to transfer inoculated broth from a tube to the ARA suspension plates. The inoculator-D set is comprised of two halves. The bottom half functions as a tray, which holds the inoculated broth once it is poured into the inoculator-D set. The top half consists of 96 small holes which correspond to the 96 wells contained within the ARA plates. The inoculated broth was first poured into the bottom half of the inoculator-D set, after which the top half was placed on top of it. The Dade Behring MicroScan Renok Pipette (Dade Behring, California) was next placed on top of the inoculator-D set, where it locked on to the top portion of the set, and siphoned up the broth through the 96 small holes. The MicroScan Renok Pipette, still attached to the top half of the inoculator-D set, was next placed on top of the ARA suspension plate (Figure 5), where it dispensed 115 ± 10 μ L of inoculated broth into each of the 96 wells simultaneously. The plates were labeled and incubated at

37° C for approximately 21 hours. The ARA plates were also inoculated with five control strains (*Staphylococcus aureus*, 2 *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*) using the same method. After incubation, the ARA plates were read using a Dade Behring MicroScan Touch Scan and the Max Flex Custom Panel System computer program (Dade Behring, 2001). On the Dade Behring MicroScan Touch Scan (Dade Behring, California), each of the 96 wells was observed for growth of bacteria. The lowest concentration of antibiotic to contain no bacterial growth in its well was recorded to determine the MIC.

Results: A total of 21 gram negative bacterial strains were isolated from the cloacal swabs. Antibiotic resistance was detected in all of the isolates. Of the 17 antibiotics which were tested by CVDC, at least 50% of the isolated bacteria displayed resistance to seven of them. The most frequent resistances displayed by the isolates were to lincomycin (100% of the isolates), clindamycin (95.2%), erythromycin (95.2%), penicillin (95.2%), and triple sulfa (95.2%). Little to no resistance was observed in the isolates to gentamicin (9.5%), amikacin (0%), enrofloxacin (0%), and neomycin (0%) (Table 1). The isolates showing resistance to the greatest amount of antibiotics tested were the *Pseudomonas* strains, which ranged from 47.1% to 70.6% resistance. Also showing significant levels of antibiotic resistance were *Stenotrophomonas maltophilia* (64.7% of the antibiotics tested), *Morganella morganii* (52.9%), *Citrobacter freundii* (52.9%), and several of the *Escherichia coli* strains (Table 2). Of the 26 antibiotics tested at NOAA, eight antibiotics had at least 50% of the isolated bacteria displaying resistances to them. The highest levels of resistances displayed by the isolates were to erythromycin (100% of the isolates), cephalixin (80%), cephalothin (80%), and penicillin (75%). Very

little resistance was noted in the isolates to amikacin (5%), apramycin (5%), ciprofloxacin (5%), gentamicin (5%), imipenem (5%), meropenem (5%), and sulfathiozole (5%). No resistance was observed to moxifloxacin or ofloxacin (Table 3). The isolates which displayed resistance to the greatest number of antibiotics tested were *Stenotrophomonas maltophilia* (69.2% of the antibiotics tested) and *Pseudomonas aeruginosa* (57.7 and 61.5%) (Table 4).

Discussion: Similar patterns of resistance were found within the two separate antibiotic resistance analyses run by CVDC and NOAA. In both tests, the isolates containing the largest variety of antibiotic resistance were *Stenotrophomonas maltophilia*, and the *Pseudomonas* strains. Additionally, both analyses found erythromycin resistance and penicillin resistance to be the most prevalent resistances displayed by the isolates, while amikacin resistance and gentamicin resistance were the least commonly observed. The results from the ARA's run at both CVDC and NOAA found the greatest percentage of resistance displayed by the isolates to be to the beta-lactam, lincosamide, macrolide, and sulfonamide (trimethoprim-sulfadiazine and triple sulfa) classes of antibiotics. The lowest percentage of resistance displayed by the isolates was to the carbapenem group of the beta-lactam class, and to the aminoglycoside, quinolone, and sulfonamide (trimethoprim-sulfamethoxazole and sulfathiazole) classes of antibiotics (Tables 5 and 6) (Beers, *et al.*, 2003; Mims, *et al.*, 1993).

Although the results of this project indicate that there may be a serious problem involving the release of antibiotics into the ocean, it is important to note when considering these data that some of the antibiotics used in the ARA panels do not selectively target gram negative bacteria. Antibiotics found in the beta-lactam,

lincosamide, and macrolide classes of antibacterials target either both gram negative and gram positive bacteria, or more selectively target gram positive bacterial strains (Mims, *et al.*, 1993). For example, while many of the isolates displayed resistance to antibiotics such as erythromycin and penicillin, this may have occurred due to the fact that erythromycin and penicillin are both designed to target gram positive bacteria, and are less effective against gram negative bacterial strains.

An valuable lesson learned from this project was the importance of preserving samples properly. Freezing the cloacal swabs slowly in a -80° C freezer caused the bacterial cells to lyse, resulting in the death of the majority of the bacteria from the samples. When the culturette swabs were stored in this way, only four out of the 21 samples collected yielded bacteria. Bacteria from the samples fared better when frozen in a broth containing glycerol, which prevented cell lysis, or when frozen quickly in liquid nitrogen. This project was greatly hindered by the inability to perform the laboratory work on the cloacal swabs immediately after collecting. The necessity of sending the samples in two large shipments also prevented any immediate feedback on how the storage of the samples affected the amount of bacteria harvested from each cloacal swab.

