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SER-2014-15433

APR 22 2015

Lieutenant Colonel John Litz
85th District Engineer and Commander
Charleston District
United States Army Corps of Engineers
69A Hagood Avenue
Charleston, South Carolina 29403

Ref.: Endangered Species Act Biological Opinion for Charleston Harbor Post 45 Project

Dear Lieutenant Colonel Litz,

The enclosed Biological Opinion ("Opinion") was prepared by the National Marine Fisheries Service (NMFS) pursuant to Section 7(a)(2) of the Endangered Species Act (ESA). The Opinion considers the effects of the proposed action on the following listed species: sea turtles (loggerhead, green, leatherback, hawksbill, and Kemp's ridley sea turtles), whales (North Atlantic right and humpback), sturgeon (Atlantic and shortnose), and the recently proposed critical habitat for the North Atlantic right whale. NMFS also concludes that the proposed action is not likely to jeopardize the continued existence of sea turtles (loggerhead, green, and Kemp's ridley sea turtles), and sturgeon (Atlantic and shortnose) and is not likely to adversely affect proposed North Atlantic right whale critical habitat, North Atlantic right and Humpback whales, and leatherback and hawksbill sea turtles.

NMFS is providing an Incidental Take Statement with the Opinion. The Incidental Take Statement (ITS) describes reasonable and prudent measures NMFS considers necessary or appropriate to minimize the impact of incidental take associated with this action. The ITS also specifies nondiscretionary terms and conditions, including monitoring and reporting requirements with which the U.S. Army Corps of Engineers (USACE) must comply to carry out the reasonable and prudent measures. Incidental take from actions described or evaluated in this Opinion that complies with these terms and conditions will be exempt from the ESA's prohibition against the take of listed species.

Please direct questions regarding this Opinion to Karla Reece, Consultation Biologist, by phone at 727-824-5312, or by email at karla.reece@noaa.gov.

Sincerely,

FOR Roy E. Crabtree, Ph.D.
Regional Administrator

Enclosure

File: 1514-22.F.2

Endangered Species Act

Biological Opinion

for the

Charleston Harbor Post 45 Project

NMFS Consultation Number: SER-2014-15433

Federal Action Agency: U.S. Army Corps of Engineers

Affected Species and Critical Habitat Determinations:

Common Name	Scientific Name	ESA Listing Status[1]	Action Agency Effect Determination[2]
Turtles			
Green	*Chelonia mydas*	E/T	LAA
Kemp's ridley	*Lepidochelys kempii*	E	LAA
Loggerhead	*Caretta caretta*	T	LAA
Hawksbill	*Eretmochelys imbricata*	E	NLAA
Leatherback	*Dermochelys coriacea*	E	NLAA
Fish			
Shortnose sturgeon	*Acipenser brevirostrum*	E	NLAA
Atlantic sturgeon	*Acipenser oxyrinchus*	E	LAA
Marine Mammals			
North Atlantic right whale	*Eubalaena glacialis*	E	NLAA
Humpback whale	*Megaptera novaeangliae*	E	NLAA
Critical Habitat			
North Atlantic right whale, Unit 2	Proposed for Designation February 20, 2015	Proposed	NLAA

Consultation Conducted By: National Marine Fisheries Service Southeast Region

Issued By: FOR Roy E. Crabtree, Ph.D. Regional Administrator

Date: April 22, 2015

[1] E = Endangered, T = Threatened

[2] NLAA = may affect, not likely to adversely affect; LAA = may affect, likely to adversely affect

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ACRONYMS AND ABBREVIATIONS

ASM	At Sea Monitoring
BA	Biological Assessment
CAFO	Concentrated Animal Feeding Operations
CCL	curved carapace length
CHP45	Charleston Harbor Post 45 Project
cm	centimeter(s)
CMP	Coastal Migratory Pelagics
CPA	Central Planning Area
CPUE	Catch Per Unit Effort
yd^3	cubic yard(s)
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
DPS	Distinct Population Segment
DQM	Dredging Quality Management
DTRU	Dry Tortugas Recovery Unit
EA	Environmental Assessment
EFDC	Environmental Fluid Dynamics Code
EPA	U.S. Environmental Protection Agency
ESA	Endangered Species Act
EWS	Early Warning System
F/SER3	NMFS Southeast Regional Office, Protected Resources Division
FMP	Fishery Management Plan
FP	Fibropapillomatosis
ft	feet/foot
FWOP	future without project
FWRI	Fish and Wildlife Research Institute
GADNR	Georgia Department of Natural Resources
GCRU	Greater Caribbean Recovery Unit
GOM	Gulf of Mexico
HSI	Habitat Suitability Index
IPCC	Intergovernmental Panel on Climate Change
ITS	Incidental Take Statement
LAA	may affect, likely to adversely affect

lb	pound(s)
MCY	million cubic yard(s)
MLLW	mean lower low water
MSA	Mixed Stock Analysis
NGMRU	Northern Gulf of Mexico Recovery Unit
NLAA	may affect, not likely to adversely affect
NMFS	National Marine Fisheries Service
NOAA	National Oceanic and Atmospheric Administration
NRU	Northern Recovery Unit
NWA	Northwest Atlantic
ODMDS	Ocean Dredged Material Disposal Site
Opinion	Biological Opinion
PCB	polychlorinated biphenyls
PED	pre-construction engineering and design
PFRU	Peninsular Florida Recovery Unit
RM	River Mile
RPAs	Reasonable and Prudent Alternatives
RPMs	Reasonable and Prudent Measures
SAB	South Atlantic Bight
SARBO	South Atlantic Regional Biological Opinion
SCDNR	South Carolina Department of Natural Resources
SCL	straight carapace length
SEFSC	Southeast Fisheries Science Center
SERO	Southeast Regional Office
STDW	Sea Turtle Data Warehouse
STSSN	Sea Turtle Stranding and Salvage Network
T&Cs	terms and conditions
TED	turtle excluder devices
TEWG	Turtle Expert Working Group
TSP	Tentatively Selected Plan
UMAM	Uniform Mitigation Assessment Method
USACE	U.S. Army Corps of Engineers
USFWS	U.S. Fish and Wildlife Service
YOY	young-of-the-year

INTRODUCTION

Section 7(a)(2) of the Endangered Species Act (ESA) of 1973, as amended (16 U.S.C. § 1531 et seq.), requires each federal agency to "insure that any action authorized, funded, or carried out by such agency is not likely to jeopardize the continued existence of any endangered or threatened species or result in the destruction or adverse modification of critical habitat of such species." To fulfill this obligation, Section 7(a)(2) requires federal agencies to consult with the appropriate Secretary on any action that "may affect" listed species or designated critical habitat. The National Marine Fisheries Service (NMFS) and the U.S. Fish and Wildlife Service (USFWS) share responsibilities for administering the ESA. Consultations on most listed marine species and their designated critical habitat are conducted between the action agency and NMFS.

Consultation is concluded after the appropriate Secretary (of the Department of Commerce if NMFS, or the Department of the Interior if USFWS) determines that the action is not likely to adversely affect listed species or critical habitat, or issues a Biological Opinion ("Opinion") that identifies whether a proposed action is likely to jeopardize the continued existence of a listed species, or destroy or adversely modify critical habitat. If either of those circumstances is expected, the Secretary identifies reasonable and prudent alternatives (RPAs) to the action as proposed that can avoid jeopardizing listed species or resulting in the destruction/adverse modification of critical habitat. In the Opinion, the Secretary states the amount or extent of incidental take of the listed species that may occur, develops reasonable and prudent measures (RPMs) to reduce the effect of take, monitors to validate the expected effects of the action, and recommends conservation measures to further conserve the species.

This document represents NMFS's Opinion based on our review of impacts associated with the Charleston Harbor Post 45 Project (CHP45). The U.S. Army Corps of Engineers (USACE) Charleston District is the permitting authority and will be carrying out the project. This Opinion analyzes project effects on ESA-listed species and proposed critical habitat in accordance with Section 7 of the ESA. This Opinion is based on project information provided by the USACE. Additional information for this Opinion was provided by the USACE, or was obtained from a variety of sources including published and unpublished literature cited herein and other sources of information including the USACE Sea Turtle Data Warehouse (STDW) (http://el.erdc.usace.army.mil/seaturtles/index.cfm).

1 CONSULTATION HISTORY

February 6, 2012 through October, 2014	Pre-consultation discussions: The scope of the project, data collection plans, sediment testing, and other project aspects were discussed in various meetings, conference calls, and emails between the USACE, NMFS, and other interested parties.
June 13, 2014	USACE sent NMFS the draft Biological Assessment (BA) for comment.
July 8, 2014	NMFS sent USACE comments, questions, and requests regarding information in the BA.
October 10, 2014	USACE formally requested Section 7 Consultation for the CHP45 and provided NMFS a copy of the Draft Integrated Feasibility Report and Environmental Impact Statement (DEIS). NMFS initiated consultation.
January 6, through February 23, 2015	NMFS requested additional information clarifying information in the DEIS and BA.
January 22, 2015	NMFS sent the project description for the Opinion to the USACE to review for accuracy.
January 23, 2015	USACE requested by email a copy of the Draft Opinion for review prior to the issuance of the Opinion.
January 30, 2015	USACE provided NMFS with a revised project description.
February 20, 2015	USACE requested conference on North Atlantic right whale proposed critical habitat.
March 16, 2015	USACE requested NMFS provide a copy of the draft Terms and Conditions and Reasonable and Prudent Measures (RPMs) from this Opinion.
March 20, 2015	NMFS provided USACE with the draft RPMS and Terms and Conditions (T&Cs).
March 25, 2015	USACE provided comments to NMFS regarding the draft RPMs and T&Cs.
March 26, 2015	NMFS and USACE held a conference call to discuss USACE concerns with the RPMS and T&Cs.
March 27, 2015	NMFS and USACE held a conference call to discuss the draft RPMs and T&Cs.
March 30, 2015	USACE notified NMFS they will propose North Atlantic right whale conservations measures (now included in Section 2.1.3.2 of this Opinion). As well, the USACE cancelled their request for a copy of the Draft Opinion.
April 3, 2015	The USACE revised the project description to include a conservation measure related to the North Atlantic right whale (Section 2.1.3.2 of this Opinion)

2 DESCRIPTION OF THE PROPOSED ACTION AND THE ACTION AREA

2.1 Proposed Action

"Action" means all activities or programs of any kind authorized, funded, or carried out, in whole or in part, by federal agencies. NMFS's determination regarding the effects of the proposed action is based on the description of the action in this section of the Opinion. Any changes to the proposed action may negate the findings of the present consultation and may require reinitiation of consultation with NMFS.

The Tentatively Selected Plan (TSP) as described in the DEIS proposes to extend and deepen the entrance channel in combination with deepening and widening the inner harbor channels that primarily serve containerships (Figure 1). The proposed navigation improvements include:

- Deepen the existing entrance channel from a project depth of -47 feet (ft) to -54 ft mean lower low water (MLLW) over the existing 800-ft bottom width, while reducing the existing stepped 1,000-ft width to 944 ft from an existing depth of -42 ft to a depth of -49 ft MLLW (fully described in section 2.1.3.1 of this Opinion and depicted in Figure 6 of that section). The proposed deepening of the entrance channel also includes 1-2 ft of required overdepth dredging and up to an additional 2 ft of allowable overdepth dredging.
- Extend the entrance channel approximately 3 miles seaward to about the -57 ft MLLW contour.
- Deepen the inner harbor from an existing project depth of -45 ft to -52 ft MLLW to the Wando Welch Terminal on the Wando River and the new SCSPA Navy Base Terminal on the Cooper River, and from -45 ft to -48 ft MLLW for the reaches above that facility to the Northern Charleston Terminal (over varying expanded bottom widths ranging from 400-1,800 ft). The proposed deepening of the inner harbor also includes overdepth dredging and advance maintenance dredging as outlined in Appendix A of the DEIS (USACE 2014).
- Enlarge the existing turning basins to a 1,800-ft diameter at the Wando Welch and new Navy base terminals to accommodate Post Panamax Generation II and Generation III container ships.
- Enlarge the North Charleston Terminal turning basin to a 1,650-ft-diameter to accommodate Post Panamax Generation II and Generation III container ships.
- Raise dikes and place dredged material from the upper harbor at the existing upland confined disposal facilities at Clouter Creek, Yellow House Creek, and Daniel Island; place material dredged from the lower harbor and sediment from the entrance channel at the expanded Ocean Dredged Material Disposal Site (ODMDS). Place some of the rock dredged from the entrance channel along the outside of the entrance channel and along the edges of the ODMDS to create hard bottom habitat.

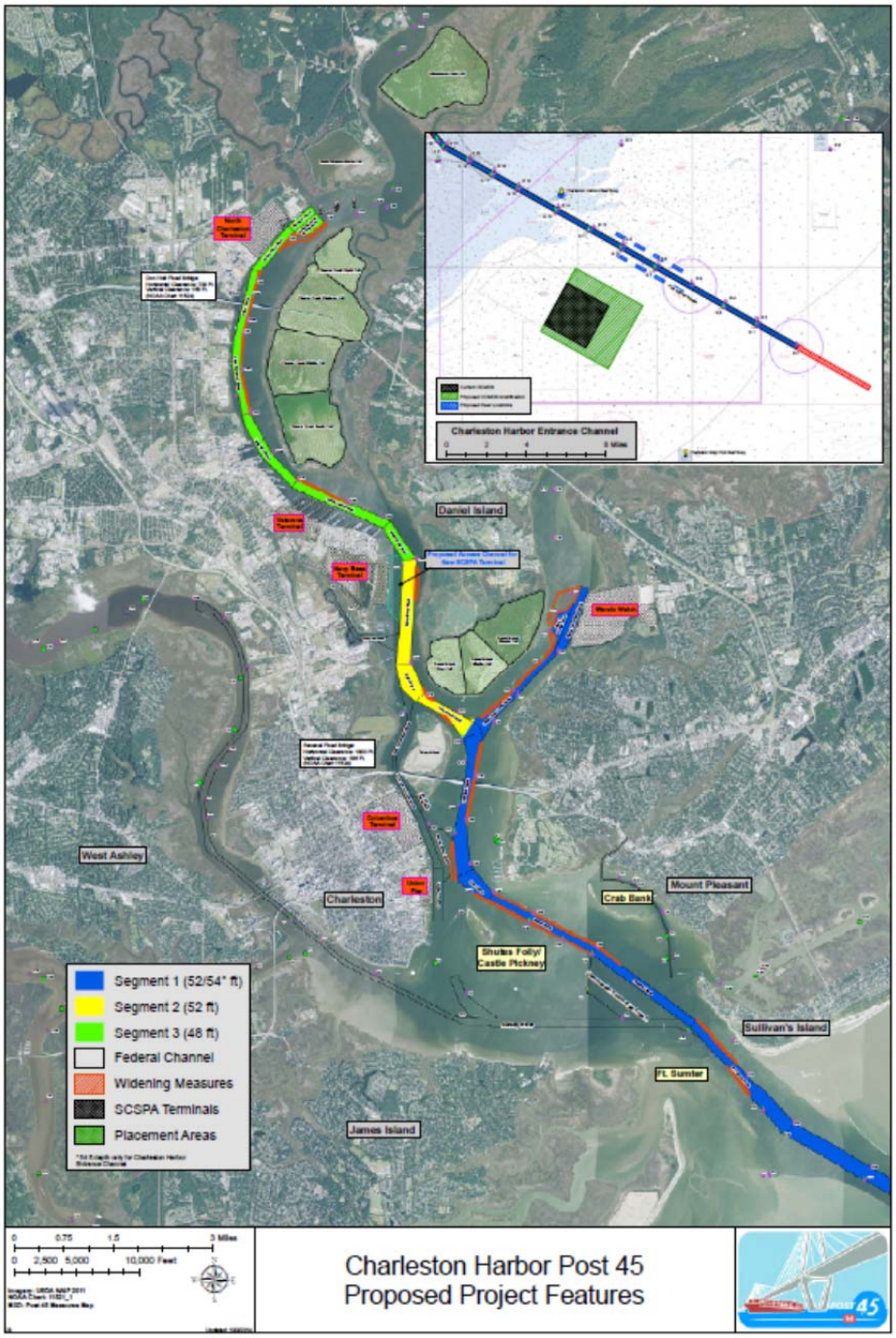


Figure 1. CHP45 Project (USACE 2014)

The TSP will indirectly impact about 281 acres of freshwater wetlands (emergent and forested) through changes in salinity, which could require compensatory mitigation in the form of preservation and conveyance of an estimated 831 acres to the U.S. Forest Service (USACE 2014). Additionally, direct impacts to about 29 acres of hard bottom habitat within the footprint of the entrance channel extension footprint require mitigation. To compensate for impacts to hard bottom habitat, rock dredged from the entrance channel will be used to construct artificial reefs. Two reefs will be constructed specifically to compensate for lost habitat in the

channel and 6 reefs will be constructed as a beneficial use of dredged material. In total, 8 new 33-acre artificial reefs will be created along the margins of the entrance channel. Additionally, at the request of the South Carolina Department of Natural Resources (SCDNR), approximately 240,000 cubic yards (yd^3) of rock material will also be placed at SCDNR's existing 25-acre Charleston Nearshore Reef. The total quantity of reef habitat created far exceeds the required mitigation. However, construction of the reefs near the entrance channel is less expensive than transporting the material to the ODMDS. The total amount of reef habitat created was limited based on conversations with SCDNR biologists in order to maintain an appropriate and productive balance of habitat types in the area.

2.1.1 Dredging

Construction of the TSP will generate about 40 million cubic yards (MCY) of dredged material. Of that, about 29 MCY will be placed in the offshore ODMDS; 2.9 MCY will be placed in Daniel Island Disposal Area; 900,000 yd^3 will be placed in Clouter Creek Disposal Area; 2.3 MCY will be placed in Yellow House Creek Disposal Area; 360,000 yd^3 will be used for artificial reef mitigation; approximately 6.3 MCY for ODMDS berm construction; 1.9 MCY for reef construction along either side of the Entrance Channel; and 240,000 yd^3 will be placed at an existing DNR artificial reef site. The proposed action would increase the area dredged from about 3,619 acres (for the current Charleston Ship Channel) to about 4,152 acres. Of the additional 533 acres that would be affected by proposed dredging, about 349 acres of the additional dredging area would result from the extension of the Entrance Channel and the remaining 184 acres of additional area to be dredged would result from channel widening. All areas to be dredged are shown in Figure 1.

The exact construction methodology will be determined by the contractor selected through contracting process. However, assumptions regarding various construction techniques that could be used were made for planning and estimating purposes as detailed in Table 1. Dredged material from widening and deepening efforts will be excavated using a hydraulic cutterhead dredge, hopper dredge, or mechanical excavator. Based on testing results (found in Appendix B of the DEIS;(USACE 2014), the rock material will not require blasting).

Table 1. Dredging areas, dredging methods, quantity, and duration (USACE 2014)

Channel Reach	Dredge Type	Number of Dredges	Estimated Number of Vessel transits	Placement Area	Dredge Quantity in Cubic Yards (yd^3)	Duration (Months)
Fort Sumter Reach, EC1	Large Hopper	1	524	ODMDS	2,357,022	4.06
Fort Sumter Reach, EC1	Medium Hopper	3	1,571	ODMDS	3,928,371	4.24
Fort Sumter Reach, EC1	Rock cutter	1	378	ODMDS Berm	2,266,766	8.72
Fort Sumter Reach, EC1	Rock cutter	1	10	DNR Site	60,000	0.34
Fort Sumter Reach, EC1	Rock cutter	1	70	Reef Placement	420,000	1.77
Fort Sumter Reach, EC1	Clamshell with bucket	1	110	ODMDS Berm	660,000	6.51
Fort Sumter Reach, EC1	Clamshell w/rock bucket	1	60	Mitigation Site	360,000	3.98

Channel Reach	Dredge Type	Number of Dredges	Estimated Number of Vessel transits	Placement Area	Dredge Quantity in Cubic Yards (yd^3)	Duration (Months)
Fort Sumter Reach, EC1	Clamshell w/rock bucket	1	30	DNR Site	180,000	1.99
Fort Sumter Reach, EC2	Large Hopper	1	432	ODMDS	1,943,512	3.54
Fort Sumter Reach, EC2	Medium Hopper	3	1,166	ODMDS	2,915,267	3.70
Fort Sumter Reach, EC2	Rock cutter	1	557	ODMDS Berm	3,346,872	12.77
Fort Sumter Reach, EC2	Rock cutter	1	70	Reef Placement	420,000	1.91
Fort Sumter Reach, EC2	Clamshell w/rock bucket	1	180	Reef Placement	1,080,000	10.97
Mount Pleasant Reach	Clamshell	1	140	ODMDS	840,083	1.52
Rebellion Reach	Clamshell	1	180	ODMDS	1,081,341	1.96
Bennis Reach	Clamshell	2	324	ODMDS	1,942,858	2.80
Horse Reach	Clamshell	2	59	ODMDS	350,996	0.53
Hog Island Reach	Clamshell	2	352	ODMDS	2,109,994	3.15
Wando River Lower Reach	Clamshell	2	295	ODMDS	1,769,070	2.55
Wando River Upper Reach	Clamshell	2	106	ODMDS	636,251	1.05
Wando River Turning Basin	Clamshell	2	547	ODMDS	3,284,633	4.52
Segment 1 Total					**31,953,036**	**82.58**
Drum Island Reach	Clamshell	2	153	ODMDS	917,473	1.45
Myers Bend	Clamshell	2	142	ODMDS	853,689	1.28
Daniel Island Reach	Pipeline	2	N/A	Daniel Island	2,211,957	2.17
Segment 2 Total					**3,983,119**	**4.9**
Daniel Island Bend	Pipeline	2	N/A	Daniel Island	74,551	0.28
Clouter Creek Reach	Pipeline	2	N/A	Daniel Island	583,150	1.23
Navy Yard Reach	Pipeline	2	N/A	Clouter Creek	358,816	0.74
North Charleston Reach	Pipeline	2	N/A	Clouter Creek	532,693	0.61
Filbin Creek Reach	Pipeline	2	N/A	Yellow House Creek	405,420	0.75
Filbin/Port Terminal Intersect	Pipeline	2	N/A	Yellow House Creek	31,692	0.08
Port Terminal Reach	Pipeline	2	N/A	Yellow House Creek	160,376	0.3
Ordnance Reach	Pipeline	2	N/A	Yellow House Creek	118,091	0.33
Ordnance Reach Turning Basin	Pipeline	2	N/A	Yellow House Creek	1,549,313	1.7
Segment 3 Total					**3,814,102**	**6.02**
North Charleston Terminal Berthing Area	Pipeline	1	N/A	Yellow House Creek	41,001	0.21

Channel Reach	Dredge Type	Number of Dredges	Estimated Number of Vessel transits	Placement Area	Dredge Quantity in Cubic Yards (yd^3)	Duration (Months)
Navy Base Terminal Berthing Area	Pipeline	1	N/A	Daniel Island	474,551	1.03
Wando Terminal Berthing Area	Pipeline	1	N/A	Daniel Island	157,633	0.32
Berthing Areas Total					**673,185**	**1.56**
Total Construction					**40,423,442**	**95**

2.1.1.1 Dredged Material Placement

Material dredged from channel deepening and widening will be distributed among the ODMDS, 2 mitigation-required reef construction sites, 6 beneficial use reef construction sites, a DNR reef construction site, and upland confined disposal areas as summarized in Table 1 and shown in Figures 2 and 3. The improvements that will be required include the raising of dikes within the footprint of the existing upland confined disposal facilities at Clouter Creek, Yellow House Creek, and/or Daniel Island and the expansion of the existing ODMDS to provide increased capacity for new work and maintenance material (Action being addressed jointly by the U.S. Environmental Protection Agency [EPA] and USACE in a Section 102 site modification Environmental Assessment [EA]). The ODMDS is in the process of being expanded by the EPA and should be available prior to project construction. Section 7 consultation for the ODMDS expansion will be carried out separate from this Opinion.

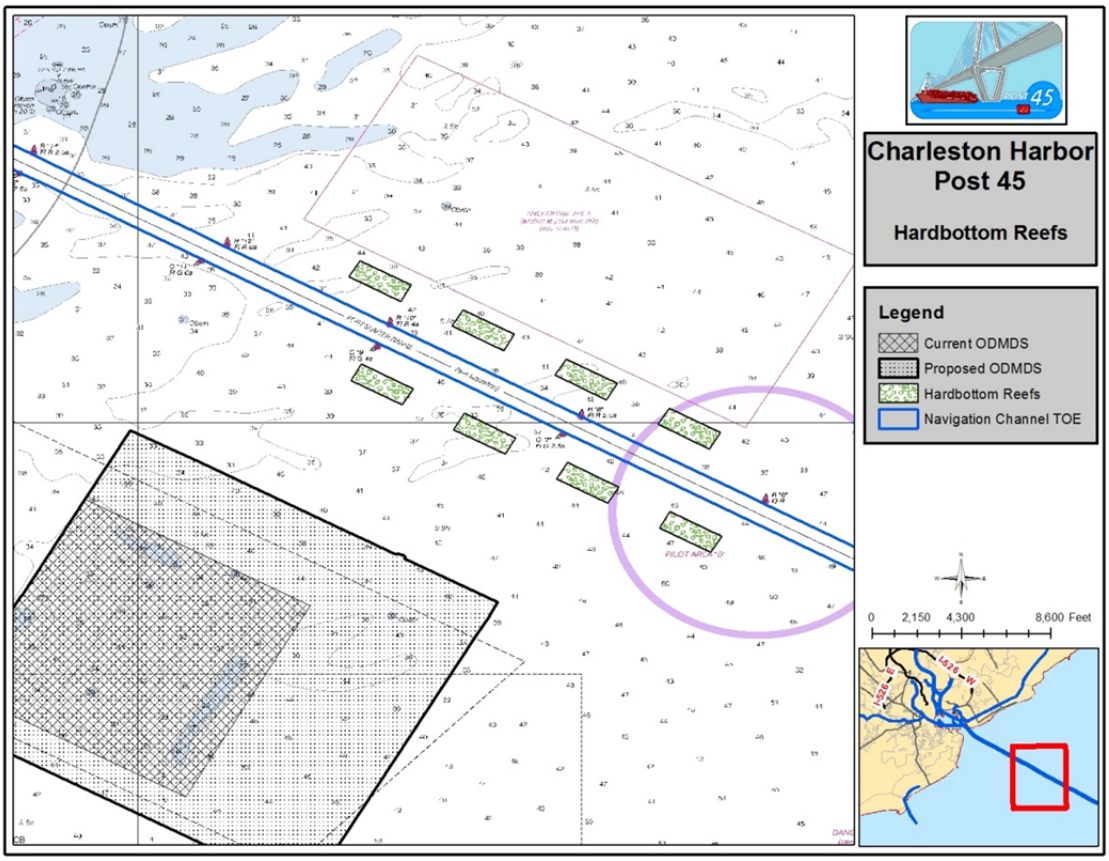


Figure 2. Location of the ODMDS (current and proposed expanded) and approximate locations for hard bottom reefs. Image from the DEIS (USACE 2014)

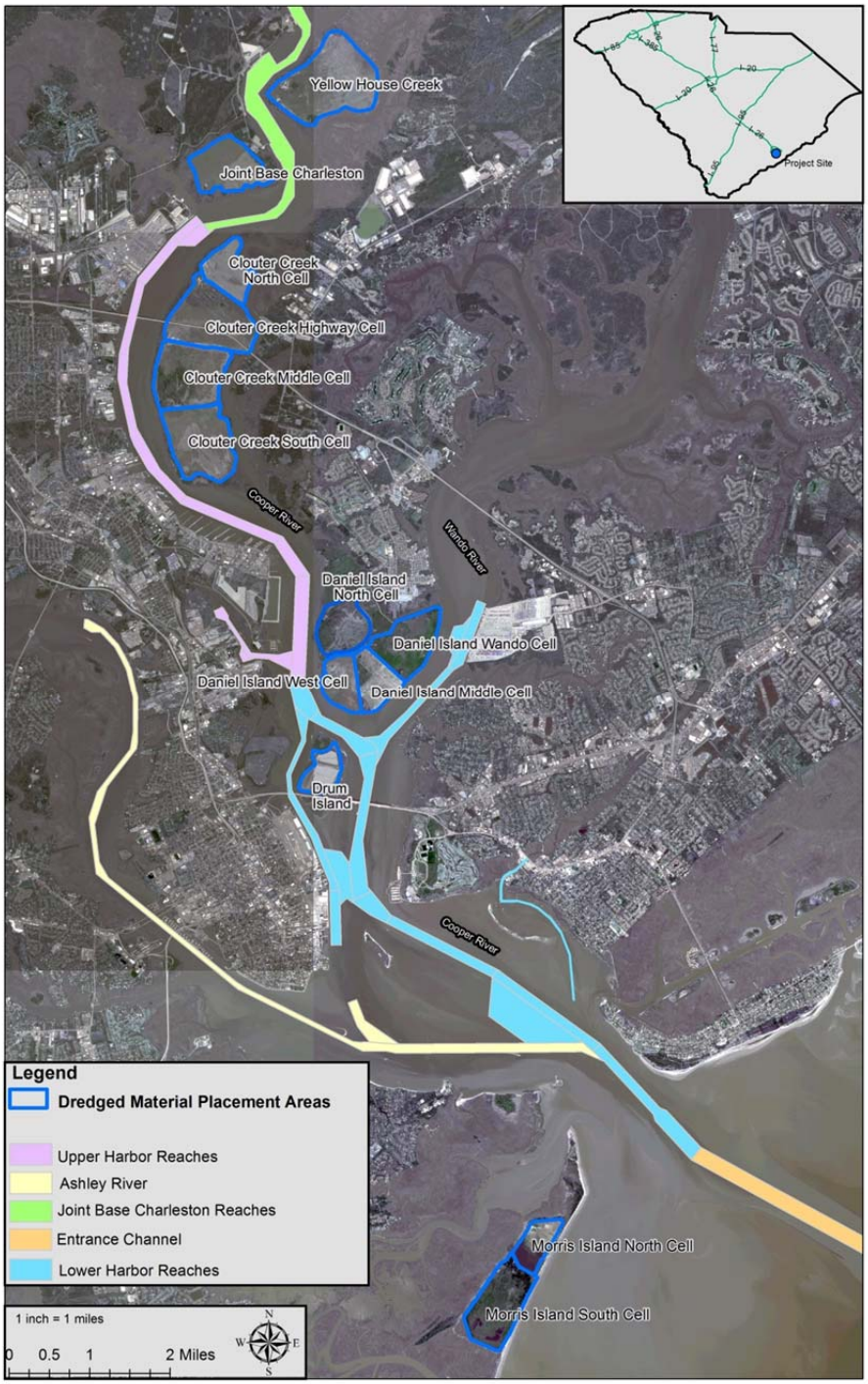


Figure 3. The locations of dredged material disposal sites in Charleston Harbor.
The Morris Island North and South cells may receive material beneficially as described in Section 2.1.1.4.3 (USACE 2014)[3].

[3] Drum Island and Morris island are depicted on Figure 3 as being a disposal area, but no dredged material will be placed in those areas, according to the DEIS (USACE 2014).

2.1.1.2 Dredge Material Transport Vessels

Three types of barges are generally used to transport dredged material to disposal sites, which include a split hull barge/scow, bottom dump barge/scow, or a flat-top barge/scow. All 3 barge types are typically pushed or pulled to the disposal site by a tug and travel at slow speeds. Medium sized hopper dredges will operate below 10 knots at all times, both fully loaded and unloaded. While large hoppers can travel at speeds of 11.1 knots while loaded and 12.8 knots while unloaded, the USACE has committed to voluntary speed reductions as described in Section 2.1.3.2, with all dredge related vessels operating in the Atlantic Ocean to travel at speeds under 10 knots from November 1 through April 30 as a conservation measure to protect right whales. Approximately 7,456 vessel transits will carry dredged material to the ODMDS site or other beneficial use sites in the ocean (Table 2).

Table 2. Number of Vessel Transits to Dispose Dredged Material

Channel Reach	Dredge Type	Placement Area	Estimated Number of Transits
Fort Sumter Reach, EC1	Large Hopper	ODMDS	524
Fort Sumter Reach, EC1	Medium Hopper	ODMDS	1,571
Fort Sumter Reach, EC1	Rock cutter	ODMDS Berm	378
Fort Sumter Reach, EC1	Rock cutter	DNR Site	10
Fort Sumter Reach, EC1	Rock cutter	Reef Placement	70
Fort Sumter Reach, EC1	Clamshell with bucket	ODMDS Berm	110
Fort Sumter Reach, EC1	Clamshell w/rock bucket	Mitigation Site	60
Fort Sumter Reach, EC1	Clamshell w/rock bucket	DNR Site	30
Fort Sumter Reach, EC2	Large Hopper	ODMDS	432
Fort Sumter Reach, EC2	Medium Hopper	ODMDS	1,166
Fort Sumter Reach, EC2	Rock cutter	ODMDS Berm	557
Fort Sumter Reach, EC2	Rock cutter	Reef Placement	70
Fort Sumter Reach, EC2	Clamshell w/rock bucket	Reef Placement	180
Mount Pleasant Reach	Clamshell	ODMDS	140
Rebellion Reach	Clamshell	ODMDS	180
Bennis Reach	Clamshell	ODMDS	324
Horse Reach	Clamshell	ODMDS	59
Hog Island Reach	Clamshell	ODMDS	352
Wando River Lower Reach	Clamshell	ODMDS	295
Wando River Upper Reach	Clamshell	ODMDS	106
Wando River Turning Basin	Clamshell	ODMDS	547
Drum Island Reach	Clamshell	ODMDS	153
Myers Bend	Clamshell	ODMDS	142
Approximate number of vessel transits:			**7,456**

2.1.1.3 Disposal Area Modifications

Disposal area modifications will occur in the uplands at Yellow House Creek Disposal Area, Daniel Island Disposal Area, and Clouter Creek Disposal Area and will increase the capacity for these disposal areas.

2.1.1.4 Beneficial Use of Dredged Material

The CHP45 will use dredged material beneficially for ODMDS berm creation, hard bottom habitat creation (reef locations), and possibly for other beneficial uses not yet determined.

2.1.1.4.1 ODMDS Berm Creation

To protect hard bottom habitat, from being buried by sediment migrating from the ODMDS, limestone rock from the entrance channel will also be used to construct an "L" shaped berm along the south and west perimeters of the ODMDS (Figure 4). This area represents approximately 427 acres of the ODMDS. The dimensions will be roughly 15,000 ft by 16,000 ft by 600 ft. The berm will be built on roughly a 3:1 slope, and will rise to about 10 ft above the natural bottom elevation but no higher than -25 ft MLLW. The reef will serve multiple purposes, including hard bottom habitat, fish habitat, and sediment containment. This beneficial use project will use smaller material to create the base of the berm and the outer portion of the berm will be created with larger rock dredged with a clamshell dredge. This will serve to increase the surface area of the reef, thereby enhancing habitat value.

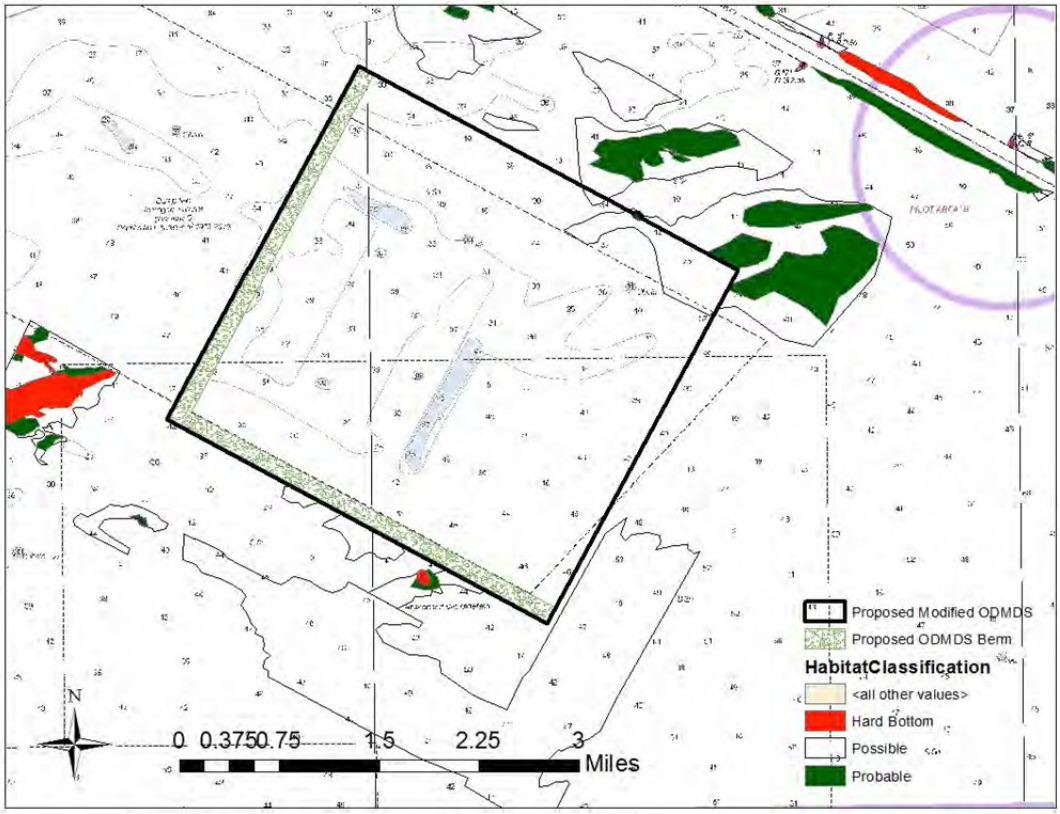


Figure 4. Proposed ODMDS and location of hard bottom habitat and the sediment containment/habitat berm (USACE 2014)

2.1.1.4.2 Hard Bottom Habitat Creation

Limestone rock will be dredged from within the entrance channel and used to create as substrate for sessile invertebrates, and structure for fish species after being placed within strategic locations nearby the channel. The USACE will construct 8 new 33-acre reef sites: 4 located along the north side of the channel and 4 located along the south side of the channel (Figure 2). Prior to construction, the locations of these reefs will be refined and coordinated with the resource agencies. These reefs will provide extensive bathymetric features located between approximately 6 nautical miles (nmi) offshore of Charleston Harbor out to approximately 10 nmi. Two of the reefs will be constructed to optimize hard bottom habitat for use as mitigation sites, and the other 6 sites will be specifically for beneficial use of dredged material. More detail on the hard bottom reef sites can be found in Appendix H (Hard Bottom Resources) and Appendix P (Mitigation) of the DEIS (USACE 2014). Additionally, at the request of the SCDNR Artificial Reef Program, approximately 240,000 yd^3 of rock material will also be deposited at the 25-acre Charleston Nearshore Reef site. The SCDNR Charleston Nearshore Reef site is discussed in Appendix M2 (404(b)(1) evaluation) (USACE 2014)(Figure 5), because it is within state waters inside of the 3-nmi limit.