The widespread use of antibiotics has already been found to present many dangers to both human and animal health. Although many studies have already been performed on terrestrial and freshwater ecosystems, very little has been discovered on how antibiotic release affects marine ecosystems. More research needs to be done on antibiotic resistance displayed by bacteria present in marine organisms. Another possible avenue for future research would be to sample sea turtles upon their entrance to a rehabilitation facility, and taking another sample just prior to release, after they have received treatment

(Tom Sheridan, 2006, personal communication). This would allow a comparison to be made between the types of antibiotics administered to a sea turtle during treatment, and the types of resistances present in the bacteria isolated from the sea turtle after treatment. It is imperative that the relationship between antibiotic release into the ocean and the development of antibiotic resistance in bacteria found in marine organisms continues to be studied, in order to gain a better understanding of its possible impacts on marine life.

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**Appendix I
(Tables and Figures)**

Table 1. Results of the ARA completed using the Kirby-Bauer method with the antibiotic panel used by CVDC. Results are based solely on isolates surviving the sampling conditions and storing process.

CVDC Results

Antibiotic	Number of Resistant Isolates	Percentage of Isolates with Resistance
Amikacin	0	0
Ampicillin	13	61.9
Augmentin	8	38.1
Ceftiofur	6	28.6
Cephalothin	13	61.9
Chloramphenicol	8	38.1
Clindamycin	20	95.2
Enrofloxacin	0	0
Erythromycin	20	95.2
Gentamicin	2	9.5
Lincomycin	21	100
Neomycin	0	0
Orbifloxacin	3	14.3
Penicillin	20	95.2
Tetracycline	6	28.6
Trimethoprim-Sulfadiazine	9	42.9
Triple Sulfa	20	95.2

Table 2. The percentage of antibiotic resistance displayed by each isolate as determined by the antibiotic panel used by CVDC. A total of 17 antibiotics were tested at CVDC. Results are based solely on isolates surviving the sampling conditions and storing process.

CVDC Results

Isolate	Number of Antibiotics to Which the Isolate is Resistant	Percentage of Resistance
<i>Citrobacter braakii</i> (SCA#8)	6	35.3
<i>Citrobacter freundii</i> (SCA#2)	9	52.9
<i>Escherichia coli</i> (CC0348)	7	41.2
<i>Escherichia coli</i> (CC0360)	9	52.9
<i>Escherichia coli</i> (CC0378)	9	52.9
<i>Escherichia coli</i> (SCA#2)	5	29.4
<i>Escherichia coli</i> (SCA#3)	10	58.8
<i>Escherichia coli</i> (SCA#5)	5	29.4
<i>Escherichia coli</i> (SCA#6)	5	29.4
<i>Escherichia coli</i> (SCA#7)	4	23.5
<i>Morganella morganii</i> (CC0382)	9	52.9
<i>Proteus vulgaris</i> (CC0356)	7	41.2
<i>Pseudomonas aeruginosa</i> (CC0380)	12	70.6
<i>Pseudomonas aeruginosa</i> (SCA#3)	11	64.7
<i>Pseudomonas spp.</i> (CC0382)	8	47.1
<i>Pseudomonas spp.</i> (SCA#4)	10	58.8
<i>Pseudomonas stutzeri</i> (SCA#7)	12	70.6
<i>Psuedomonas stutzeri</i> (CC0364)	10	58.8
<i>Salmonella spp., Poly D</i> (CC0380)	4	23.5
<i>Shewanella algae/putrefaciens</i> (CC0384)	6	35.3
<i>Stenotrophomonas maltophilia</i> (SCA#7)	11	64.7

Table 3. Results of the ARA completed using the tube dilution method with the antibiotic panel utilized by NOAA. Results are based solely on isolates surviving the sampling conditions and storing process.

NOAA Results

Antibiotic	Number of Resistant Isolates	Percentage of Isolates with Resistance
Amikacin	1	5
Amoxicillin	12	60
Ampicillin	11	55
Apramycin	1	5
Azithromycin	10	50
Cefoxitin	10	50
Ceftriaxone	3	15
Cephalexin	16	80
Cephalothin	16	80
Chloramphenicol	4	20
Ciprofloxacin	1	5
Erythromycin	20	100
Gentamicin	1	5
Imipenem	1	5
Meropenem	1	5
Moxifloxacin	0	0
Nalidixic Acid	3	15
Nitrofurantoin	9	45
Ofloxacin	0	0
Oxytetracycline	8	40
Penicillin	15	75
Streptomycin	3	15
Sulfathiazole	1	5
Tetracycline	4	20
Trimethoprim	8	40
Trimethoprim/Sulfamethoxazole	3	15

Table 4. The percentage of antibiotic resistance displayed by each isolate as determined by the antibiotic panel used by NOAA. Twenty-six different antibiotics were tested using the antibiotic panel designed by Dade Behring for NOAA. Results are based solely on isolates surviving the sampling conditions and storing process.

NOAA Results

Isolate	Number of Antibiotics to Which the Isolate is Resistant	Percentage of Resistance
<i>Citrobacter braakii</i> (SCA#8)	5	19.2
<i>Citrobacter freundii</i> (SCA#2)	11	42.3
<i>Escherichia coli</i> (CC0348)	6	23.1
<i>Escherichia coli</i> (CC0360)	8	30.8
<i>Escherichia coli</i> (CC0378)	9	34.6
<i>Escherichia coli</i> (SCA#2)	1	3.8
<i>Escherichia coli</i> (SCA#3)	9	34.6
<i>Escherichia coli</i> (SCA#5)	3	11.5
<i>Escherichia coli</i> (SCA#6)	6	23.1
<i>Escherichia coli</i> (SCA#7)	2	7.7
<i>Morganella morganii</i> (CC0382)	7	26.9
<i>Proteus vulgaris</i> (CC0356)	10	38.5
<i>Pseudomonas aeruginosa</i> (CC0380)	16	61.5
<i>Pseudomonas aeruginosa</i> (SCA#3)	15	57.7
<i>Pseudomonas spp.</i> (CC0382)	9	34.6
<i>Pseudomonas spp.</i> (SCA#4)	7	26.9
<i>Pseudomonas stutzeri</i> (SCA#7)	10	38.5
<i>Salmonella spp., Poly D</i> (CC0380)	1	3.8
<i>Shewanella algae/putrefaciens</i> (CC0384)	9	34.6
<i>Stenotrophomonas maltophilia</i> (SCA#7)	18	69.2

Table 5. Results of the ARA completed using the Kirby-Bauer method with the antibiotic panel used by CVDC. Antibiotics are grouped into classes. Results are based solely on isolates surviving the sampling conditions and storing process.

CVDC Results		
Antibiotic	Number of Resistant Isolates	Percentage of Isolates with Resistance
Aminoglycosides		
Amikacin	0	0
Gentamicin	2	9.5
Neomycin	0	0
Beta-lactams		
Cephalosporins		
Ceftiofur	6	28.6
Cephalothin	13	61.9
Penicillins		
Ampicillin	13	61.9
Augmentin	8	38.1
Penicillin	20	95.2
Chloramphenicols		
Chloramphenicol	8	38.1
Lincosamides		
Clindamycin	20	95.2
Lincomycin	21	100
Macrolides		
Erythromycin	20	95.2
Quinolones		
Enrofloxacin	0	0
Orbifloxacin	3	14.3
Sulfonamides		
Trimethoprim-Sulfadiazine	9	42.9
Triple Sulfa	20	95.2
Tetracyclines		
Tetracycline	6	28.6

Table 6. Results of the ARA completed using the tube dilution method with the antibiotic panel utilized by NOAA. The antibiotics are grouped into their respective classes. Results are based solely on isolates surviving the sampling conditions and storing process.

NOAA Results

Antibiotic	Number of Resistant Isolates	Percentage of Isolates with Resistance
Aminoglycosides		
Amikacin	1	5
Apramycin	1	5
Gentamicin	1	5
Streptomycin	3	15
Beta-lactams		
Carbapenems		
Imipenem	1	5
Meropenem	1	5
Cephalosporins		
Cefoxitin	10	50
Ceftriaxone	3	15
Cephalexin	16	80
Cephalothin	16	80
Penicillins		
Amoxicillin	12	60
Ampicillin	11	55
Penicillin	15	75
Chloramphenicols		
Chloramphenicol	4	20
Macrolides		
Azithromycin	10	50
Erythromycin	20	100
Nitrofurantoin		
Nitrofurantoin	9	45
Quinolones		
Ciprofloxacin	1	5
Moxifloxacin	0	0
Nalidixic Acid	3	15
Ofloxacin	0	0
Sulfonamides		

Sulfathiazole	1	5
Trimethoprim/Sulfamethoxazole	3	15
Tetracyclines		
Oxytetracycline	8	40
Tetracycline	4	20
Trimethoprim		
Trimethoprim	8	40

Table 7. The minimal inhibitory concentration (MIC) for the isolates as determined by the ARA panel used at NOAA. The “R” or “S” next to the MIC denotes whether the isolate is resistant (R) or susceptible (S) to each antibiotic. Isolate numbers GSTP12, 27853, and 29212 (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*, respectively) were used as positive controls, and isolates 29213 and 25922 (*Staphylococcus aureus* and *Escherichia coli*, respectively) were used as negative controls.

GSTP12/*Escherichia coli* is a positive control used specifically by NOAA, and was isolated from the Greenwood Sewage Treatment Plant. The antibiotic dilution units are in $\mu\text{g/mL}$. All panels were considered “NOAA Custom Panel”.