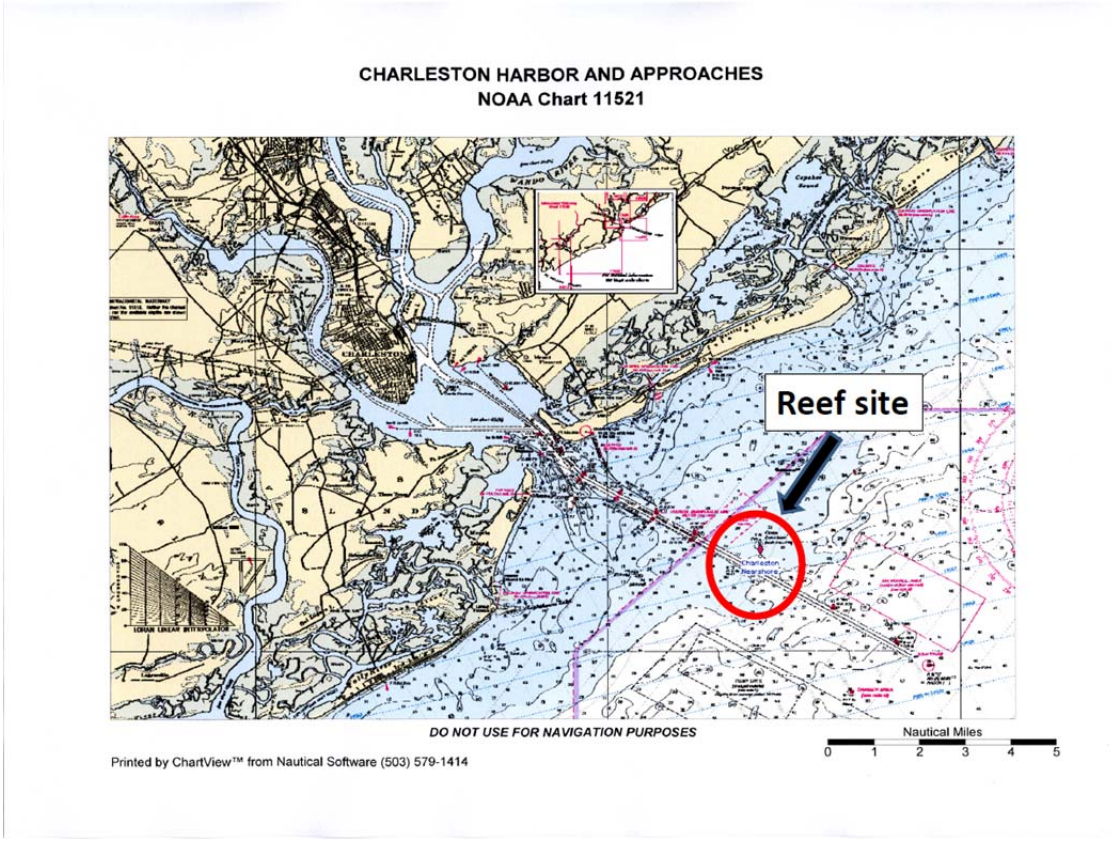


Figure 5. SCDNR Nearshore Reef Site Location (USACE 2014)

Two Mitigation Sites: A grid-based approach will be used to construct the reef structures at the mitigation sites. Each site will consist of sixteen (16) 300-ft by 300-ft cells that combine to create a 33-acre patch reef area about 600 ft wide and 2,400 ft long. The cell arrangement will be 2 across by 8 long. The 16 cells will each require 8,000-12,000 yd^3, or approximately 128,000-192,000 yd^3 total of fill material to create the desired peak vertical relief of 3.5-4.5 ft

(after settling) and the desired aerial coverage within each cell of 75%. All of the material used to construct the mitigation sites will be excavated using a clamshell dredge to maximize the size of the material used to construct the reef and minimize dispersal of the material.

6 Placement Sites: The 6 new 33-acre placement sites will each have the same dimensions as the mitigation sites (600 ft wide by 2,400 ft long). Dredged material will be placed to cover the entire area to a peak relief height of about 10 ft (after settlement) and tapering to natural contours/conditions at the site margins. Each site will utilize about 320,000 yd^3 of material. Smaller material generated by the hopper dredges will be used to create a base that will be covered with larger material dredged using clamshell dredges to create the desired habitat. To estimate volumes, it was assumed that the average height of material will be about 6 ft based on a peak relief height of about 10 ft and tapering to 0 ft at the margins of the sites.

2.1.1.4.3 Other Potential Beneficial Use Sites

Other locations identified as possible beneficial use sites are: Crab Bank enhancement, Shutes Folly Enhancement, bird nesting island creation, and nearshore placement off Morris Island. In all cases, the precise size and scope of the projects will be determined during the pre-construction, engineering, and design (PED) phase, and will be dependent on a source of suitable material. Because detailed information about the size, scope, and construction methodology is not available for these beneficial use sites, they cannot be analyzed in a meaningful way in this Opinion and therefore are not analyzed as part of the action. The USACE may need to reinitiate this consultation should they opt to move forward with these beneficial use sites.

Post-Dredging Operations

Since dredging equipment does not typically result in a perfectly smooth and even channel bottom (see discussion above), a drag bar, chain, or other item may be pulled along the channel bottom to smooth down high spots and fill in low spots. This finishing technique also reduces the need for additional dredging to remove any high spots that may have been missed by the dredging equipment. Historically, these types of activities have not been required in the project area; however they may occur from Myers Bend and the Wando River downstream to Segment 1 in the entrance channel. The USACE has not yet determined the extent of post-dredging operations that will be required.

Operations and Maintenance Considerations

Maintenance dredging will generally be conducted by hopper, clamshell, and cutterhead dredges and will operate essentially the same as current practices documented in the Charleston Harbor Dredged Material Management Program Preliminary Assessment. Maintenance dredging will use the same placement areas as those used for existing conditions, and the duration and frequency of dredging events will not change due to the proposed project. All future maintenance dredging in Charleston Harbor and the entrance channel will be carried out under the NMFS South Atlantic Regional Biological Opinion (SARBO)(NMFS 1997b) and will not be discussed further in this Opinion[4].

[4] SARBO is currently being revised and updated and will address the impacts of dredging a bigger and deeper channel.

2.1.2 Freshwater Wetlands Mitigation

Indirect wetland impacts are predicted to occur through a shift from fresh/brackish wetland vegetation to brackish/salt wetland vegetation. The impacts result from converting one dominant type of wetland vegetation to another (freshwater to salt tolerant species). The USACE has used the Uniform Mitigation Assessment Method (UMAM), as defined in Florida Administrative Code (FAC) 62-345, to determine the necessary amount of compensatory mitigation. Using the UMAM tool, the proposed project will require approximately 831 acres of freshwater forested and emergent wetlands throughout these parcels. The Charleston District has determined that preservation of land within the proclamation boundary of the Francis Marion National Forest best meets of the compensatory mitigation requirements. The preserved lands will provide important physical, chemical, and biological functions for the Cooper River Basin and will contribute to the sustainability of the watershed by ensuring the functions of bottomland hardwood wetlands and emergent wetlands on these properties are sustained in perpetuity.

2.1.3 Conservation Measures

Below are the conservation measures the USACE will implement during the construction of the CHP45 to avoid impacts to ESA-listed species and associated habitats. All conservation measures are described fully in Appendix P of the DEIS (USACE 2014).

2.1.3.1 General Conservation Measures:

1. No Anchoring in Hard Bottom Habitat: As a means to avoid or minimize effects of anchorage during dredging on hard bottom habitat, the design specifications will be written to require the contractor to avoid anchoring of equipment within adjacent hard bottom habitat. The approximate locations of these resources will be shown in the contract drawings. If the contractor is required to anchor outside the channel to utilize a cutterhead dredge, the anchor(s) shall be placed to avoid affecting any of the identified hard bottom habitat or any of the created hard bottom habitat reefs.

2. Hard Bottom Habitat Impact Minimization: The existing channel side slopes will be maintained by extending them downward, rather than the more typical approach of maintaining the existing bottom width and extending the side slopes outward. The measure would avoid all direct impacts to hard bottom habitat along the margins of the entrance channel (Figure 6).

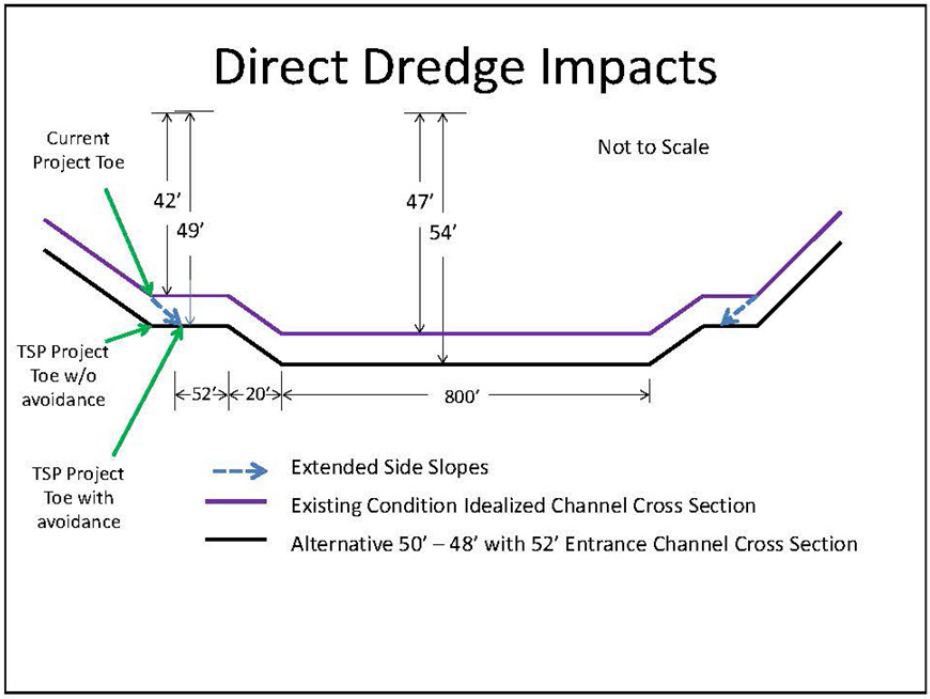


Figure 6. Proposed side slope extension to avoid hard bottom areas (USACE 2014)

3. No Blasting: Geotechnical investigations involving rock strength analysis indicates the rock that requires removal to obtain the project depth can be removed with either a cutterhead dredge or a rock bucket clamshell dredge and will not require blasting. As a result of their analysis, blasting will not be used, therefore eliminating any potential effects resulting from noise impacts to marine mammals and fish that blasting may cause.

4. Dredging Quality Management (DQM) will be used to monitor dredged material placement in the ODMDS and other nearshore disposal sites.

5. Observer Requirement: For the construction of the proposed project, during transit to and from offshore disposal areas, an observer will monitor from the bridge during daylight hours for the presence of endangered species.

2.1.3.2 Right Whale Avoidance Measures

The USACE has established precautionary collision avoidance measures to be implemented during dredging and disposal operations that take place during the time North Atlantic right whales are present in waters offshore of USACE projects (between November 1 and April 30). For the construction of the proposed project, these precautionary measures include[5]:

[5] The Corps of Engineers (Corps) has assessed these conditions specific to resource protection within the Charleston Harbor area and their effect on the ability of the Corps to execute this project considering the anticipated additional costs associated with these measures. The Corps has indicated that while these conditions are acceptable for this project at this time, every Corps project is evaluated independently and the Corps does not endorse a generic application of these conditions to other Corps projects. Further, the Corps' inclusion of these conditions is not an endorsement of the application of these conditions to any project of any other federal agency.

1. Before the initiation of each project, at the pre-construction/partnering meeting, the USACE will brief the contractor on the presence of the species, and review the requirements for right whale protection.

2. Each contractor will be required to instruct all personnel associated with the dredging/construction project about the possible presence of endangered North Atlantic right whales in the area and the need to avoid collisions. Each contractor will also be required to brief his or her personnel concerning the civil and criminal penalties for harming, harassing, or killing species that are protected under the ESA and the Marine Mammal Protection Act of 1972. Dredges and all other disposal and attendant vessels are required to stop, alter course, or otherwise maneuver to avoid approaching the known location of a North Atlantic right whale. The contractor will be required to submit an endangered species watch plan that is adequate to protect North Atlantic right whales from the impacts of the proposed work.

3. Dredge-related vessel speed reductions to protect whales: From November 1 through April 30, all project vessels operating in the Atlantic Ocean that are greater than or equal to 65 ft in overall length will maintain a speed of 10-knots or less during right whale migration/calving season while in specified areas designated as proposed right whale critical habitat and slow to 5 knots or the minimum safe navigable speed when visibility is reduced by night, fog, precipitation, or if sea state is greater than 3 ft. As set forth in this proposed action, the speed limits for project vessels shall only apply until a new SARBO is signed, at which time the project would abide by the conditions set forth in the new SARBO.

4. Whale observers: From November 1 through April 30, one observer with at-sea large whale identification experience will be on watch 100% during daylight hours (30 mins before sunrise to 30 mins after sunset).

5. Operational AIS transmitters powered on and transmitting: The USACE shall provide NMFS's Southeast Regional Office (takereport.nmfsser@noaa.gov with reference to this Opinion) with an end-of-project report including all AIS data with any deviances from (a) within 30 days of completion of the North Atlantic right whale migration and calving season (April 30). This report may be incorporated into the final report summarizing the results of the hopper dredging project.

2.1.3.3 Sea Turtles

1. Relocation Trawling: USACE will coordinate with NMFS staff to determine if relocation and abundance trawling during hopper dredging is necessary if and when an excessive level of take has occurred during project construction[6].

2. Environmental Windows: The environmental windows for turtle-safe hopper dredging have targeted the winter months since sea turtle abundance is dramatically reduced at

[6] Because the USACE does not specify a trigger for relocation trawling, NMFS must specify what the trigger is and how relocation trawling will be implemented in the T&Cs of this Opinion in order to authorize any relocation trawling incidental take.

water temperatures below 13°C and typically absent during temperatures below 11°C (Moon et al. 1997; STAC 2006). The typical environmental window for Charleston Harbor maintenance hopper dredging is December 1 through March 31[7]. During construction of the proposed project, the USACE will follow this window; however, if conditions are such that it's beneficial to continue dredging, the window may be expanded to April 30. No environmental windows are necessary for mechanical or hydraulic cutterhead dredging.

3. Hopper Dredging: The USACE will conduct all CHP45 hopper dredging consistent with Reasonable and Prudent Measures (RPMs) outlined in the current SARBO or any subsequent revisions of SARBO (NMFS 1997b). These measures include but are not limited to inflow/overflow screening, observers, etc.

2.1.4 Monitoring and Adaptive Management:

The Mitigation Monitoring and Adaptive Management Plan is detailed in Appendix P of the DEIS (USACE 2014). Listed below are the components specific to dredging, disposal, and ecosystem changes. These descriptions are found in the DEIS is full detail.

2.1.4.1 Monitoring during Construction

A real-time placement monitoring/verification system, or DQM, will be used to monitor placement within specific patterns and tolerances as well as to monitor how the placement actually occurred. The use of DQM is required for USACE federal navigation projects that use a scow or hopper dredge to dispose of material in an ODMDS. Information regarding vessel loads, vessel tracks, and discharge time and location records is recorded and maintained in the DQM system and will provide 24/7 coverage of operations. Bathymetric surveys will be completed twice during construction of the reef to ensure that each of the cells in the mitigation reef plan are obtaining a peak vertical relief of 4-5 ft. If the cells are not reaching the desired relief with 1 scow load, additional scows will be directed to those sites.

2.1.4.2 Post-Construction Monitoring

Approximately 20% (~ 6 cells) of the mitigation reef cells will be analyzed similar to the methods described in Appendix P of the DEIS, Section 5.1.1.1 "Pre-Construction Impact Refinement." The cells will be chosen either randomly or strategically based on input from SCDNR and NMFS. Monitoring will occur within 6 months of completion of the reef and will continue once a year for 4 years in order to fully account for the anticipated 3.5 years until recovery. If the ecological success criteria, based on the abundance and diversity of sessile invertebrates at the impact site, are met prior to the completion of 4 years of monitoring, a meeting will be held with the resource agencies and monitoring efforts will be ceased. If success criteria are not met at the end of 4 years, USACE will meet with SCDNR and NMFS to determine corrective actions.