Test Date	Isolate	Organism	Amikacin	Ampicillin	Amoxicillin	Apramycin	Azithromycin	Chloramphenicol	Ceftriaxone	Cephalexin	Cephalothin	Cefoxitin
12/14/2005	CC0348	<i>Escherichia coli</i>	<=8 (S)	16 (S)	32 (R)	<=8 (S)	4 (S)	<=8 (S)	<=8 (S)	64 (R)	32 (R)	>32 (R)
12/14/2005	SCA #3	<i>Escherichia coli</i>	<=8 (S)	32 (R)	>32 (R)	<=8 (S)	4 (S)	<=8 (S)	64 (R)	64 (R)	>32 (R)	<=1 (S)
12/14/2005	SCA #8	<i>Citrobacter braakii</i>	<=8 (S)	<=4 (S)	8 (S)	<=8 (S)	<=2 (S)	<=8 (S)	<=8 (S)	32 (R)	32 (R)	16 (S)
12/14/2005	CC0382	<i>Pseudomonas spp.</i>	<=8 (S)	32 (R)	16 (S)	<=8 (S)	>8 (R)	16 (S)	<=8 (S)	>128 (R)	>128 (R)	>32 (R)
12/14/2005	SCA #7	<i>Pseudomonas stutzeri</i>	<=8 (S)	>32 (R)	32 (R)	<=8 (S)	4 (S)	32 (R)	<=8 (S)	>128 (R)	>128 (R)	>32 (R)
12/14/2005	CC0384	<i>Shewanella algae/putrefaciens</i>	<=8 (S)	>32 (R)	>32 (R)	16 (S)	>8 (R)	<=8 (S)	<=8 (S)	>128 (R)	>128 (R)	<=8 (S)
12/14/2005	SCA #5	<i>Escherichia coli</i>	<=8 (S)	<=4 (S)	8 (S)	<=8 (S)	<=2 (S)	<=8 (S)	<=8 (S)	<=16 (S)	<=16 (S)	32 (R)
12/14/2005	SCA #7	<i>Stenotrophomonas maltophilia</i>	64 (R)	>32 (R)	>32 (R)	>32 (R)	8 (R)	<=8 (S)	>64 (R)	>128 (R)	>128 (R)	>32 (R)
12/14/2005	SCA #6	<i>Escherichia coli</i>	<=8 (S)	16 (S)	32 (R)	<=8 (S)	4 (S)	<=8 (S)	<=8 (S)	128 (R)	32 (R)	>32 (R)
12/14/2005	CC0380	<i>Salmonella spp.</i>	<=8 (S)	<=4 (S)	<=4 (S)	<=8 (S)	4 (S)	<=8 (S)	<=8 (S)	<=16 (S)	<=16 (S)	<=8 (S)
12/14/2005	CC0380	<i>Pseudomonas aeruginosa</i>	<=8 (S)	>32 (R)	>32 (R)	16 (S)	>8 (R)	>32 (R)	64 (R)	>128 (R)	>128 (R)	>32 (R)
12/14/2005	SCA #3	<i>Pseudomonas aeruginosa</i>	<=8 (S)	>32 (R)	>32 (R)	<=8 (S)	>8 (R)	>32 (R)	16 (S)	>128 (R)	>128 (R)	>32 (R)
12/14/2005	SCA #4	<i>Pseudomonas spp.</i>	<=8 (S)	16 (S)	8 (S)	<=8 (S)	>8 (R)	<=8 (S)	<=8 (S)	>128 (R)	>128 (R)	>32 (R)
12/14/2005	SCA #2	<i>Escherichia coli</i>	<=8 (S)	<=4 (S)	<=4 (S)	<=8 (S)	<=2 (S)	<=8 (S)	<=8 (S)	<=16 (S)	<=16 (S)	<=8 (S)
12/14/2005	SCA #7	<i>Escherichia coli</i>	<=8 (S)	<=4 (S)	<=4 (S)	<=8 (S)	4 (S)	<=8 (S)	<=8 (S)	<=16 (S)	32 (R)	16 (S)
12/14/2005	SCA #2	<i>Citrobacter freundii</i>	<=8 (S)	16 (S)	16 (S)	<=8 (S)	4 (S)	>32 (R)	<=8 (S)	32 (R)	<=16 (S)	16 (S)
12/14/2005	CC0382	<i>Morganella morganii</i>	<=8 (S)	>32 (R)	>32 (R)	<=8 (S)	>8 (R)	<=8 (S)	<=8 (S)	>128 (R)	>128 (R)	<=8 (S)
12/14/2005	CC0356	<i>Proteus vulgaris</i>	<=8 (S)	>32 (R)	>32 (R)	<=8 (S)	>8 (R)	<=8 (S)	<=8 (S)	>128 (R)	>128 (R)	<=8 (S)
12/14/2005	CC0360	<i>Escherichia coli</i>	<=8 (S)	>32 (R)	>32 (R)	<=8 (S)	8 (R)	<=8 (S)	<=8 (S)	>128 (R)	64 (R)	>32 (R)
12/15/2005	CC0378	<i>Escherichia coli</i>	<=8 (S)	>32 (R)	>32 (R)	<=8 (S)	>8 (R)	<=8 (S)	<=8 (S)	32 (R)	64 (R)	<=8 (S)
12/15/2005	29213	CONTROL- <i>S. aureus</i>	<=8 (S)	<=4 (S)	<=4 (S)	16 (S)	<=2 (S)	<=8 (S)	<=8 (S)	<=16 (S)	<=16 (S)	<=8 (S)
12/15/2005	GSTP12	CONTROL-GSTP-12/ <i>E. coli</i>	<=8 (S)	>32 (R)	>32 (R)	<=8 (S)	>8 (R)	>32 (R)	<=8 (S)	<=16 (S)	64 (R)	<=8 (S)
12/15/2005	27853	CONTROL- <i>P. aeruginosa</i>	<=8 (S)	>32 (R)	>32 (R)	<=8 (S)	>8 (R)	>32 (R)	<=8 (S)	>128 (R)	>128 (R)	>32 (R)
12/15/2005	25922	CONTROL- <i>E. coli</i>	<=8 (S)	<=4 (S)	<=4 (S)	<=8 (S)	<=2 (S)	<=8 (S)	<=8 (S)	<=16 (S)	<=16 (S)	<=8 (S)
12/15/2005	29212	CONTROL- <i>E. faecalis</i>	64 (R)	<=4 (S)	<=4 (S)	>32 (R)	4 (S)	<=8 (S)	>64 (R)	128 (R)	32 (R)	>32 (R)

Table 7 cont'd

Test Date	Isolate	Organism	Ciprofloxacin	Erythromycin	Nitrofurantoin	Gentamicin	Imipenem	Meropenem	Moxifloxacin	Nalidixic Acid	Ofloxacin
12/14/2005	CC0348	<i>Escherichia coli</i>	<=1 (S)	64 (R)	<=16 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/14/2005	SCA #3	<i>Escherichia coli</i>	64 (R)	32 (R)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)	<=4 (S)
12/14/2005	SCA #8	<i>Citrobacter braakii</i>	<=1 (S)	32 (R)	<=16 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/14/2005	CC0382	<i>Pseudomonas spp.</i>	<=1 (S)	64 (R)	>128 (R)	<=2 (S)	<=2 (S)	<=2 (S)	0.5 (S)	8 (S)	<=1 (S)
12/14/2005	SCA #7	<i>Pseudomonas stutzeri</i>	<=1 (S)	64 (R)	>128 (R)	<=2 (S)	<=2 (S)	<=2 (S)	1 (S)	16 (S)	<=1 (S)
12/14/2005	CC0384	<i>Shewanella algae/putrefaciens</i>	<=1 (S)	128 (R)	64 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/14/2005	SCA #5	<i>Escherichia coli</i>	<=1 (S)	32 (R)	<=16 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/14/2005	SCA #7	<i>Stenotrophomonas maltophilia</i>	<=1 (S)	64 (R)	>128 (R)	>16 (R)	>16 (R)	>16 (R)	<=0.25 (S)	16 (S)	<=1 (S)
12/14/2005	SCA #6	<i>Escherichia coli</i>	<=1 (S)	64 (R)	<=16 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	8 (S)	<=1 (S)
12/14/2005	CC0380	<i>Salmonella spp.</i>	<=1 (S)	64 (R)	32 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/14/2005	CC0380	<i>Pseudomonas aeruginosa</i>	<=1 (S)	128 (R)	>128 (R)	<=2 (S)	<=2 (S)	<=2 (S)	1 (S)	>32 (R)	<=1 (S)
12/14/2005	SCA #3	<i>Pseudomonas aeruginosa</i>	<=1 (S)	128 (R)	>128 (R)	<=2 (S)	<=2 (S)	<=2 (S)	1 (S)	>32 (R)	<=1 (S)
12/14/2005	SCA #4	<i>Pseudomonas spp.</i>	<=1 (S)	32 (R)	>128 (R)	<=2 (S)	<=2 (S)	<=2 (S)	0.5 (S)	8 (S)	<=1 (S)
12/14/2005	SCA #2	<i>Escherichia coli</i>	<=1 (S)	32 (R)	<=16 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/14/2005	SCA #7	<i>Escherichia coli</i>	<=1 (S)	32 (R)	<=16 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/14/2005	SCA #2	<i>Citrobacter freundii</i>	2 (S)	64 (R)	128 (R)	<=2 (S)	<=2 (S)	<=2 (S)	4 (S)	>32 (R)	4 (S)
12/14/2005	CC0382	<i>Morganella morganii</i>	<=1 (S)	128 (R)	64 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/14/2005	CC0356	<i>Proteus vulgaris</i>	<=1 (S)	128 (R)	128 (R)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/14/2005	CC0360	<i>Escherichia coli</i>	<=1 (S)	64 (R)	<=16 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/15/2005	CC0378	<i>Escherichia coli</i>	<=1 (S)	>128 (R)	128 (R)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/15/2005	29213	CONTROL- <i>S. aureus</i>	<=1 (S)	<=16 (S)	<=16 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	32 (R)	<=1 (S)
12/15/2005	GSTP12	CONTROL-GSTP-12/ <i>E. coli</i>	>4 (R)	>128 (R)	<=16 (S)	8 (S)	<=2 (S)	<=2 (S)	>4 (S)	>32 (R)	>8 (R)
12/15/2005	27853	CONTROL- <i>P. aeruginosa</i>	<=1 (S)	128 (R)	>128 (R)	<=2 (S)	<=2 (S)	<=2 (S)	2 (S)	>32 (R)	2 (S)
12/15/2005	25922	CONTROL- <i>E. coli</i>	<=1 (S)	32 (R)	<=16 (S)	<=2 (S)	<=2 (S)	<=2 (S)	<=0.25 (S)	<=4 (S)	<=1 (S)
12/15/2005	29212	CONTROL- <i>E. faecalis</i>	<=1 (S)	<=16 (S)	<=16 (S)	4 (S)	<=2 (S)	8 (S)	<=0.25 (S)	>32 (R)	<=1 (S)