2.1.4.3 Water Quality Monitoring

The objective of the water quality monitoring effort for this study will be to determine if there is a significant difference between pre- and post-construction water quality data (Appendix P, Section 5.3 of the DEIS). If there is a significant increase beyond the model-predicted changes,

[7] The SARBO dredging window for hopper dredging in the Charleston area is November 1 through May 31.

consultation with resource agencies will be used to develop adaptive management measures for dissolved oxygen (DO) and indirect wetland impacts from salinity changes. Continuous data collection of mid-depth and bottom salinity and DO at high and low tides will be collected for at least 1 year before construction, during construction, and after construction throughout the Charleston Harbor estuary, including the Ashley, Cooper, and Wando Rivers.

2.1.4.4 Monitoring for Beneficial Use of Dredged Material Projects

Beneficial uses have been proposed for this project including expanding Crab Bank, expanding/protecting Shutes Folly, nearshore placement off Morris Island, and/or a new bird nesting island off the south jetty. Since details related to beneficial use have been moved to the PED phase of the project, details have not yet been established for monitoring plans.. Monitoring for any of these projects will be coordinated with the resource agencies and will be consistent with the goals of the project and USACE's Engineering With Nature Program principles.[8]

2.2 Summary of the Proposed Action

Because some of the exact details for construction of the project are not yet determined, we plan to complete our analysis with the available information, resolving any remaining uncertainties in a precautionary manner to protect the species. Therefore, in the absence of finalized project plans, we will complete this Opinion based on the following assumptions that could be considered the worst case scenario of all options:

1. Approximately 40 MCY of material will be removed from the Port of Charleston and the Charleston entrance channel.

2. Hopper dredging (the most harmful means of dredging to the species affected by the action) will be carried out only in the Fort Sumter Reach (areas EC1 and EC2). Approximately 11.15 MCY of material will be dredged using a hopper dredge.

3. Dredging will be conducted year-round for a period of 6 years with hopper dredging carried out only during the appropriate environmental window spelled out in Section 2.1.3.3, item 2.

4. Approximately 7,456 loads (depending on the size of the transport barges or hopper dredges used) of dredged material will be transported to offshore ODMDS and beneficial use locations.

5. General plans for the beneficial use of dredged material have been spelled out in Section 2.1.1.4 of this Opinion. The USACE has committed to choosing beneficial use locations that will not interfere with existing hard bottom. Should beneficial use locations impact hard bottom, potentially affect ESA-listed species, or any designated critical habitat under the purview of NMFS, the USACE will need to reinitiate consultation.

[8] The Engineering With Nature Program enables more sustainable delivery of economic, social, and environmental benefits associated with water resources infrastructure. http://el.erdc.usace.army.mil/ewn/

6. The USACE has not yet determined the extent of post-dredging operations (bed-leveling or similar activities) that will be required, but it has stated that they may occur from Myers Bend and the Wando River downstream to Segment 1 in the entrance channel. In the absence of a description of the extent of effort expected, we must assume that they will occur in this entire area.

2.3 Action Area

The action area is defined by regulation as "all areas to be affected directly or indirectly by the federal action and not merely the immediate area involved in the action" (50 CFR 402.02). Charleston Harbor is located in a natural tidal estuary, formed by the confluence of the Cooper, Ashley, and Wando Rivers. The project area encompasses the offshore entrance channel, offshore and landside confined dredged material disposal sites, inner harbor channels, and any extension of the water bodies and shorelines that could be impacted by proposed improvements (Figure 1). For purposes of this consultation, NMFS will consider the action area to be all areas to be widened and/or dredged from the North Charleston Terminal down the Cooper River and from the Wando Welch Terminal down the Wando River to the confluence with the Cooper River, out the entrance channel to a point approximately 17.3 miles offshore to the sea buoy and from the entrance channel to the hard bottom habitat creation (reef) areas, the SCDNR reef, and the ODMDS location offshore. Additionally, the action area will include any areas upstream of the project that experience changes in salinity, or approximately 3 miles south of "The Tee" (Figure 7), with the red line denoting the upper extent of the action area. The full extent of the action area is shown in Figure 7.

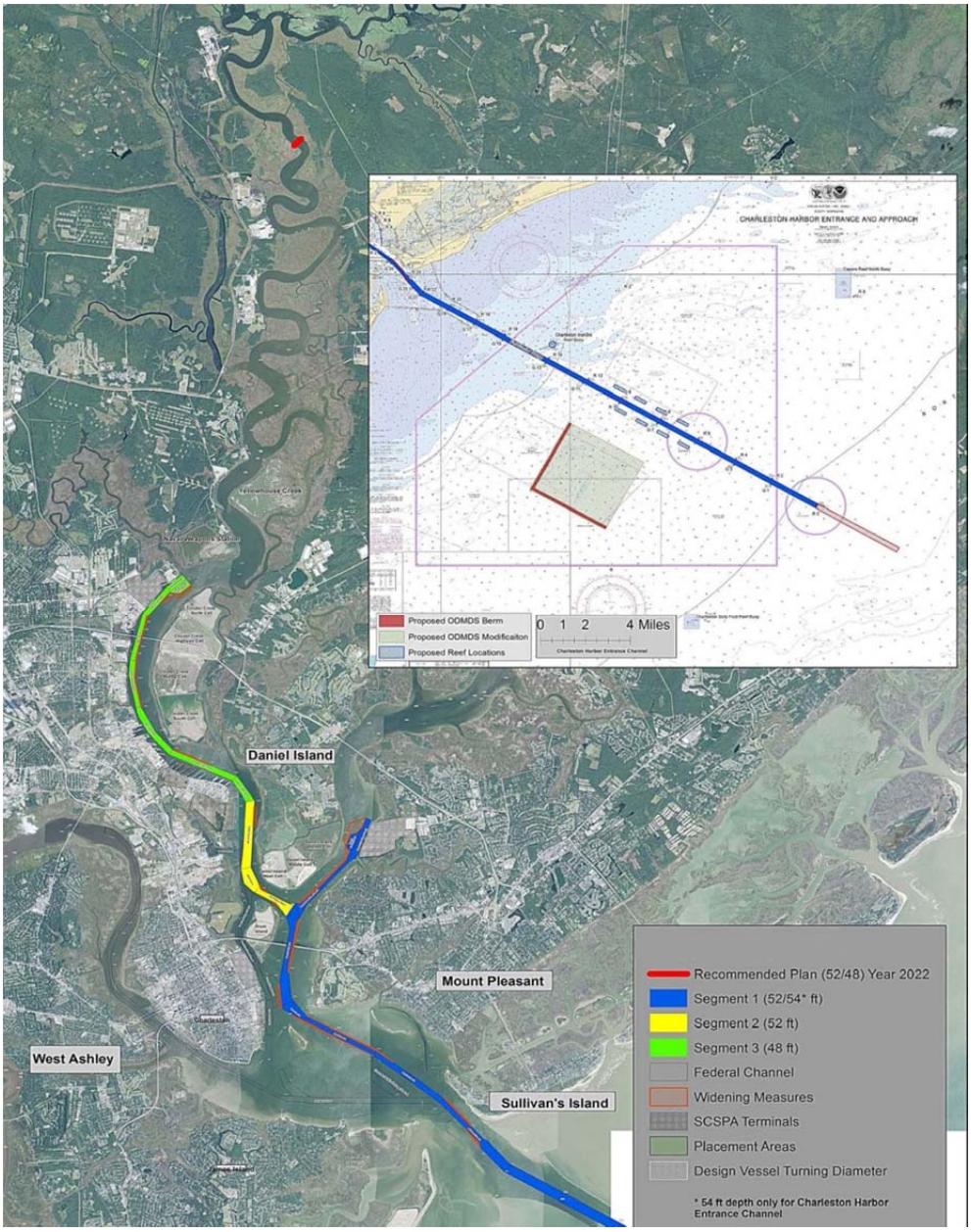


Figure 7. The action area of the project (USACE 2014)

3 STATUS OF SPECIES AND CRITICAL HABITATS

Listed species occurring within the action area that may be affected by the proposed action are itemized in Table 3 with their respective scientific names and status. The action area includes proposed designated critical habitat for the North Atlantic right whale.

Table 3. Status of Listed Species in the Action Area

Common Name	Scientific Name	ESA Listing Status[9]
Turtles		
Green	*Chelonia mydas*[10]	E/T
Kemp's ridley	*Lepidochelys kempii*	E
Loggerhead	*Caretta caretta*[11]	T
Hawksbill	*Eretmochelys imbricata*	E
Leatherback	*Dermochelys coriacea*	E
Fish		
Shortnose sturgeon	*Acipenser brevirostrum*	E
Atlantic sturgeon	*Acipenser oxyrinchus*[12]	E
Marine Mammals		
North Atlantic right whale	*Eubalaena glacialis*	E
Humpback whale	*Megaptera novaeangliae*	E
Critical Habitat		
North Atlantic right whale, Unit 2	Proposed for Designation February 20, 2015 (NMFS 2015)	Proposed

3.1 Analysis Species and Critical Habitats Not Likely to be Adversely Affected

We have determined that the proposed action being considered in this Opinion is not likely to adversely affect the North Atlantic right whale, its designated or proposed critical habitat, humpback whale, shortnosed sturgeon, leatherback sea turtles, and hawksbill sea turtles. These species are excluded from further analysis and consideration in this Opinion. The following discussion summarizes our rationale for this determination.

3.1.1 North Atlantic Right Whale

Year round, right whales can be found from Cape Cod to Nova Scotia, an area the whales use for feeding and mating. Each fall, pregnant females and others travel from this area to their only known calving area in the warm, calm coastal waters off the Southeast Atlantic Bight (SAB) which extends roughly from Cape Hatteras, North Carolina, to West Palm Beach, Florida. Recent information reveals the South Carolina coast to be part of this calving area, including the waters surrounding the Port of Charleston (Good 2008). Non-calving whales are moving between habitats continuously during the calving season (B. Zoodsma, NMFS, pers. comm. to K. Reece, NMFS, January 29, 2015). When spring arrives, the whales make the long journey back north. Aerial survey data shows regular observations or these whales off the Port of Charleston (Figure 8). Sightings off the Southeast Atlantic Coast include primarily adult females and calves, but juveniles and adult males are also commonly observed. Annual right whale migration

[9] E = Endangered, T = Threatened
[10] Green turtles are listed as threatened except for the Florida and Pacific coast of Mexico breeding populations, which are listed as endangered.
[11] NWA DPS
[12] River and in-shore habitats within the action area may affect Atlantic sturgeon from the Carolina and South Atlantic DPS; however, Atlantic sturgeon from all DPS may be affected in off-shore waters within the action area.

past the CHP45 action area (both to and from) as well as the use of calving grounds off the southeastern U.S. coast occurs from November 1 through April 30.

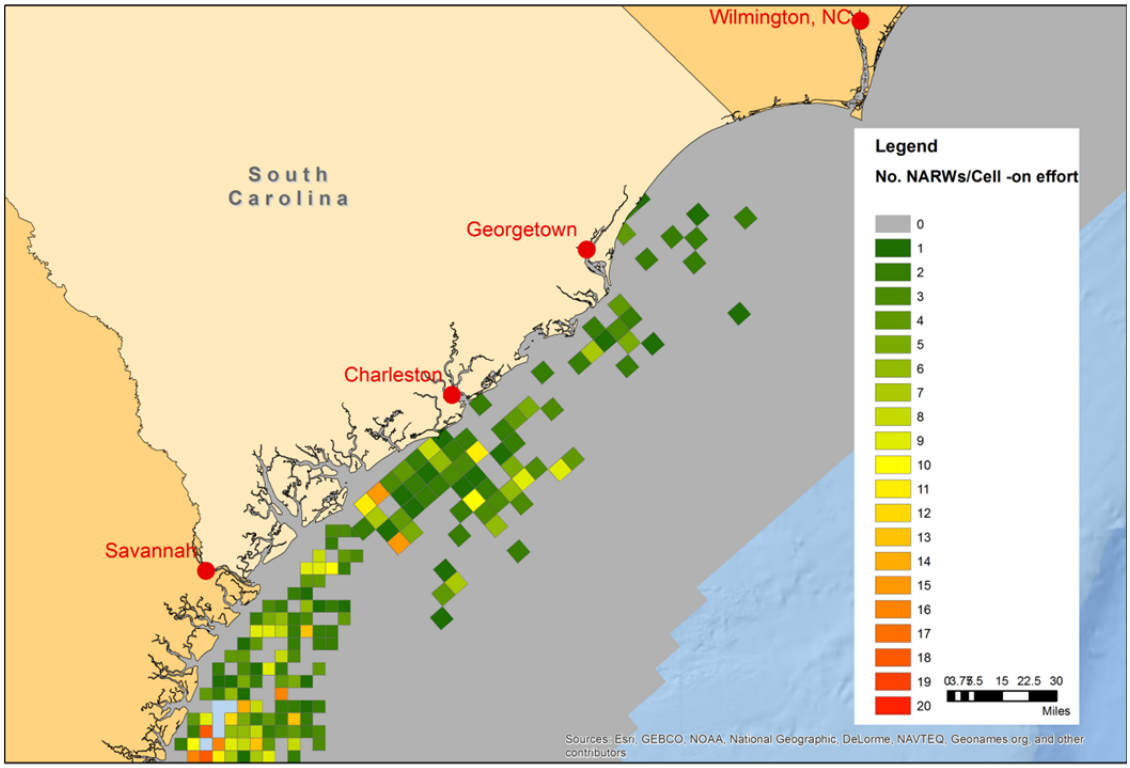


Figure 8. Cumulative North Atlantic Right Whale sightings in 3 x 3 nmi and 4.06 x 4.06 nmi grid cells for 2004/2005 through 2012/2013 seasons in the southeast United States. (Source: North Atlantic Right Whale Consortium, unpublished data and analysis by Florida Fish and Wildlife Conservation Commission)

Ship collisions caused 38% of confirmed right whale deaths from 1985-2005 (Kraus et al. 2005). Seven percent of the population exhibit scars indicative of additional, nonlethal vessel interactions (Kraus 1990). In 2011, of 4 deceased right whales encountered, half were associated with rope entanglement, 1 had multiple skull and vertebral fractures that are consistent with ship strike, and a fourth was found floating offshore with no evidence of entanglement. In January 2011, a live right whale was observed with approximately 14 propeller cuts across its body; it had been observed 5 days earlier with no injuries.

Various types and sizes of vessels have been involved in ship strikes with large whales, including container/cargo ships/freighters, tankers, steamships, U.S. Coast Guard vessels, Navy vessels, cruise ships, ferries, recreational vessels, fishing vessels, whale-watching vessels, and other vessels (Jensen and Silber 2003d). In March 2008, a 43-ft vessel traveling at 18-19 knots (20.7-21.86 mph) struck and seriously injured an adult female right whale, Eg No. 2324, about 8 nmi off the north end of Cumberland Island, Georgia (George and Naessig 2006; Zoodsma 2005). This animal was last seen in September 2005 when she was spotted in Massachusetts Bay in exceptionally poor health (Waring et al. 2012) and is now presumed dead. In May 2009, a 33.7-ft vessel reportedly struck and killed a 21.3-ft southern right whale calf in New South Wales, Australia (NSWNPS 2009).

Records of right whale ship strikes (Knowlton and Kraus 2001) and large whale ship-strike records (Jensen and Silber 2003a; Laist et al. 2001) have been compiled, and all indicate vessel speed is a principal factor in ship strikes. In assessing records in which vessel speed was known, Laist et al. (2001) found "a direct relationship between the occurrence of a whale strike and the speed of the vessel involved in the collision." The authors concluded that most deaths occurred when a vessel was traveling in excess of 14 knots. Jensen and Silber (2003) identified 292 records of known or probable ship strikes of all large whale species from 1975-2002. In 58 of the records, ship speed at the time of collision was known: Speed ranged from 2-51 knots, with an average of 18.1 knots. A majority (79%) of ship strikes occurred at speeds of 13 knots or greater. Of the 58 cases where speed was known, 19 (32.8%) resulted in serious injury to the whale. The mean vessel speed that resulted in serious injury or death to the whale was 18.6 knots (Jensen and Silber 2003a).

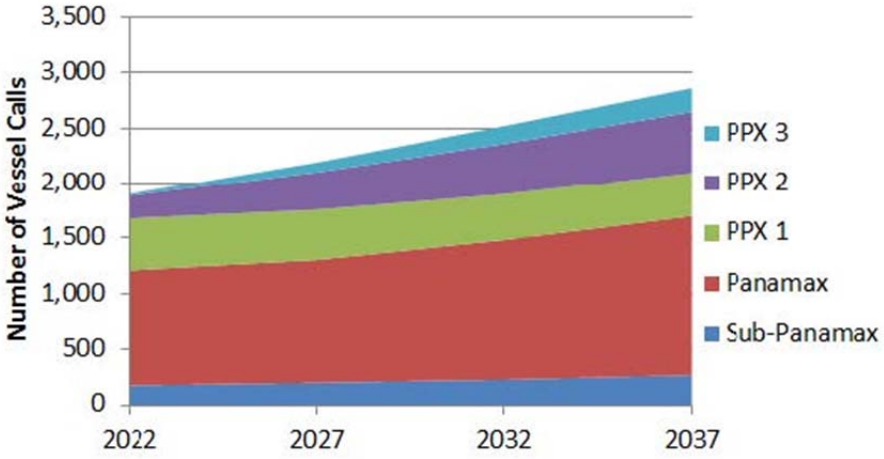
Using a total of 64 records of ship strikes in which vessel speed was known, Pace and Silber (2005) tested speed as a predictor of the probability of death or serious injury. The authors concluded that there was strong evidence that the probability of death or serious injury increased rapidly with increasing speed. Specifically, the predicted probability of serious injury or death increased from 45% to 75% as vessel speed increased from 10 knots to 14 knots, and exceeded 90% at 17 knots. Interpretation of the logistic regression curve (Jensen and Silber 2003a) implies injury or death at around 25 knots and faster. In a related study, Vanderlaan and Taggart (2007) analyzed all published historical data on vessels striking large whales. The authors found that the probability of a lethal injury resulting from a strike ranged from 20% at 9 knots to 80% at 15 knots and 100% at 21 knots or more.

Related studies of the occurrence and severity of strikes relative to vessel speed have been conducted for other species and locations. Panigada et al. (2006) concluded that vessel speed restrictions and the relocation of vessel routes in high cetacean density areas would reduce the likelihood of ship strikes of fin whales in the Mediterranean Sea. Speed zones were adopted in Florida in the early 2000s to reduce the numbers of collisions and manatee injuries resulting from collisions with boats. Laist and Shaw (2006) assessed the effectiveness of these speed zones at reducing watercraft-related manatee deaths. Watercraft-related manatee deaths did decline in the areas assessed in the paper, and the authors reported that this decline reflected the fact that well-designed speed restrictions could be effective if properly enforced. They further stated that "reduced speed allows time for animals to detect and avoid oncoming boats, and that similar measures may be useful for other marine mammal species vulnerable to collision impacts with vessels (e.g., North Atlantic right whales)" (Laist and Shaw 2006).