Table 7 cont'd

Test Date	Isolate	Organism	Oxytetracycline	Penicillin	Streptomycin	Sulfathiazole	Trimethoprim	Trimethoprim/ Sulfamethoxazole	Tetracycline
12/14/2005	CC0348	<i>Escherichia coli</i>	<=4 (S)	>128 (R)	<=16 (S)	<=250 (S)	<=2 (S)	<=2/38 (S)	<=4 (S)
12/14/2005	SCA #3	<i>Escherichia coli</i>	>128 (R)	<=16 (S)	<=250 (R)	<=2 (S)	<=4 (S)	<=2/38 (S)	
12/14/2005	SCA #8	<i>Citrobacter braakii</i>	<=4 (S)	128 (R)	64 (R)	<=250 (S)	<=2 (S)	<=2/38 (S)	<=4 (S)
12/14/2005	CC0382	<i>Pseudomonas spp.</i>	<=4 (S)	>128 (R)	<=16 (S)	<=250 (S)	>16 (R)	<=2/38 (S)	<=4 (S)
12/14/2005	SCA #7	<i>Pseudomonas stutzeri</i>	<=4 (S)	>128 (R)	<=16 (S)	<=250 (S)	>16 (R)	<=2/38 (S)	<=4 (S)
12/14/2005	CC0384	<i>Shewanella algae/putrefaciens</i>	16 (R)	>128 (R)	<=16 (S)	<=250 (S)	16 (R)	<=2/38 (S)	8 (S)
12/14/2005	SCA #5	<i>Escherichia coli</i>	<=4 (S)	64 (R)	<=16 (S)	<=250 (S)	<=2 (S)	<=2/38 (S)	<=4 (S)
12/14/2005	SCA #7	<i>Stenotrophomonas maltophilia</i>	16 (R)	>128 (R)	64 (R)	<=250 (S)	16 (R)	<=2/38 (S)	8 (S)
12/14/2005	SCA #6	<i>Escherichia coli</i>	<=4 (S)	>128 (R)	<=16 (S)	<=250 (S)	<=2 (S)	<=2/38 (S)	<=4 (S)
12/14/2005	CC0380	<i>Salmonella spp.</i>	<=4 (S)	<=16 (S)	<=16 (S)	<=250 (S)	<=2 (S)	<=2/38 (S)	<=4 (S)
12/14/2005	CC0380	<i>Pseudomonas aeruginosa</i>	16 (R)	>128 (R)	<=16 (S)	<=250 (S)	>16 (R)	>4/76 (R)	>32 (R)
12/14/2005	SCA #3	<i>Pseudomonas aeruginosa</i>	32 (R)	>128 (R)	<=16 (S)	<=250 (S)	>16 (R)	>4/76 (R)	>32 (R)
12/14/2005	SCA #4	<i>Pseudomonas spp.</i>	<=4 (S)	32 (S)	<=16 (S)	<=250 (S)	16 (R)	<=2/38 (S)	<=4 (S)
12/14/2005	SCA #2	<i>Escherichia coli</i>	<=4 (S)	32 (S)	<=16 (S)	<=250 (S)	<=2 (S)	<=2/38 (S)	<=4 (S)
12/14/2005	SCA #7	<i>Escherichia coli</i>	<=4 (S)	32 (S)	<=16 (S)	<=250 (S)	<=2 (S)	<=2/38 (S)	<=4 (S)
12/14/2005	SCA #2	<i>Citrobacter freundii</i>	>32 (R)	64 (R)	<=16 (S)	>500 (R)	>16 (R)	>4/76 (R)	>32 (R)
12/14/2005	CC0382	<i>Morganella morganii</i>	<=4 (S)	>128 (R)	<=16 (S)	<=250 (S)	<=2 (S)	<=2/38 (S)	<=4 (S)
12/14/2005	CC0356	<i>Proteus vulgaris</i>	>32 (R)	>128 (R)	<=16 (S)	<=250 (S)	8 (S)	<=2/38 (S)	16 (R)
12/14/2005	CC0360	<i>Escherichia coli</i>	<=4 (S)	>128 (R)	<=16 (S)	<=250 (S)	<=2 (S)	<=2/38 (S)	<=4 (S)
12/15/2005	CC0378	<i>Escherichia coli</i>	16 (R)	64 (R)	<=16 (S)	<=250 (S)	<=2 (S)	<=2/38 (S)	8 (S)
12/15/2005	29213	CONTROL- <i>S. aureus</i>	<=4 (S)	<=16 (S)	<=16 (S)	500 (R)	<=2 (S)	<=2/38 (S)	<=4 (S)
12/15/2005	GSTP12	CONTROL-GSTP-12/ <i>E. coli</i>	>32 (R)	>128 (R)	128 (R)	>500 (R)	>16 (R)	>4/76 (R)	>32 (R)
12/15/2005	27853	CONTROL- <i>P. aeruginosa</i>	16 (R)	>128 (R)	<=16 (S)	>500 (R)	>16 (R)	>4/76 (R)	32 (R)
12/15/2005	25922	CONTROL- <i>E. coli</i>	<=4 (S)	32 (S)	<=16 (S)	<=250 (S)	<=2 (S)	<=2/38 (S)	<=4 (S)
12/15/2005	29212	CONTROL- <i>E. faecalis</i>	16 (R)	<=16 (S)	32 (S)	>500 (R)	<=2 (S)	<=2/38 (S)	16 (R)

Figure 1. Dade Behring MicroScan Turbidity Meter (Dade Behring, California).

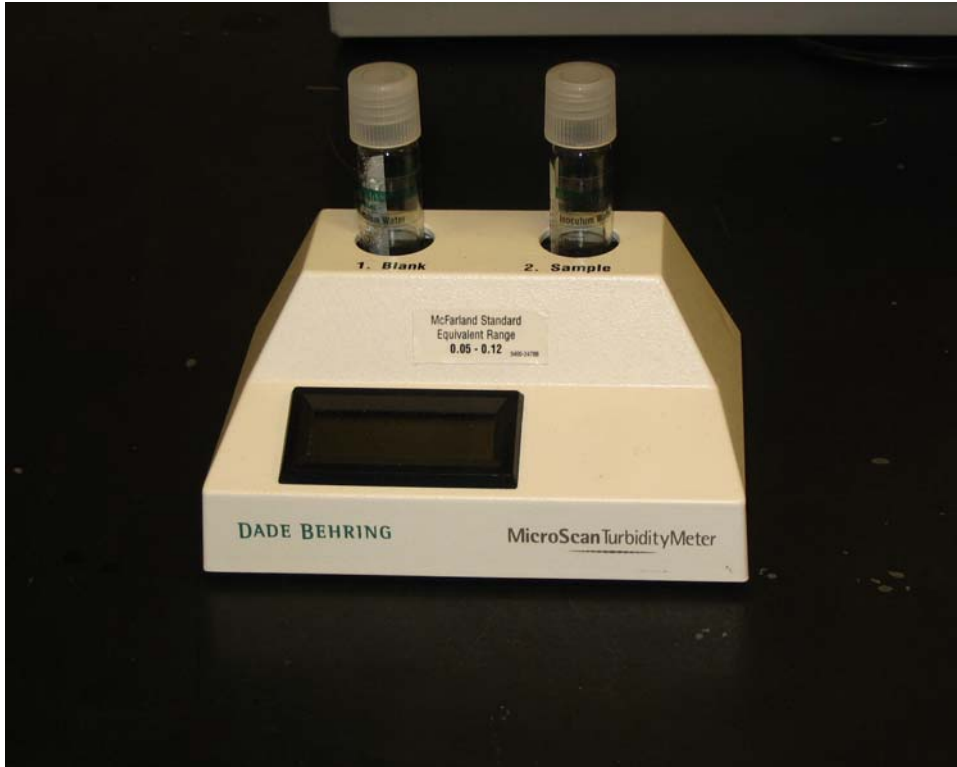


Figure 2. Dade Behring Inoculator D Set with the lid on (Dade Behring, California).

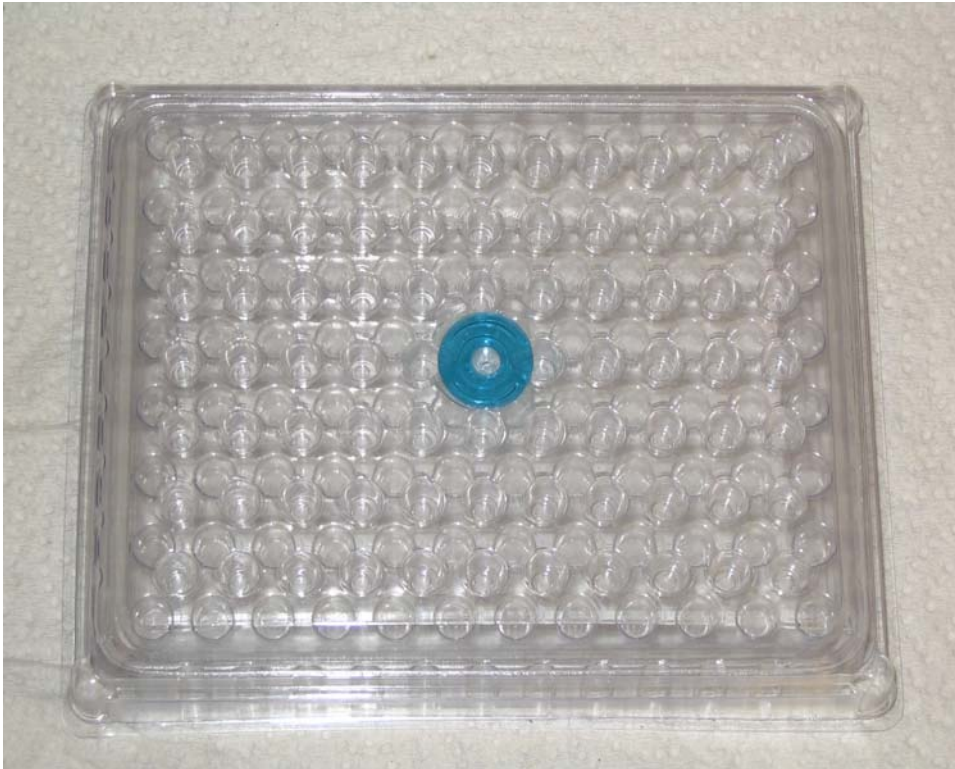


Figure 3. Both halves of the Dade Behring Inoculator D Set (Dade Behring, California).

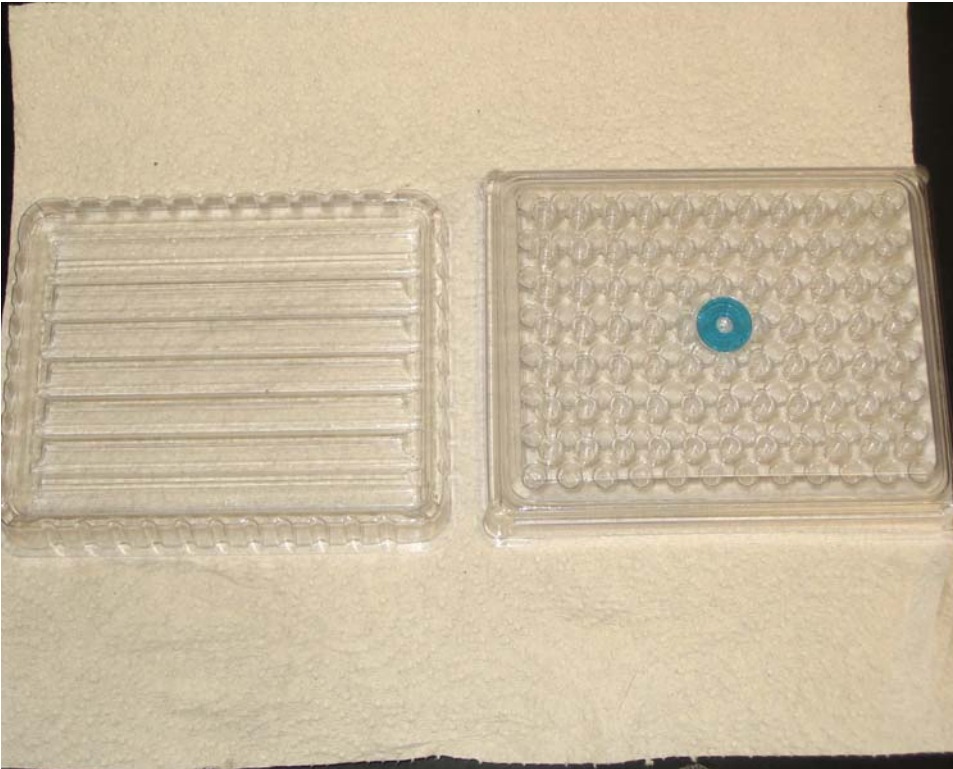


Figure 4. Dade Behring MicroScan Renok Pipette (Dade Behring, California).