The behavior of whales in the path of approaching ships is uncertain, but in some cases, last-second flight responses may occur. If a whale attempts to avoid an oncoming vessel at the last minute, a burst of speed coupled with a push from the bow wave could mean that mere seconds might determine whether the whale is struck (Laist et al. 2001). A reduction in speed from 18 knots to 10 knots would give whales an additional 8.6 seconds (at a distance of 100 m) to avoid the vessel in this flight response scenario (Laist 2005, unpublished data). In a separate study involving whale behavior, Kite-Powell et al. (2007) developed a model that analyzed ship-strike risk with respect to vessel speed and whale avoidance behavior. The authors of the ship-strike

analysis assert that ship-strike risk decreases as speed decreases and the distance that the whale detects the vessel increases. Assuming certain whale behavior, the model suggests that the ship-strike risk posed by a conventional ship (e.g., containership) traveling at 20-25 knots can be reduced by 30% at a speed of 12 or 14 knots, and by 40% at a speed of 10 knots, due to the whales' increased ability to detect and avoid approaching vessels. If a whale detects and reacts to an oncoming vessel at a distance of 820 ft (250 m) or greater, it will likely avoid a ship strike, whereas at detection distances less than 328 ft (100 m), the probability of ship strike is almost 100% at speeds of 15 knots or faster. However, research on vessel-whale collisions indicates that of 3 speeds considered—10, 12, and 14 knots—adopting a speed limit of 10 knots would be the most beneficial to the recovery of the right whale population. Historically, only a small percentage of ship strikes occurred at 10 knots, and those that did usually resulted in injury rather than death (Laist et al. 2001). Nonetheless, it is important to note of the 3 speeds considered above, while a 10-knot speed restriction is most effective at reducing the risk of ship strikes, it will not eliminate the risk; there is still a 45% predicted probability of serious injury or mortality at 10 knots (Pace and Silber 2005).

The proposed action allows for larger and more fully loaded vessels to call on the port. Larger ships currently experience transportation delays due to insufficient Federal channel depths in Charleston. To reach port terminals, these larger ships must either light load, experience delays while waiting for favorable tide conditions, or both, which require more vessels to carry the same amount of goods (USACE 2104). The construction of the TSP would result in larger ships with fewer ship transits because larger ships carry more goods, thus requiring a smaller number of ships to transport the same amount of goods. However, with the increasing demands for imported goods and materials it is projected that the port will continue to receive increasing vessel calls over time (Figure 9) (USACE 2014) even without this port deepening project. Separate from port-related vessel traffic, NMFS is expecting an increase in vessel traffic related to dredge activities transiting between the navigational channel and the disposal sites during the deepening project only. The DEIS (USACE 2014) states that the duration and frequency of dredging events would be within the range of the current practices, therefore, we anticipate maintenance dredging in the future to occur at a similar interval as it has in the past.



Vessel Class	2022	2027	2032	2037
Sub-Panamax	176	202	229	269
Panamax	1,035	1,103	1,255	1,434
PPX 1	470	456	416	392
PPX 2	206	342	460	555
PPX 3	16	89	161	214
Total	**1,903**	**2,192**	**2,520**	**2,863**

Figure 9. Expected vessel calls in the Port of Charleston (USACE 2014)[13]

During the construction of CHP45, NMFS-approved endangered species observers will be required to be present to watch for marine mammals during all daytime hopper dredging and any vessel transits from the CHP45 to the ODMDS locations that occur during the right whale migration/calving season as described in Section 2.1.3.2. Observers will reduce the chances of an inadvertent collision with a right whale by increasing the likelihood of detection of a right whale, which allows for more reaction time by both the vessel and the whale. Depending on the size of the vessel used, there will be an estimated 7,456 dredge trips during the project to dispose of the dredged material in the offshore disposal areas. Right whales occur in low densities and in irregular distribution within the SAB including the areas between the entrance channel and the disposal areas (B. Zoodsma, NMFS, personal communication to K. Reece, NMFS, January 29, 2015). Given their reduced numbers and irregular habitat usage patterns, it is unlikely that right whales will be adversely impacted by dredge-related vessel transits. The likelihood of interaction is further reduced by the precautions stipulated for vessel avoidance and the USACE proposed conservation measures (Section 2.1.3.2) of this Opinion, which require project-related vessels (i.e., dredges and towed or self-propelled barges) to abide travel no faster than 10-knots during right whale migration/calving season (November 1 thought April 30) while traveling in the Atlantic Ocean between dredging and disposal areas. NMFS believes that the conservation measures limiting vessel speeds during the right whale migration/calving season of no greater than 10 knots (no greater than 5 knots at night and during periods of limited visibility) will

[13] Containerships are classified as sub-Panamax, Panamax, Post-Panamax Generation I (PPX1), Post-Panamax Generation II (PPX2), and Generation III (PPX3)

reduce the chance of an inadvertent collision with a right whale by (1) significantly increasing the likelihood of detection of a right whale that may be in, near, or approaching the path of the vessel, (2) significantly increasing the watch stander's reaction time (i.e., the time between when she or he detects the whale and takes action to avoid it), and (3) significantly increasing the likelihood that the whale may detect the oncoming vessel and possibly move out of the way to avoid being struck by it. Thus, NMFS concludes that the project's vessel-related effects on North Atlantic right whales are discountable based on the rarity of the species and on the implementation of the suite of whale conservation measures discussed above

Entrance channel extension and the construction of reefs will not cause any habitat impacts that will impact the activities of right whales. While actual construction activities may cause a whale to move away, there are ample available habitats nearby. Given the slow speed and low number of individual dredge-related vessels right whales will not be prevented from moving about in or migrating through the area. Thus, construction impacts, the extension of the entrance channel, and the construction of reefs are considered insignificant to right whales.

3.1.2 North Atlantic Right Whale Proposed Critical Habitat

Modifications to designated critical habitat for the North Atlantic right whale were proposed on February 20, 2015. Critical habitat consists of specific areas on which are found those physical or biological features essential to the conservation of the species called essential features.

North Atlantic right whales are observed calving off the southeastern U.S. coast, in an area designated as Unit 2 of the proposed North Atlantic right whale critical habitat (Figure 10). The entrance channel, the ODMDS, and the offshore reef areas are located in Unit 2. The essential features of right whale calving habitat are calm sea surface conditions, sea surface temperature, and depth. These features are dynamic in their distributions throughout the SAB in that they vary over both time and space, and their variations do not necessarily correlate with each other. As such, calving right whales likely select areas containing varying combinations of the preferred ranges of the essential features available within the SAB, as identified previously, depending on factors such as the weather (e.g., storms, prevailing winds) and the age of the calves (e.g., neonate or more mature calf).

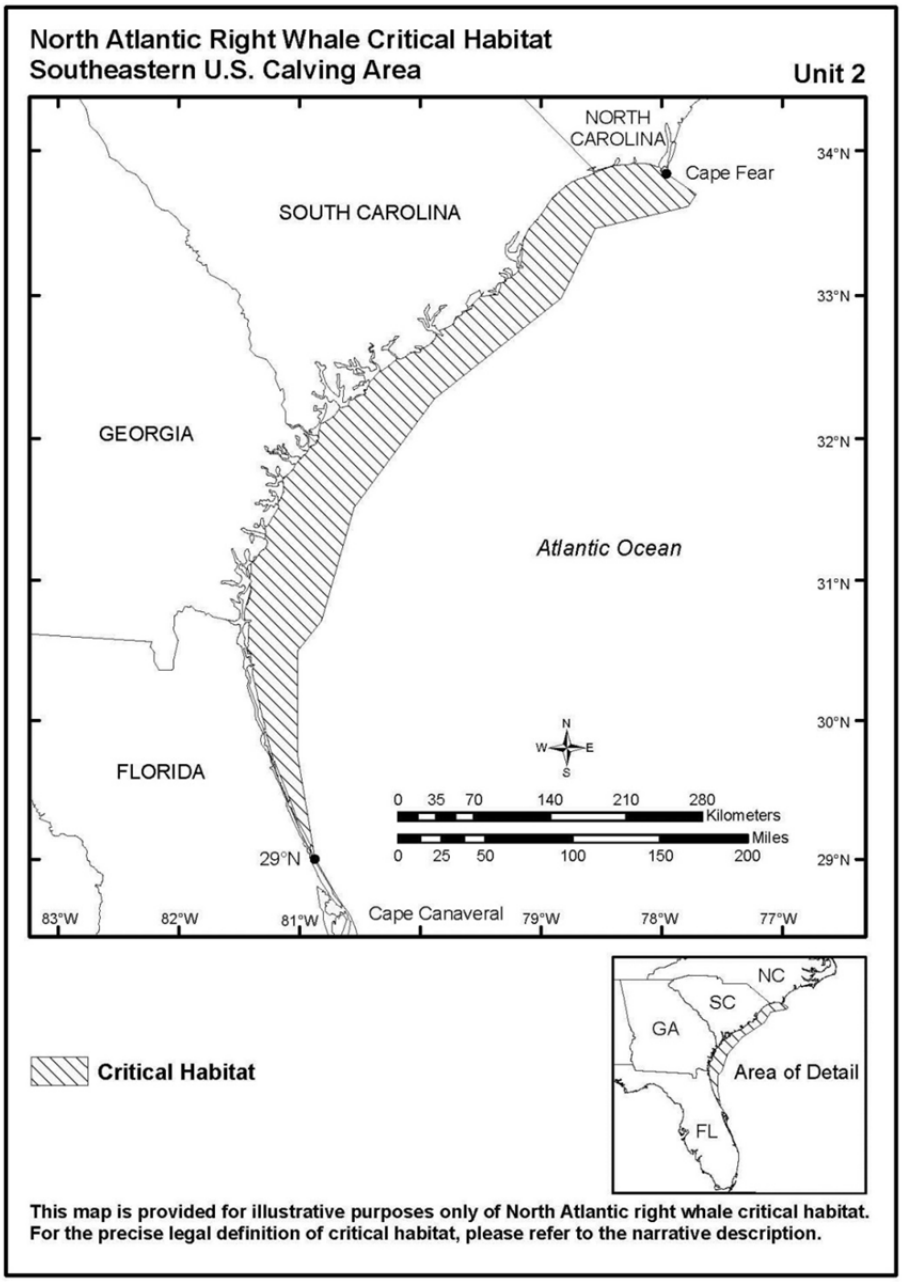


Figure 10. Proposed North Atlantic right whale critical habitat, Unit 2 (NMFS 2015)

Neither the dredging, related vessel operations, nor the disposal of dredged material will significantly impact water depth, sea surface conditions, or the temperature of the ocean. While the ODMDS and the reef mitigation sites will decrease water depths by as much as 10 ft, elevated sea bottom will not impede whales in any way. Water depths will still be sufficient for the animals to move freely throughout the habitat.

The likelihood of interaction which may impact the distribution of right whale calf/cow pairs is further reduced by the precautions stipulated for vessel avoidance. Thus, the proposed action will have insignificant effects on the physical and biological features (water depth, surface

conditions, and water temperature), which were the bases for determining this habitat to be critical.

3.1.3 Humpback Whale

Humpback whales live in all major oceans from the equator to subpolar latitudes. They typically migrate between tropical/subtropical and temperate/polar latitudes. In the Atlantic Ocean, humpback whales feed in the northwestern Atlantic during the summer months and migrate to calving and mating areas in the Caribbean. They utilize 6 separate feeding areas in northern waters after their return. These areas are within the biologically important area defined by the 200-m isobath[14] on the North American east coast. These areas are outside of the project's potential impact area. The best available estimate for the number of individuals in the North Atlantic is 11,750 humpback whales. Recent estimates of abundance in the North Atlantic stock indicate continued population growth; however, the size of the humpback whale stock may be below the optimum sustainable population in the U.S. Atlantic Exclusive Economic Zone (Waring et al. 2014).

Humpback whales face many threats due to human activity. They may become entangled in fishing gear; either swimming away with the gear after entanglement or by becoming anchored by it. Inadvertent vessel strikes can injure or kill humpbacks. Whale watching vessels may harass, stress, or strike whales. Traffic through shipping channels, fisheries, and aquaculture may displace whales that normally aggregate in that area. Vessel speed limits (described in Section 2.1.3.2), and using dedicated observers will diminish the potential interactions between large whales and dredging equipment (NMFS 1997b). Thus, NMFS concludes that the project's vessel related effects on humpback whales are discountable based on the rarity of the species and on the implementation of the suite of whale conservation measures discussed above and in the RPMs of this Opinion.

3.1.4 Shortnose Sturgeon

The shortnose sturgeon is the smallest of the 3 sturgeon species that occur in eastern North America. They attain a maximum length of about 6 ft, and a weight of about 55 pounds. Shortnose sturgeon inhabit large coastal rivers of eastern North America. Although considered an anadromous species,[15] shortnose sturgeon are more properly characterized as "freshwater amphidromous," meaning that they move between fresh and salt water during some part of their life cycle, but not necessarily for spawning. Early life stages of shortnose sturgeon (eggs, larvae, and young-of-the-year) are extremely sensitive to salinity. Zeigeweid et al. (2008) conducted experiments on 66- to 144-day-old juvenile shortnose sturgeon between 0.4-42.8 grams in body weight. At those ages and weights, 50% of juvenile sturgeon died after a 48-hour exposure to salinities between 14.8 and 20.9 parts per thousand (ppt). Laboratory experiments conducted by Jenkins et al. (1993) showed that 76-day-old juvenile shortnose sturgeon experienced 100% mortality during a 96-hour exposure test to salinities equal to or greater than 15 ppt. However, 330-day-old sturgeon tolerated salinities up to 20 ppt for 18 hours, but 100% died when exposed to 30 ppt. Both studies found that salinity tolerance increased with age and body weight (Ziegeweid et al. 2008; Jenkins et al. 1993). There are no studies indicating that adult shortnose

[14] An isobaths is an imaginary line or a line on a map or chart that connects all points having the same depth below a water surface (as of an ocean, sea, or lake).
[15] One that lives primarily in marine waters and breeds in freshwater

sturgeon are sensitive to salinity. Shortnose sturgeon generally stay in the rivers where they were born ("natal rivers"), though recent research indicates they stray into non-natal (and sometimes non-adjacent) riverine systems via the marine environment more frequently than previously thought (Post et al. 2014; Zydlewski et al. 2011).

Southern populations of shortnose sturgeon usually spawn at least 125 miles (200 km) upriver (Kynard 1997) or throughout the fall line[16] zone, if they are able to reach it. Shortnose sturgeon found in the action area are unable to access the fall-line zone because of dams located along the river, the nearest being Pinopolis Dam, at River Mile (RM) 48. The dam has isolated the shortnose sturgeon population in the Cooper River, blocking upstream access to sturgeon below the Pinopolis Dam. Historically, telemetry studies have indicated that shortnose sturgeon do not pass upriver through the vessel lock in the Pinopolis Dam on the Cooper River. In 2012, however, 2 shortnose sturgeon were recorded travelling through the Pinopolis Lock and were later recorded in the Wateree and Congaree Rivers (B. Post, SCDNR, pers. comm. to K. Reece, NMFS, April 11, 2012).

The population of shortnose sturgeon downstream of Pinopolis have been documented in the tailrace area immediately below the dam since 1997 (Cooke and Leach 1999). Fertilized shortnose sturgeon eggs collected in the Pinopolis Dam tailrace verified spawning despite non-traditional spawning habitat (i.e., barren hard bottom with scattered pockets of clam shell and marl pieces) (Cooke and Leach 2004; Duncan et al. 2004). This spawning in the tailrace in atypical habitat supports the hypothesis that a blockage in spawning migration can force new spawning areas (Kynard et al. 1999). Kynard (1997) reported that for sturgeon spawning directly below a dam in tailrace flows, the facility's operation controls the suitability of water velocities for spawning and rearing of eggs and embryos. It is likely that the variable operation of the dam results in eggs being removed from the substrate by high velocity flows or being compromised due to the lack of adequate water and oxygen-providing aeration during low velocity flows. Cook and Leach (2004) reported that the tailrace of the Pinopolis Dam experiences a combination of tidal influence, highly variable discharge, high current velocities (alternating with no current), and limited spawning substrates which limit early-life survival.

Laboratory studies of larvae found most ceased downstream migration after only 2 days, though some continued for 14 (C. Cauthron & B. Kynard unpublished data). This timeframe is sufficient to move downstream but not sufficient to move to the estuary. Tolerance of early life stages to increasing salinity and low dissolved oxygen increases with age. Twenty- two day old larvae from the Savannah River tolerated a maximum of 9 ppt salinity and required more than 3 mg l-1 oxygen, while fish about 300 days old tolerated 25 ppt salinity for 18 hours and most survived short periods of 3 mg l-1 oxygen (Jenkins et al. 1993). No larvae have been found downstream of the Pinopolis Dam (B. Post, SCDNR, pers. comm. to K. Reece, NMFS, April 14, 2015). However, any eggs that made it to the larval stage would not be viable because of exposure to salinity downstream of the dam. Larvae would not have enough time to mature to a stage that was tolerant of increased estuarine salinities. Typical spawning occurs further upriver allowing sufficient time for larvae to develop increased salinity tolerances. No known collections of early life-stage shortnose sturgeon, other than fertilized in the tailrace, have been

[16] The fall line is the boundary between an upland region of continental bedrock and an alluvial coastal plain, sometimes characterized by waterfalls or rapids.

made to date with limited survey efforts. The absence of early life stage shortnose sturgeon indicates that recruitment failure is occurring as smaller fish are not present to grow and replace the reproducing adults. This finding led Cooke and Leach (2004) to determine that the Cooper River subpopulation of shortnose sturgeon is recruitment-limited. Thus, the status quo does not allow for shortnose sturgeon eggs to develop into fry that subsequently mature into adult sturgeon. The CHP45 project will not alter these current conditions faced by early life stage shortnose sturgeon and will not change their likelihood of survival.

Recent research conducted from 2011-2014 documented 40 shortnose sturgeon that were detected by a receiver in the Cooper River moving as far upstream as the Pinopolis Dam (RM 48) and as far downstream as RM 0 (Post et al. 2014) with only 3 shortnose sturgeon (all adult) observed in the Wando River (B. Post, SCDNR, pers. comm. to K. Reece, NMFS, April 8, 2015). All 40 of these sturgeon demonstrated upstream and downstream movements with movement patterns that were similar over each year of the study period (Post et al. 2014). Shortnose sturgeon that were tagged in other waterbodies (the Great Pee Dee and Edisto Rivers in South Carolina and another tagged in North Carolina waters) occupied the same freshwater water tidal zone, but they did not make presumed spawning runs to Pinopolis Dam and were never detected upstream of RM 28.148. Prior research carried out by Palmer (2001) found shortnose sturgeon migrated seasonally within a 25.4-mile stretch of the river between the Pinopolis Dam at RM 48 and the Naval Weapons Station at about RM 22.6 (Palmer 2001)(Table 4). In the winter, the shortnose sturgeon aggregate around RM 27; this structurally diverse area is thought to be a productive foraging site and to provide shelter to the fish from high river flow (Palmer 2001). Direction of shortnose sturgeon movement was not affected by tidal flow (Palmer 2001). Direction of movement by shortnose sturgeon is also independent of tidal flow in the Altamaha and Cape Fear Rivers (Collins et al. 2000a; Moser and Ross 1995).

Shortnose sturgeon do not frequently utilize the lower 22 miles (approximate) of the Cooper River (Palmer 2001). This area between the Naval Weapons Station (about RM 22) and Charleston Harbor is dredged about every 18 months (removing ~ 1 MCY) to allow safe passage of deep-draft vessels, thereby removing substrate and prey. Therefore, the shortnose sturgeon in the Cooper River exist in an abbreviated ecosystem: available habitat is restricted within the upper 26 miles of the Cooper River between Pinopolis Dam at RM 48 and about RM 22. Sturgeon were tagged and tracked by Palmer (2001) in the Cooper River by season and River Mile. Pinopolis Dam is located at RM 48.0 and provides a barrier to upstream movement. The Naval Weapons Station is at about RM 22.

Table 4. Seasonal Distribution of 14 Shortnose Sturgeon

Season	River Mile
Spring	25.5-48.0
Summer	30.6-48.0; primarily 44.2-45.9 and 39.4-41.4
Fall	27.2-48.0
Winter	22.6-48.0

Shortnose sturgeon are not expected to be in the entrance channel in the Atlantic ocean where hopper dredging will occur. NMFS has previously determined in Opinions evaluating the effects of dredging that, while oceangoing hopper-type dredges may lethally entrain protected species, non-hopper type dredging methods (e.g., mechanical, clamshell or bucket dredging, hydraulic

cutterhead dredges) are slower and unlikely to overtake or adversely affect them. As well, taking by mechanical dredges appears to be exceedingly rare with none observed occurring in the Charleston area. Thus, NMFS concludes that the project's dredge related physical effects on shortnose sturgeon are discountable based on the rarity of the species in the project area, hopper dredging will not be used in areas where Atlantic sturgeon are likely to occur, and the lack of observed sturgeon takes resulting from mechanical and hydraulic cutterhead dredging near the CHP45 project area.

The proposed channel deepening will increase the salinity concentrations in the action area. The Environmental Fluid Dynamics Code[17] (EFDC) model predicted salinity changes resulting from the project. Figure 11 depicts the overall changes in salinity that are predicted the result from the proposed action. The greatest changes in salinity are projected in the Wando River. However, shortnose sturgeon rarely utilize the Wando River (B. Post, SCDNR, pers. comm. to K. Reece, NMFS, April 8, 2015), and those that do are adults and not sensitive to variations in salinity. Based on information in the DEIS, all projected salinity increases in the Cooper River will be less than one ppt, with the average change estimated to be less than 0.40 ppt. Additionally, in the upper Cooper River (where younger, less salt tolerant shortnose sturgeon would be found), the model results indicate no salinity increase is projected during average weather/climatic conditions. In the extreme climatic conditions such as drought (99% exceedance) the projected salinity increases in the upper river are projected to be 0.25 ppt or less. Finally, results indicate the location of the Cooper River brackish-freshwater transition zone will move approximately one-half mile upstream shown in Figure 12. Due to the very small magnitude of the anticipated changes in salinity throughout portions of the action area which are used by shortnose sturgeon, and the related conclusion that the effects will not alter the current conditions that already impede successful recruitment, we anticipate that any direct effects to shortnose sturgeon will be insignificant.

[17] The Environmental Fluid Dynamics Code is a state-of-the-art hydrodynamic model that can be used to simulate aquatic systems in one, two, and three dimensions.

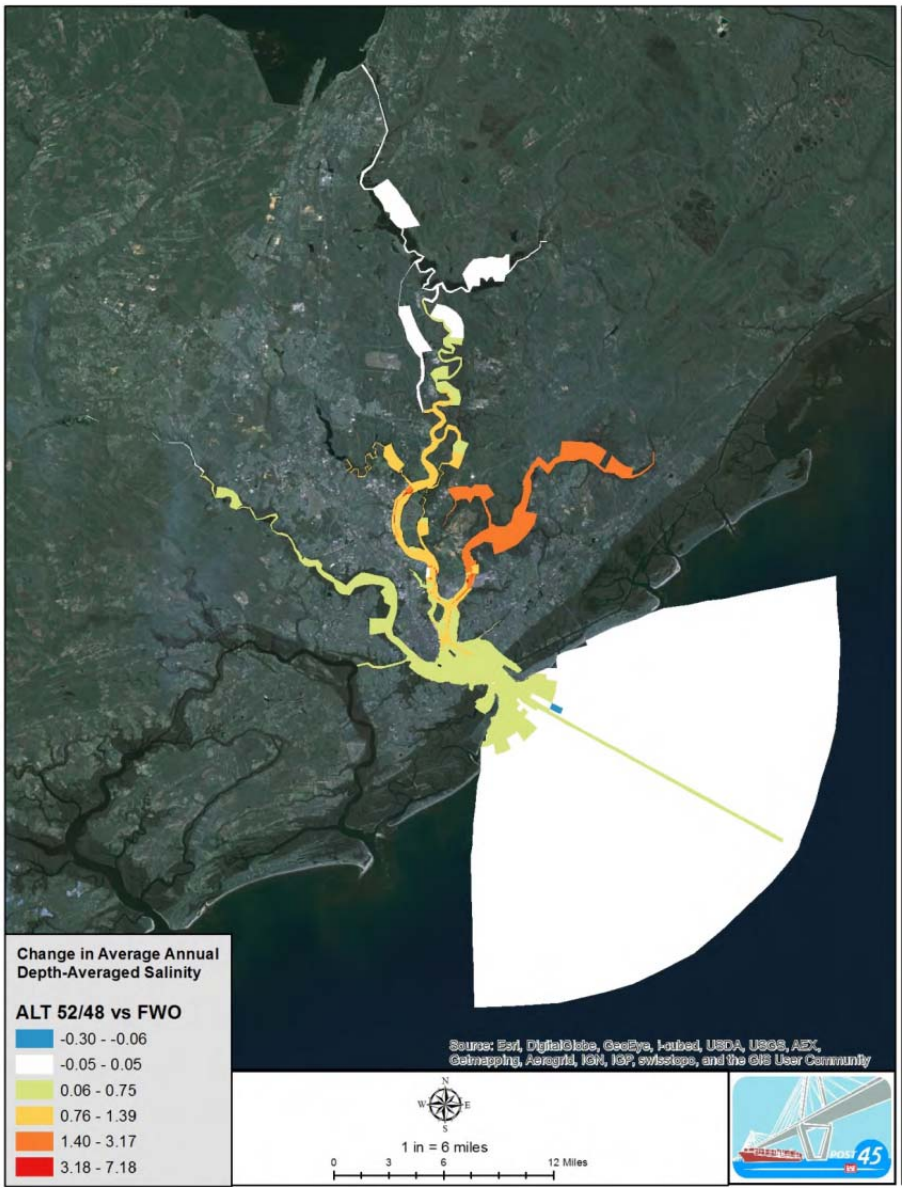


Figure 11. Changes in Average Annual Salinity Predicted to Result from the Proposed Action (USACE 2014).

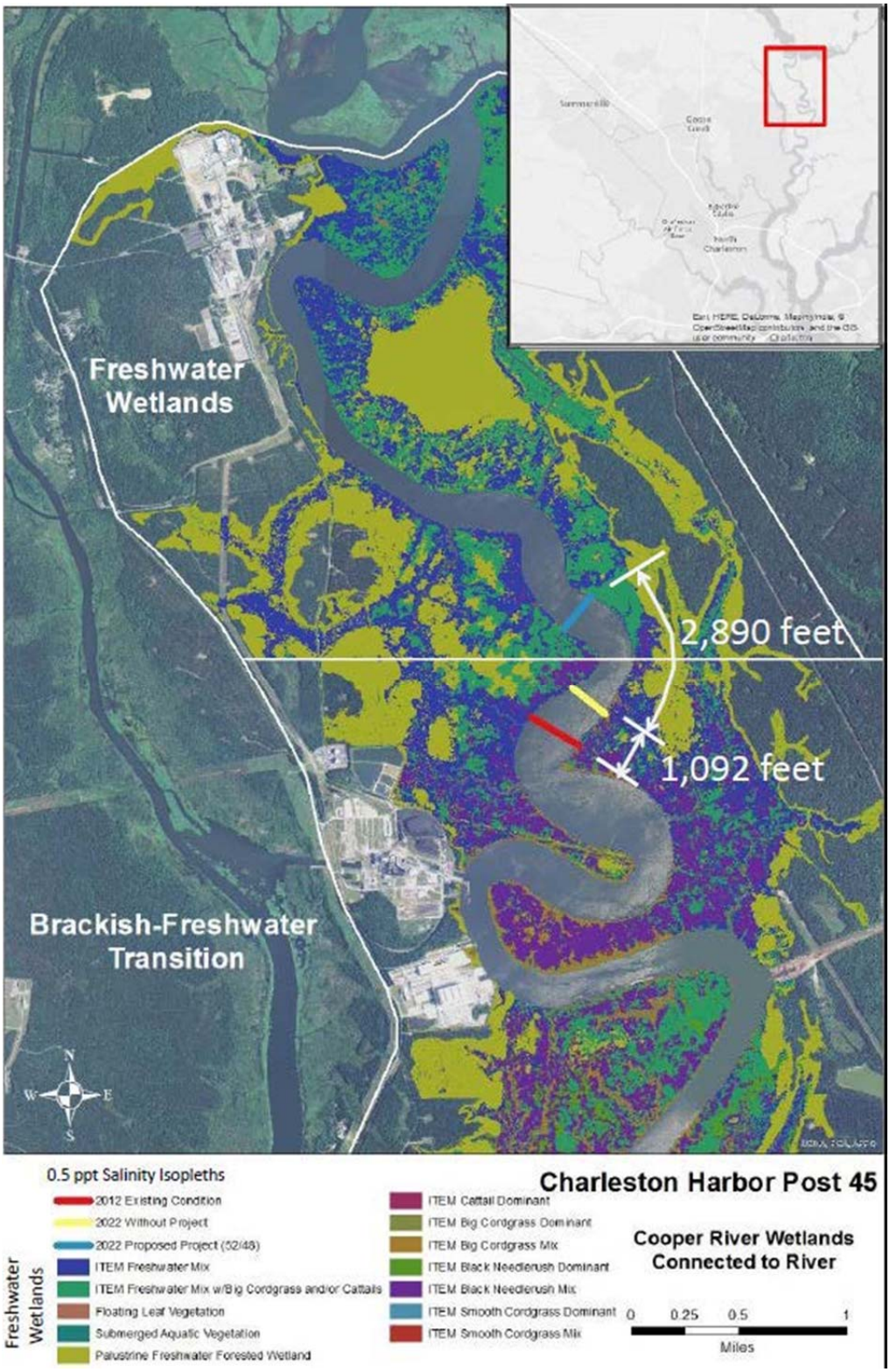


Figure 12. Projected upriver movement of the brackish-freshwater transition in the Cooper River following the CHP45 project (USACE 2014).

The proposed channel deepening may also have an effect on dissolved oxygen (DO). The current average DO level in the Cooper River is 5.77 mg/L. Overall, the current values in the river range from average low values of 4.7 mg/L to average high levels of 7.6 mg/L. None of the data for the current conditions indicates any DO levels in the Cooper River less than 4.4 mg/L. The EFDC model was used to forecast future effect of the proposed project combined with the effects of all anticipated discharges to predict all future changes in DO. The model results indicate very minor DO reductions throughout the action area (average reduction of 0.03mg/L). Additionally, this analysis indicates that the combined effect of the proposed project with on-going and future unrelated discharges would result in Cooper River DO reductions ranging from less than 0.02 mg/L to 0.1256 mg/L. The average anticipated total DO reduction in the areas occupied by shortnose sturgeon (i.e., above mile 20) is less than 0.03 mg/L. Due to the very small magnitude of the anticipated changes in DO throughout portions of the action area which are used by shortnose sturgeon, we anticipate that any direct effects to shortnose sturgeon will be insignificant.

In order to better understand the implications of deepening the harbor on fishery habitats, the USFWS Habitat Suitability Index (HSI) models for representative species were applied in order to evaluate effects of project alternatives (USACE 2014). These models assess potential changes to habitat quantity and quality. The model results indicate that the quantity of suitable shortnose sturgeon foraging habitat is projected to slightly increase from about 20,977 acres to 21,017 acres. Foraging habitat quality is affected by substrate, velocity, and temperature in the model. Since substrate stays constant outside of dredged areas, velocity, and temperature become the influencing variables. Since the bottom temperatures are slightly lower in the alternative conditions compared to the FWOP, temperature positively benefits shortnose sturgeon foraging in the HSI within many cells, and negatively in fewer cells. The anticipated changes in habitat quality (i.e., HSI) are projected to be very small. The combined effect of increased quantity and minor qualtitative changes result in a projected net 0.19% increase in habitat units from the proposed project when compared to the future without project (FWOP) condition (USACE 2014). Results of HSI modeling indicate that shortnose sturgeon foraging habitat will not be adversely affected by the proposed project.

The CHP45 project includes enlarging existing turning basins. The Wando Welch, Navy base, and North Charleston Terminal turning basin will all be expanded to accommodate Post Panamax Generation II and Generation III container ships. All 3 terminals are located in the bottom 13 miles of the Cooper and in the Wando River. Impacts from enlarging the turning basins are limited to temporary loss of prey species as a result of dredging operations disturbing sediments in the areas. This effect is considered insignificant for shortnose sturgeon because these fish do not regularly utilize the Wando River or the lower 22 miles (approximate) of the Cooper River (Palmer 2001).

The elevation of dikes around upland disposal areas will impact a very small portion of tidal fringing saltmarsh at the southern end of Daniel Island across from the Wando Welch Terminal. Impacts from elevating the existing dikes considered insignificant for Shortnose sturgeon because these fish do not utilize the Wando River or the lower 22 miles (approximate) of the Cooper River (Palmer 2001).

3.1.1 Leatherback Sea Turtle

Leatherback sea turtles may be found in the action area, particularly when onshore winds and/or currents push jellyfish, their preferred prey, into inshore waters. However, leatherbacks are primarily a pelagic species, preferring deeper waters than those of the action area (the deepest portions of the offshore action area are less than 60 feet deep). Furthermore, in over 30 years of NMFS consultations with the USACE on hopper dredging projects carried out in the Charleston Harbor area, there has never been a documented take of a leatherback sea turtle by a hopper dredge. Because of this and their very large size (compared to hopper dredge dragheads or mechanical dredge equipment), pelagic nature (surface and mid-water), preference for deeper waters located beyond the project area further offshore, and feeding habits (which make it unlikely they would ever encounter a bottom-hugging hopper dredge draghead), NMFS believes the possibility that they would be adversely affected by a hopper dredge is discountable.

3.1.2 Hawksbill Sea Turtle

With respect to the United States, nesting occurs in Puerto Rico, the U.S. Virgin Islands, and along the southeast coast of Florida. Outside of the nesting areas, hawksbills have been seen off the U.S. Gulf of Mexico states and along the Eastern Seaboard as far north as Massachusetts, although sightings north of Florida are rare (NMFS and USFWS 1993). They are closely associated with coral reefs and other hard-bottom habitats, but they are also found in other habitats including inlets, bays, and coastal lagoons (NMFS and USFWS 1993). The hawksbill's diet is highly specialized and consists primarily of sponges (Meylan 1999). During the past 30 years of NMFS consultations with the USACE on hopper dredging projects carried out in the Charleston Harbor area there has never been a documented take of a hawksbill sea turtle by a hopper dredge.[18] Due to hawksbill sea turtles' preferred habitat and diet, it is considered extremely unlikely that the species would be encountered in the action; therefore, NMFS believes the possibility that they would be adversely affected is discountable.

3.2 Status of Species Likely to be Adversely Affected

The following discussion focuses on the species of sea turtles and sturgeon that NMFS believes are likely to be adversely affected by the proposed action. The sea turtle subsections focus primarily on the natural history of Atlantic Ocean populations of these species because these are the populations that may be directly affected by the proposed action. As sea turtles are highly migratory, potentially affected species in the action area may make migrations in other areas of the Gulf of Mexico, Atlantic Ocean, and Caribbean Sea. The following subsections are synopses of the best available information on the life history, distribution, population trends, and current status of the 3 species of sea turtles that are likely to be adversely affected by 1 or more components of the proposed action. Additional background information on the status of sea turtle species can be found in a number of published documents: recovery plans for the Atlantic green sea turtle (NMFS and USFWS 1991b), Kemp's ridley sea turtle (NMFS and USFWS 1992), and loggerhead sea turtle (NMFS and USFWS 2008b); and status reviews, stock assessments, and biological reports (NMFS-SEFSC 2001; NMFS-SEFSC 2009e; NMFS and USFWS 1995; NMFS and USFWS 2007a; NMFS and USFWS 2007m; NMFS and USFWS 2007q; NMFS and USFWS 2007v; NMFS and USFWS 2007w; TEWG 1998; TEWG 2000a; TEWG 2007; TEWG 2009).

[18] USACE Sea Turtle Data Warehouse: http://el.erdc.usace.army.mil/seaturtles/index.cfm

3.2.1 Loggerhead

The loggerhead sea turtle was listed as a threatened species throughout its global range on July 28, 1978. NMFS and USFWS published a Final Rule designating 9 DPSs for loggerhead sea turtles (76 FR 58868, September 22, 2011, and effective October 24, 2011). This rule listed the following DPSs: (1) Northwest Atlantic Ocean (threatened), (2) Northeast Atlantic Ocean (endangered), (3) South Atlantic Ocean (threatened), (4) Mediterranean Sea (endangered), (5) North Pacific Ocean (endangered), (6) South Pacific Ocean (endangered), (7) North Indian Ocean (endangered), (8) Southeast Indo-Pacific Ocean (endangered), and (9) Southwest Indian Ocean (threatened). The Northwest Atlantic (NWA) DPS is the only population that occurs within the action area and therefore is the only one considered in this Opinion.