Figure 5. The 96-well suspension plate, containing a preset panel of desiccated antibiotics, designed by Dade Behring specifically for the National Oceanic and Atmospheric Administration (NOAA) (Dade Behring, California).



Appendix II
(List of Acronyms)

Acronym	Definition
ARA	Antibiotic Resistance Analysis
CAMHB	Cation-Adjusted Mueller-Hinton Broth
CVDC	Clemson Veterinary Diagnostic Center
DTS	Debilitated Turtle Syndrome
MIC	Minimal Inhibitory Concentration
NOAA	National Oceanic and Atmospheric Administration
OD	Optical Density
PCV	Packed Cell Volume
SCDNR	South Carolina Department of Natural Resources
TP	Total Protein
TSA	Tryptic Soy Agar
TSB	Bacto Tryptic Soy Broth

Appendix III

(Hours Spent at the South Carolina Aquarium Sea Turtle Rescue Program)

A total of 487.5 hours were accumulated while working with the South Carolina Aquarium Sea Turtle Rescue Program. In addition to the tasks that were mentioned in the body of the report, I also performed several other duties, which I will now list.

- ❖ Participated in the “Head-Start” Program- The South Carolina Aquarium is permitted to receive a specified number of hatchlings each year to raise in the “Head-Start Program.” The hatchlings are maintained at the South Carolina Aquarium for approximately four years, before they are released into the open ocean. This program benefits sea turtle populations by releasing the turtles when they are large enough in size to preclude their being prey to many marine species, and thus theoretically increase their survivability. I assisted in this program by feeding and maintaining some of the juveniles currently involved in this program.
- ❖ Transferred sea turtles from the interior of the South Carolina Aquarium to the outside environment in large plastic buckets. This allowed the sea turtles to receive some exposure to sunlight to assist them in re-calcifying their weakened carapaces.
- ❖ Force-fed sea turtles that were uninterested in eating-When sea turtles were uninterested in eating, or too weak to eat, we had to devise an alternative method for getting them to ingest oral medications. First, we pulverized the medications into a powder and mixed them with some mineral oil (to assist in absorption) and Boost (Novartis Nutrition Corporation, 2005). We then placed the turtle on an incline to reduce regurgitation. Next, we pried its mouth open and placed a small piece of a PVC pipe in its mouth to keep it opened. We then fed a lubricated tube

down its esophagus and into its stomach. Once the tube was in place, we used a syringe to shoot approximately 40-50 cc's of the mixture through the tube and into the turtle's stomach. In most cases, the turtle regurgitated approximately half of this mixture. Generally, the turtles that were sick enough to be force fed were too far gone, and did not survive.

- ❖ Attended some of the necropsies performed on the turtles that had died while undergoing treatment at the South Carolina Aquarium, which were used to determine the possible causes of the turtles' demise.
- ❖ Under proper supervision, extracted blood samples from the dorsal cervical sinus of the sea turtles and analyzed it to monitor the turtles' packed cell volume, total protein, and glucose levels. We used this data to ascertain how each turtle was responding to their respective treatments, and to approximate when each turtle would be ready for release.
- ❖ Administered medications intramuscularly.

The following chart lists all of the sea turtles that were admitted to the South Carolina Aquarium Sea Turtle Rescue Program:

Sea Turtles Admitted to the South Carolina Aquarium Sea Turtle Rescue Program

Number	Name	Date Admitted	Location Found	Diagnosis	Outcome
SCA # 1	"Jetty"	5-19-05	Charleston, SC	DTS	Released 8-19-05 on Seabrook Island
SCA # 2	"Gardner"	6-2-05	Garden City, SC	DTS	Released 8-19-05 on Seabrook Island
	"Myrtle"	6-9-05	North Myrtle Beach, SC	DTS	Not involved in study; died during treatment at SCA (6-16-05)
SCA # 3	"Hunter"	6-10-05	Huntington Beach State Park, SC	DTS	Died during treatment at SCA (7-11-05)
SCA # 4	"Surfside"	6-24-05	Surfside Beach	DTS	Released 8-30-05 on the Isle of Palms
SCA # 5	"Sullivan"	6-30-05	Sullivan's Island, SC	DTS	Died during treatment at SCA (7-6-05)
SCA # 6	"Horry"	7-7-05	Myrtle Beach State Park, SC	DTS	Died during treatment at SCA (7-10-05)
SCA # 7	"Little Cumberland"	7-20-05	Little Cumberland Island, GA	DTS	Died during treatment at SCA (7-26-05)
SCA # 8	"Deweese"	8-6-05	Deweese Island, SC	DTS	Released 11-17-05 on Deweese Island
	"St. Simons"	8-12-05	St. Simon's Island, SC	DTS	Set to be released this summer; not involved in study because received antibiotics 3 days before a sample could be obtained

In addition to the above mentioned sea turtles, I also assisted in caring for seven hatchlings and juveniles, all of which were involved in the "Head Start" Program.

Appendix IV
(Hours Spent at the National Oceanic and Atmospheric Administration-NOAA)

A total of 59.5 hours were accumulated while performing the laboratory work necessary to complete this project at NOAA. In addition to storing my bacterial samples and preparing my samples for ARA and to determine the MIC of each isolate, I also assisted in making media and cleaning and sterilizing laboratory equipment.

Individuals with whom I interacted to complete this project include: Dr. Dave Owens, Dr. Tom Sheridan, Dr. Susan Morrison, Dr. Kem Fronabarger, Dr. Al Segars, Mike Arendt, Brian Thompson, Kelly Thorvalson, Dr. Jan Gooch, Dr. Pamela Parnell, Dr. Craig Harms, and Dr. Terry Norton.