Species Description and Distribution

Loggerheads are large sea turtles. Adults in the southeast United States average about 3 ft (92 centimeters [cm]) long, measured as a straight carapace length (SCL), and weigh approximately 255 lb (116 kg) (Ehrhart and Yoder 1978). Adult and subadult loggerhead sea turtles typically have a light yellow plastron and a reddish brown carapace covered by non-overlapping scutes that meet along seam lines. They typically have 11 or 12 pairs of marginal scutes, 5 pairs of costals, 5 vertebrals, and a nuchal (precentral) scute that is in contact with the first pair of costal scutes (Dodd 1988).

The loggerhead sea turtle inhabits continental shelf and estuarine environments throughout the temperate and tropical regions of the Atlantic, Pacific, and Indian Oceans (Dodd 1988). Habitat uses within these areas vary by life stage. Juveniles are omnivorous and forage on crabs, mollusks, jellyfish, and vegetation at or near the surface (Dodd 1988). Subadult and adult loggerheads are primarily found in coastal waters and eat benthic invertebrates such as mollusks and decapod crustaceans in hard bottom habitats.

The majority of loggerhead nesting occurs at the western rims of the Atlantic and Indian Oceans concentrated in the north and south temperate zones and subtropics (NRC 1990). For the NWA DPS, most nesting occurs along the coast of the United States, from southern Virginia to Alabama. Additional nesting beaches for this DPS are found along the northern and western Gulf of Mexico, eastern Yucatán Peninsula, at Cay Sal Bank in the eastern Bahamas (Addison 1997; Addison and Morford 1996), off the southwestern coast of Cuba (Gavilan 2001), and along the coasts of Central America, Colombia, Venezuela, and the eastern Caribbean Islands.

Non-nesting, adult female loggerheads are reported throughout the U.S. Atlantic, Gulf of Mexico, and Caribbean Sea. Little is known about the distribution of adult males who are seasonally abundant near nesting beaches. Aerial surveys suggest that loggerheads as a whole are distributed in U.S. waters as follows: 54% off the southeast U.S. coast, 29% off the northeast U.S. coast, 12% in the eastern Gulf of Mexico, and 5% in the western Gulf of Mexico (TEWG 1998).

Within the NWA DPS, most loggerhead sea turtles nest from North Carolina to Florida and along the Gulf Coast of Florida. Previous Section 7 analyses have recognized at least 5 western Atlantic subpopulations, divided geographically as follows: (1) a Northern nesting subpopulation, occurring from North Carolina to northeast Florida at about 29ºN; (2) a South

Florida nesting subpopulation, occurring from 29°N on the east coast of the state to Sarasota on the west coast; (3) a Florida Panhandle nesting subpopulation, occurring at Eglin Air Force Base and the beaches near Panama City, Florida; (4) a Yucatán nesting subpopulation, occurring on the eastern Yucatán Peninsula, Mexico (Márquez M 1990; TEWG 2000b); and (5) a Dry Tortugas nesting subpopulation, occurring in the islands of the Dry Tortugas, near Key West, Florida (NMFS-SEFSC 2001).

The recovery plan for the Northwest Atlantic population of loggerhead sea turtles concluded that there is no genetic distinction between loggerheads nesting on adjacent beaches along the Florida Peninsula. It also concluded that specific boundaries for subpopulations could not be designated based on genetic differences alone. Thus, the recovery plan uses a combination of geographic distribution of nesting densities, geographic separation, and geopolitical boundaries, in addition to genetic differences, to identify recovery units. The recovery units are as follows: (1) the Northern Recovery Unit (Florida/Georgia border north through southern Virginia), (2) the Peninsular Florida Recovery Unit (Florida/Georgia border through Pinellas County, Florida), (3) the Dry Tortugas Recovery Unit (islands located west of Key West, Florida), (4) the Northern Gulf of Mexico Recovery Unit (Franklin County, Florida, through Texas), and (5) the Greater Caribbean Recovery Unit (Mexico through French Guiana, the Bahamas, Lesser Antilles, and Greater Antilles) (NMFS and USFWS 2008b). The recovery plan concluded that all recovery units are essential to the recovery of the species. Although the recovery plan was written prior to the listing of the NWA DPS, the recovery units for what was then termed the Northwest Atlantic population apply to the NWA DPS.

Life History Information

The Northwest Atlantic Loggerhead Recovery Team defined the following 8 life stages for the loggerhead life cycle, which include the ecosystems those stages generally use: (1) egg (terrestrial zone), (2) hatchling stage (terrestrial zone), (3) hatchling swim frenzy and transitional stage (neritic zone[19]), (4) juvenile stage (oceanic zone), (5) juvenile stage (neritic zone), (6) adult stage (oceanic zone), (7) adult stage (neritic zone), and (8) nesting female (terrestrial zone) (NMFS and USFWS 2008). Loggerheads are long-lived animals. They reach sexual maturity between 20-38 years of age, although age of maturity varies widely among populations (Frazer and Ehrhart 1985; NMFS 2001b). The annual mating season occurs from late March to early June, and female turtles lay eggs throughout the summer months. Females deposit an average of 4.1 nests within a nesting season (Murphy and Hopkins 1984), but an individual female only nests every 3.7 years on average (Tucker 2010). Each nest contains an average of 100-126 eggs (Dodd 1988) which incubate for 42-75 days before hatching (NMFS and USFWS 2008b). Loggerhead hatchlings are 1.5-2 in long and weigh about 0.7 ounces (20 grams).

As post-hatchlings, loggerheads hatched on U.S. beaches enter the "oceanic juvenile" life stage, migrating offshore and becoming associated with *Sargassum* habitats, driftlines, and other convergence zones (Carr 1986; Conant et al. 2009; Witherington 2002). Oceanic juveniles grow at rates of 1-2 in (2.9-5.4 cm) per year (Bjorndal et al. 2003; Snover 2002) over a period as long as 7-12 years (Bolten et al. 1998) before moving to more coastal habitats. Studies have suggested that not all loggerhead sea turtles follow the model of circumnavigating the North

[19] Neritic refers to the nearshore marine environment from the surface to the sea floor where water depths do not exceed 200 meters.

Atlantic Gyre as pelagic juveniles, followed by permanent settlement into benthic environments (Bolten and Witherington 2003; Laurent et al. 1998). These studies suggest some turtles may either remain in the oceanic habitat in the North Atlantic longer than hypothesized, or they move back and forth between oceanic and coastal habitats interchangeably (Witzell 2002). Stranding records indicate that when immature loggerheads reach 15-24 in (40-60 cm) SCL, they begin to reside in coastal inshore waters of the continental shelf throughout the U.S. Atlantic and Gulf of Mexico (Witzell 2002).

After departing the oceanic zone, neritic juvenile loggerheads in the Northwest Atlantic inhabit continental shelf waters from Cape Cod Bay, Massachusetts, south through Florida, The Bahamas, Cuba, and the Gulf of Mexico. Estuarine waters of the United States, including areas such as Long Island Sound, Chesapeake Bay, Pamlico and Core Sounds, Mosquito and Indian River Lagoons, Biscayne Bay, Florida Bay, and numerous embayments fringing the Gulf of Mexico, comprise important inshore habitat. Along the Atlantic and Gulf of Mexico shoreline, essentially all shelf waters are inhabited by loggerheads (Conant et al. 2009).

Like juveniles, non-nesting adult loggerheads also use the neritic zone. However, these adult loggerheads do not use the relatively enclosed shallow-water estuarine habitats with limited ocean access as frequently as juveniles. Areas such as Pamlico Sound, North Carolina, and the Indian River Lagoon, Florida, are regularly used by juveniles but not by adult loggerheads. Adult loggerheads do tend to use estuarine areas with more open ocean access, such as the Chesapeake Bay in the U.S. mid-Atlantic. Shallow-water habitats with large expanses of open ocean access, such as Florida Bay, provide year-round resident foraging areas for significant numbers of male and female adult loggerheads (Conant et al. 2009).

Offshore, adults primarily inhabit continental shelf waters, from New York south through Florida, The Bahamas, Cuba, and the Gulf of Mexico. Seasonal use of mid-Atlantic shelf waters, especially offshore New Jersey, Delaware, and Virginia during summer months, and offshore shelf waters, such as Onslow Bay (off the North Carolina coast), during winter months has also been documented (Hawkes et al. 2007; Georgia Department of Natural Resources, unpublished data; South Carolina Department of Natural Resources, unpublished data). Satellite telemetry has identified the shelf waters along the west Florida coast, The Bahamas, Cuba, and the Yucatán Peninsula as important resident areas for adult female loggerheads that nest in Florida (Foley et al. 2008; Girard et al. 2009; Hart et al. 2012). The southern edge of the Grand Bahama Bank is important habitat for loggerheads nesting on the Cay Sal Bank in The Bahamas, but nesting females are also resident in the bights of Eleuthera, Long Island, and Ragged Islands. They also reside in Florida Bay in the United States, and the north coast of Cuba (A. Bolten and K. Bjorndal, University of Florida, unpublished data). Moncada et al. (2010) report the recapture in Cuban waters of 5 adult female loggerheads originally flipper-tagged in Quintana Roo, Mexico, indicating that Cuban shelf waters likely also provide foraging habitat for adult females that nest in Mexico.

Status and Population Dynamics

A number of stock assessments and similar reviews (Conant et al. 2009; Heppell et al. 2003; NMFS-SEFSC 2001; NMFS-SEFSC 2009a; NMFS and USFWS 2008b; TEWG 1998; TEWG

2000b; TEWG 2009) have examined the stock status of loggerheads in the Atlantic Ocean, but none have been able to develop a reliable estimate of absolute population size.

Numbers of nests and nesting females can vary widely from year to year. Nesting beach surveys, though, can provide a reliable assessment of trends in the adult female population, due to the strong nest site fidelity of female loggerhead sea turtles, as long as such studies are sufficiently long and survey effort and methods are standardized (e.g., (NMFS and USFWS 2008b). NMFS and USFWS (2008b) concluded that the lack of change in 2 important demographic parameters of loggerheads, remigration interval and clutch frequency, indicate that time series on numbers of nests can provide reliable information on trends in the female population.

Peninsular Florida Recovery Unit

The Peninsular Florida Recovery Unit (PFRU) is the largest loggerhead nesting assemblage in the Northwest Atlantic. A near-complete nest census (all beaches including index nesting beaches) undertaken from 1989-2007 showed an average of 64,513 loggerhead nests per year, representing approximately 15,735 nesting females per year (NMFS and USFWS 2008b). The statewide estimated total for 2013 was 77,975 nests (FWRI nesting database).

In addition to the total nest count estimates, the Florida Fish and Wildlife Research Institute (FWRI) uses an index nesting beach survey method. The index survey uses standardized data-collection criteria to measure seasonal nesting and allow accurate comparisons between beaches and between years. This provides a better tool for understanding the nesting trends (Figure 13). FWRI performed a detailed analysis of the long-term loggerhead index nesting data (1989-2013) (http://myfwc.com/research/wildlife/sea-turtles/nesting/loggerhead-trends/). Over that time period, 3 distinct trends were identified. From 1989-1998 there was a 30% increase that was then followed by a sharp decline over the subsequent decade. Large increases in loggerhead nesting occurred since then. FWRI examined the trend from the 1998 nesting high through 2013 and found the decade-long post-1998 decline had reversed and there was no longer a demonstrable trend. Looking at the data from 1989 through 2014 (an increase of over 32%), FWRI concluded that there was an overall positive change in the nest counts (http://myfwc.com/research/wildlife/sea-turtles/nesting/loggerhead-trends/).

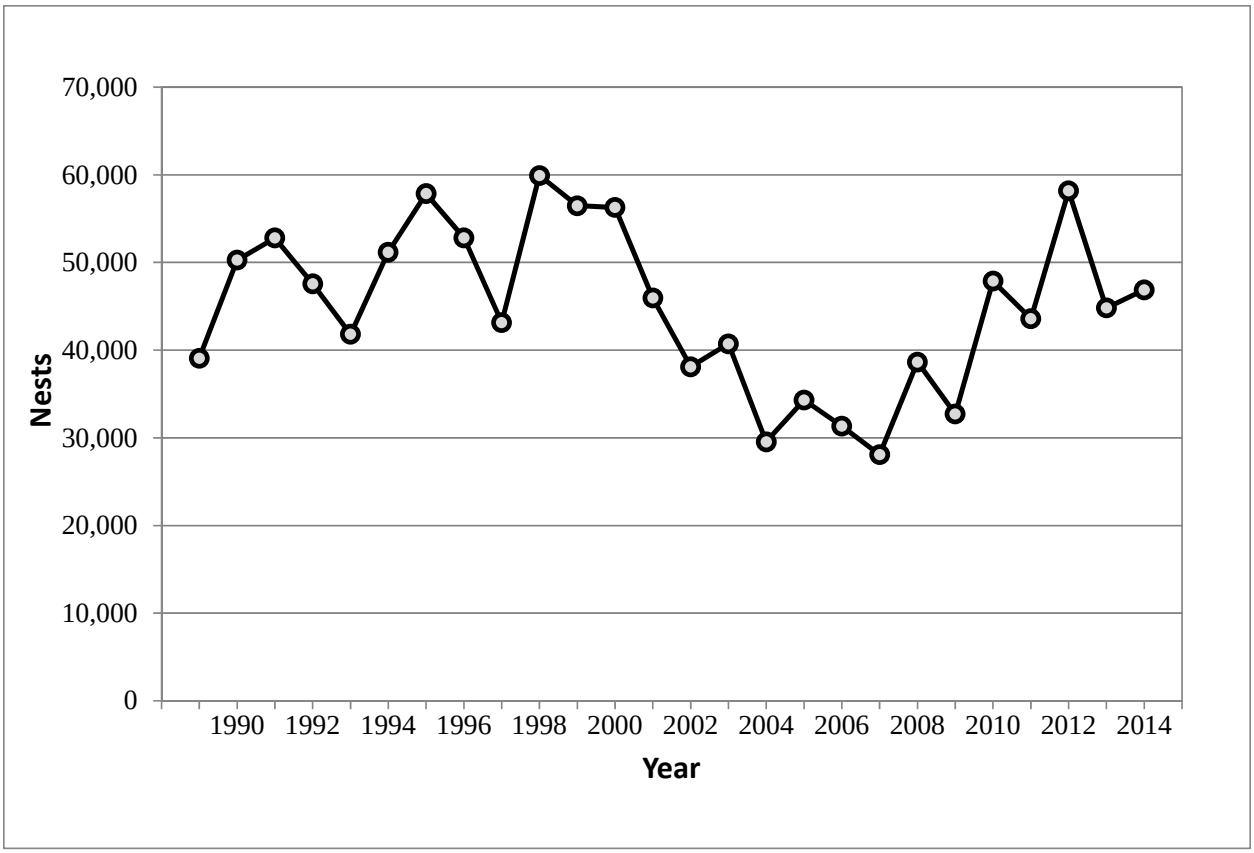


Figure 13. Loggerhead sea turtle nesting at Florida index beaches since 1989

Northern Recovery Unit

Annual nest totals from beaches within the Northern Recovery Unit (NRU) averaged 5,215 nests from 1989-2008, a period of near-complete surveys of NRU nesting beaches (Georgia Department of Natural Resources [GADNR] unpublished data, North Carolina Wildlife Resources Commission [NCWRC] unpublished data, South Carolina Department of Natural Resources [SCDNR] unpublished data), and represent approximately 1,272 nesting females per year, assuming 4.1 nests per female (Murphy and Hopkins 1984). The loggerhead nesting trend from daily beach surveys showed a significant decline of 1.3% annually from 1989-2008. Nest totals from aerial surveys conducted by SCDNR showed a 1.9% annual decline in nesting in South Carolina from 1980-2008. Overall, there are strong statistical data to suggest the NRU had experienced a long-term decline over that period of time.

Data since that analysis (Table 5 showing data from GADNR, SCDNR, and NCWRC nesting datasets) are showing improved nesting numbers and a departure from the declining trend. Georgia nesting has rebounded to show the first statistically significant increasing trend since comprehensive nesting surveys began in 1989 (Mark Dodd, GADNR press release, http://www.georgiawildlife.com/node/3139). South Carolina and North Carolina nesting have also begun to show a shift away from the declining trend of the past.

Table 5. Total Number of NRU Loggerhead Nests

Nests Recorded	2008	2009	2010	2011	2012	2013	2014
Georgia	1,649	998	1,760	1,992	2,241	2,289	1,196
South Carolina	4,500	2,182	3,141	4,015	4,615	5,193	2,083
North Carolina	841	302	856	950	1,074	1,260	542
Total	6,990	3,472	5,757	6,957	7,930	8,742	3,821

South Carolina also conducts an index beach nesting survey similar to the one described for Florida. Although the survey only includes a subset of nesting, the standardized effort and locations allow for a better representation of the nesting trend over time. Increases in nesting were seen for the period from 2009-2012, with 2012 showing the highest index nesting total since the start of the program (Figure 14).

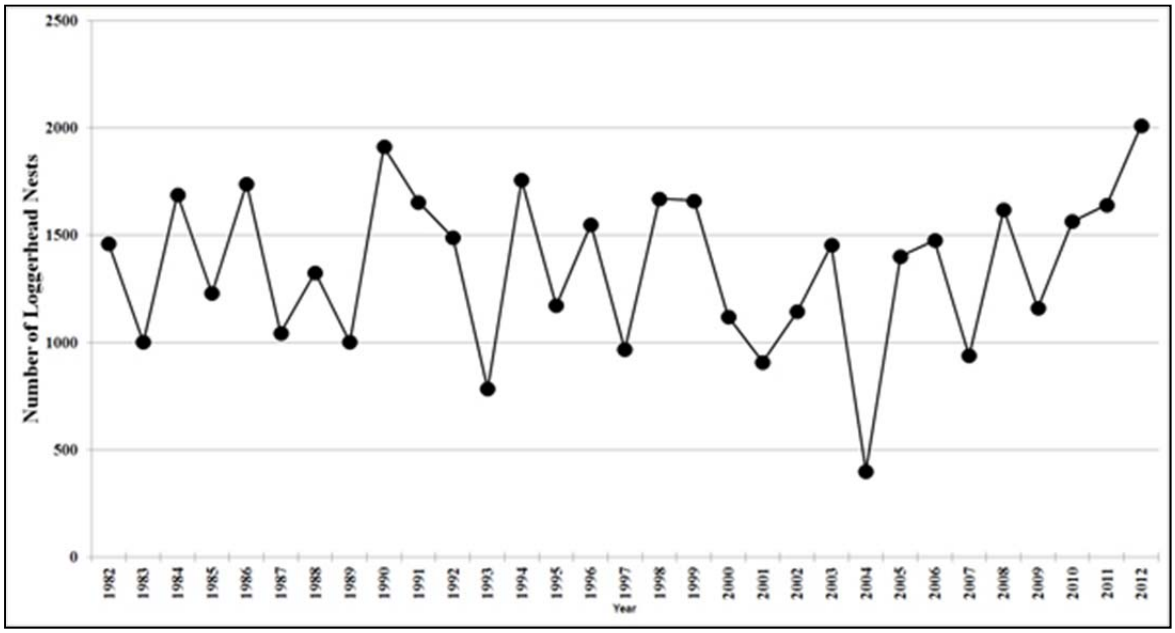


Figure 14. South Carolina index nesting beach counts for loggerhead sea turtles (from the SCDNR website, http://www.dnr.sc.gov/seaturtle/nest.htm)

Other NW Atlantic DPS Recovery Units

The remaining 3 recovery units—Dry Tortugas (DTRU), Northern Gulf of Mexico (NGMRU), and Greater Caribbean (GCRU)—are much smaller nesting assemblages, but they are still considered essential to the continued existence of the species. Nesting surveys for the DTRU are conducted as part of Florida's statewide survey program. Survey effort was relatively stable during the 9-year period from 1995-2004, although the 2002 year was missed. Nest counts ranged from 168-270, with a mean of 246, but there was no detectable trend during this period (NMFS and USFWS 2008b). Nest counts for the NGMRU are focused on index beaches rather than all beaches where nesting occurs. Analysis of the 12-year dataset (1997-2008) of index nesting beaches in the area shows a statistically significant declining trend of 4.7% annually. Nesting on the Florida Panhandle index beaches, which represents the majority of NGMRU nesting, had shown a large increase in 2008, but then declined again in 2009 and 2010 before rising back to a level similar to the 2003-2007 average in 2011. Nesting survey effort has been inconsistent among the GCRU nesting beaches, and no trend can be determined for this

subpopulation (NMFS and USFWS 2008b). Zurita et al. (2003) found a statistically significant increase in the number of nests on 7 of the beaches on Quintana Roo, Mexico, from 1987-2001, where survey effort was consistent during the period. Nonetheless, nesting has declined since 2001, and the previously reported increasing trend appears to not have been sustained (NMFS and USFWS 2008b).

In-water Trends

Nesting data are the best current indicator of sea turtle population trends; but, in-water data also provide some insight. In-water research suggests the abundance of neritic juvenile loggerheads is steady or increasing. Although Ehrhart et al. (2007) found no significant regression-line trend in a long-term dataset, researchers have observed notable increases in catch per unit effort (CPUE) (Arendt et al. 2009; Ehrhart et al. 2007; Epperly et al. 2007). Researchers believe that this increase in CPUE is likely linked to an increase in juvenile abundance, although it is unclear whether this increase in abundance represents a true population increase among juveniles or merely a shift in spatial occurrence. Bjorndal et al. (2005), cited in NMFS and USFWS (2008b), caution about extrapolating localized in-water trends to the broader population and relating localized trends in neritic sites to population trends at nesting beaches. The apparent overall increase in the abundance of neritic loggerheads in the southeastern United States may be due to increased abundance of the largest oceanic/neritic juveniles (historically referred to as small benthic juveniles), which could indicate a relatively large number of individuals around the same age may mature in the near future (TEWG 2009). In-water studies throughout the eastern United States, however, indicate a substantial decrease in the abundance of the smallest oceanic/neritic juvenile loggerheads, a pattern corroborated by stranding data (TEWG 2009).

Population Estimate

The NMFS Southeast Fisheries Science Center developed a preliminary stage/age demographic model to help determine the estimated impacts of mortality reductions on loggerhead sea turtle population dynamics (NMFS-SEFSC 2009a). The model uses the range of published information for the various parameters including mortality by stage, stage duration (years in a stage), and fecundity parameters such as eggs per nest, nests per nesting female, hatchling emergence success, sex ratio, and remigration interval. Resulting trajectories of model runs for each individual recovery unit, and the western North Atlantic population as a whole, were found to be very similar. The model run estimates, from the adult female population size for the western North Atlantic (from the 2004-2008 time frame), suggest the adult female population size is approximately 20,000 to 40,000 individuals, with a low likelihood of being up to 70,000 (NMFS-SEFSC 2009a). A less robust estimate for total benthic females in the western North Atlantic was also obtained, yielding approximately 30,000-300,000 individuals, up to less than 1 million (NMFS-SEFSC 2009a). A preliminary regional abundance survey of loggerheads within the northwestern Atlantic continental shelf for positively identified loggerhead in all strata estimated about 588,000 loggerheads (interquartile range of 382,000-817,000). When correcting for unidentified turtles in proportion to the ratio of identified turtles, the estimate increased to about 801,000 loggerheads (interquartile range of 521,000-1,111,000) (NEFSC 2011).

General Threats Faced by All Sea Turtle Species

Sea turtles face numerous natural and man-made threats that shape their status and affect their ability to recover. Many of the threats are either the same or similar in nature for all listed sea

turtle species, those identified in this section are discussed in a general sense for all sea turtles and will not be repeated for Green (section 3.2.3) or Kemp's Ridley (Section 3.2.4). Threat information specific to a particular species are then discussed in the corresponding status sections where appropriate.

Fisheries

Incidental bycatch in commercial fisheries is identified as a major contributor to past declines, and threat to future recovery, for all of the sea turtle species (NMFS and USFWS 1991b; NMFS and USFWS 1992; NMFS and USFWS 1993; NMFS and USFWS 2008a; NMFS et al. 2011). Domestic fisheries often capture, injure, and kill sea turtles at various life stages. Sea turtles in the pelagic environment are exposed to U.S. Atlantic pelagic longline fisheries. Sea turtles in the benthic environment in waters off the coastal United States are exposed to a suite of other fisheries in federal and state waters. These fishing methods include trawls, gillnets, purse seines, hook-and-line gear (including bottom longlines and vertical lines [e.g., bandit gear, handlines, and rod-reel]), pound nets, and trap fisheries. Refer to the Environmental Baseline section of this opinion for more specific information regarding federal and state managed fisheries affecting sea turtles within the action area). The Southeast U.S. shrimp fisheries have historically been the largest fishery threat to benthic sea turtles in the southeastern United States, and continue to interact with and kill large numbers of sea turtles each year.

In addition to domestic fisheries, sea turtles are subject to direct as well as incidental capture in numerous foreign fisheries, further impeding the ability of sea turtles to survive and recover on a global scale. For example, pelagic stage sea turtles, especially loggerheads and leatherbacks, circumnavigating the Atlantic are susceptible to international longline fisheries including the Azorean, Spanish, and various other fleets (Aguilar et al. 1994; Bolten et al. 1994; Crouse 1999). Bottom longlines and gillnet fishing is known to occur in many foreign waters, including (but not limited to) the northwest Atlantic, western Mediterranean, South America, West Africa, Central America, and the Caribbean. Shrimp trawl fisheries are also occurring off the shores of numerous foreign countries and pose a significant threat to sea turtles similar to the impacts seen in U.S. waters. Many unreported takes or incomplete records by foreign fleets make it difficult to characterize the total impact that international fishing pressure is having on listed sea turtles. Nevertheless, international fisheries represent a continuing threat to sea turtle survival and recovery throughout their respective ranges.

Non-Fishery In-Water Activities

There are also many non-fishery impacts affecting the status of sea turtle species, both in the ocean and on land. In nearshore waters of the United States, the construction and maintenance of federal navigation channels has been identified as a source of sea turtle mortality. Hopper dredges, which are frequently used in ocean bar channels and sometimes in harbor channels and offshore borrow areas, move relatively rapidly and can entrain and kill sea turtles (NMFS 1997a). Sea turtles entering coastal or inshore areas have also been affected by entrainment in the cooling-water systems of electrical generating plants. Other nearshore threats include harassment and/or injury resulting from private and commercial vessel operations, military detonations and training exercises, in-water construction activities, and scientific research activities.

Coastal Development and Erosion Control
Coastal development can deter or interfere with nesting, affect nesting success, and degrade nesting habitats for sea turtles. Structural impacts to nesting habitat include the construction of buildings and pilings, beach armoring and renourishment, and sand extraction (Bouchard et al. 1998; Lutcavage et al. 1997). These factors may decrease the amount of nesting area available to females and change the natural behaviors of both adults and hatchlings, directly or indirectly, through loss of beach habitat or changing thermal profiles and increasing erosion, respectively. (Ackerman 1997; Witherington et al. 2003; Witherington et al. 2007). In addition, coastal development is usually accompanied by artificial lighting which can alter the behavior of nesting adults (Witherington 1992) and is often fatal to emerging hatchlings that are drawn away from the water (Witherington and Bjorndal 1991). In-water erosion control structures such as breakwaters, groins, and jetties can impact nesting females and hatchling as they approach and leave the surf zone or head out to sea by creating physical blockage, concentrating predators, creating longshore currents, and disrupting of wave patterns.

Environmental Contamination
Multiple municipal, industrial, and household sources, as well as atmospheric transport, introduce various pollutants such as pesticides, hydrocarbons, organochlorides (e.g., dichlorodiphenyltrichloroethane [DDT], polychlorinated biphenyls [PCB], and perfluorinated chemicals [PFC]), and others that may cause adverse health effects to sea turtles (Garrett 2004; Grant and Ross 2002; Hartwell 2004; Iwata et al. 1993). Acute exposure to hydrocarbons from petroleum products released into the environment via oil spills and other discharges may directly injure individuals through skin contact with oils (Geraci 1990), inhalation at the water's surface and ingesting compounds while feeding (Matkin and Saulitis 1997). Hydrocarbons also have the potential to impact prey populations, and therefore may affect listed species indirectly by reducing food availability in the action area.

The April 20, 2010, explosion of the Deepwater Horizon oil rig affected sea turtles in the Gulf of Mexico. There is an on-going assessment of the long-term effects of the spill on Gulf of Mexico marine life, including sea turtle populations. Following the spill, juvenile Kemp's ridley, green, and loggerhead sea turtles were found in *Sargassum* algae mats in the convergence zones, where currents meet and oil collected. Sea turtles found in these areas were often coated in oil and/or had ingested oil. Approximately 536 live adult and juvenile sea turtles were recovered from the Gulf and brought into rehabilitation centers; of these, 456 were visibly oiled (these and the following numbers were obtained from http://www.nmfs.noaa.gov/pr/health/oilspill/). To date, 469 of the live recovered sea turtles have been successfully returned to the wild, 25 died during rehabilitation, and 42 are still in care but will hopefully be returned to the wild eventually.

During the clean-up period, 613 dead sea turtles were recovered in coastal waters or on beaches in Mississippi, Alabama, Louisiana, and the Florida Panhandle. As of February 2011, 478 of these dead turtles had been examined. Many of the examined sea turtles showed indications that they had died as a result of interactions with trawl gear, most likely used in the shrimp fishery, and not as a result of exposure to or the ingestion of oil.

During the spring and summer of 2010, nearly 300 sea turtle nests were relocated from the northern Gulf to the east coast of Florida with the goal of preventing hatchlings from entering the

oiled waters of the northern Gulf. From these relocated nests, 14,676 sea turtles, including 14,235 loggerheads, 125 Kemp's ridleys, and 316 greens, were ultimately released from Florida beaches.

A thorough assessment of the long-term effects of the spill on sea turtles has not yet been completed. However, the spill resulted in the direct mortality of many sea turtles and may have had sublethal effects or caused environmental damage that will impact other sea turtles into the future. The population level effects of the spill and associated response activity are likely to remain unknown for some period into the future.

Marine debris is a continuing problem for sea turtles. Sea turtles living in the pelagic environment commonly eat or become entangled in marine debris (e.g., tar balls, plastic bags/pellets, balloons, and ghost fishing gear) as they feed along oceanographic fronts where debris and their natural food items converge. This is especially problematic for sea turtles that spend all or significant portions of their life cycle in the pelagic environment (i.e., leatherbacks, juvenile loggerheads, and juvenile green turtles).

Climate Change

There is a large and growing body of literature on past, present, and future impacts of global climate change, exacerbated and accelerated by human activities. Some of the likely effects commonly mentioned are sea level rise, increased frequency of severe weather events, and change in air and water temperatures. NOAA's climate information portal provides basic background information on these and other measured or anticipated effects (see http://www.climate.gov).

Climate change impacts on sea turtles currently cannot be predicted with any degree of certainty; however, significant impacts to the hatchling sex ratios of sea turtles may result (NMFS and USFWS 2007q). In sea turtles, sex is determined by the ambient sand temperature (during the middle third of incubation) with female offspring produced at higher temperatures and males at lower temperatures within a thermal tolerance range of 25°-35°C (Ackerman 1997). Increases in global temperature could potentially skew future sex ratios toward higher numbers of females (NMFS and USFWS 2007q).

The effects from increased temperatures may be intensified on developed nesting beaches where shoreline armoring and construction have denuded vegetation. Erosion control structures could potentially result in the permanent loss of nesting beach habitat or deter nesting females (NRC 1990). These impacts will be exacerbated by sea level rise. If females nest on the seaward side of the erosion control structures, nests may be exposed to repeated tidal overwash (NMFS and USFWS 2007q). Sea level rise from global climate change is also a potential problem for areas with low-lying beaches where sand depth is a limiting factor, as the sea may inundate nesting sites and decrease available nesting habitat (Baker et al. 2006; Daniels et al. 1993; Fish et al. 2005). The loss of habitat as a result of climate change could be accelerated due to a combination of other environmental and oceanographic changes such as an increase in the frequency of storms and/or changes in prevailing currents, both of which could lead to increased beach loss via erosion (Antonelis et al. 2006; Baker et al. 2006).

Other changes in the marine ecosystem caused by global climate change (e.g., ocean acidification, salinity, oceanic currents, dissolved oxygen levels, nutrient distribution, etc.) could influence the distribution and abundance of lower trophic levels (e.g., phytoplankton, zooplankton, submerged aquatic vegetation, crustaceans, mollusks, forage fish, etc.) which could ultimately affect the primary foraging areas of sea turtles.

Other Threats

Predation by various land predators is a threat to developing nests and emerging hatchlings. The major natural predators of sea turtle nests are mammals, including raccoons, dogs, pigs, skunks, and badgers. Emergent hatchlings are preyed upon by these mammals as well as ghost crabs, laughing gulls, and the exotic South American fire ant (*Solenopsis invicta*). In addition to natural predation, direct harvest of eggs and adults from beaches in foreign countries continues to be a problem for various sea turtle species throughout their ranges (NMFS and USFWS 2008b).

Diseases, toxic blooms from algae and other microorganisms, and cold stunning events are additional sources of mortality that can range from local and limited to wide-scale and impacting hundreds or thousands of animals.

Threats (Specific to Loggerhead Sea Turtles)

The threats faced by loggerhead sea turtles are well-summarized in the general discussion of threats in the Section above. The impact of fishery interactions is a point of further emphasis for this species. The joint NMFS and USFWS Loggerhead Biological Review Team determined that the greatest threats to the NWA DPS of loggerheads result from cumulative fishery bycatch in neritic and oceanic habitats (Conant et al. 2009).

Regarding the impacts of pollution, loggerheads may be particularly affected by organochlorine contaminants; they have the highest organochlorine concentrations (Storelli et al. 2008) and metal loads (D'Ilio et al. 2011) in sampled tissues among the sea turtle species. It is thought that dietary preferences were likely to be the main differentiating factor among sea turtle species. Storelli et al. (2008) analyzed tissues from stranded loggerhead sea turtles and found that mercury accumulates in sea turtle livers while cadmium accumulates in their kidneys, as has been reported for other marine organisms like dolphins, seals, and porpoises (Law et al. 1991).

Specific information regarding potential climate change impacts on loggerheads is also available. Modeling suggests an increase of 2°C in air temperature would result in a sex ratio of over 80% female offspring for loggerheads nesting near Southport, North Carolina. The same increase in air temperatures at nesting beaches in Cape Canaveral, Florida, would result in close to 100% female offspring. Such highly skewed sex ratios could undermine the reproductive capacity of the species. More ominously, an air temperature increase of 3°C is likely to exceed the thermal threshold of most nests, leading to egg mortality (Hawkes et al. 2007). Warmer sea surface temperatures have also been correlated with an earlier onset of loggerhead nesting in the spring (Hawkes et al. 2007; Weishampel et al. 2004), short inter-nesting intervals (Hays et al. 2002), and shorter nesting seasons (Pike et al. 2006).

3.2.2 Green Sea Turtle

The green sea turtle was listed as threatened under the ESA on July 28, 1978, except for the Florida and Pacific coast of Mexico breeding populations, which were listed as endangered.

Species Description and Distribution

The green sea turtle is the largest of the hardshell marine turtles, growing to a weight of 350 pound (lb) (159 kg) and a straight carapace length of greater than 3.3 ft (1 m). Green sea turtles have a smooth carapace with 4 pairs of lateral (or costal) scutes and a single pair of elongated prefrontal scales between the eyes. They typically have a black dorsal surface and a white ventral surface, although the carapace of green sea turtles in the Atlantic Ocean has been known to change in color from solid black to a variety of shades of grey, green, or brown and black in starburst or irregular patterns (Lagueux 2001).

With the exception of post-hatchlings, green sea turtles live in nearshore tropical and subtropical waters where they generally feed on marine algae and seagrasses. They have specific foraging grounds and may make large migrations between these forage sites and natal beaches for nesting (Hays et al. 2001). Green sea turtles nest on sandy beaches of mainland shores, barrier islands, coral islands, and volcanic islands in more than 80 countries worldwide (Hirth and USFWS 1997). The 2 largest nesting populations are found at Tortuguero, on the Caribbean coast of Costa Rica, and Raine Island, on the Pacific coast of Australia along the Great Barrier Reef.

Differences in mitochondrial Deoxyribonucleic Acid (DNA) properties of green sea turtles from different nesting regions indicate there are genetic subpopulations (Bowen et al. 1992; Fitzsimmons et al. 2006). Despite the genetic differences, sea turtles from separate nesting origins are commonly found mixed together on foraging grounds throughout the species' range. Such mixing occurs at extremely low levels in Hawaiian foraging areas, perhaps making this central Pacific population the most isolated of all green sea turtle populations occurring worldwide (Dutton et al. 2008).

In U.S. Atlantic and Gulf of Mexico waters, green sea turtles are distributed throughout inshore and nearshore waters from Texas to Massachusetts. Principal benthic foraging areas in the southeastern United States include Aransas Bay, Matagorda Bay, Laguna Madre, and the Gulf inlets of Texas (Doughty 1984; Hildebrand 1982; Shaver 1994), the Gulf of Mexico off Florida from Yankeetown to Tarpon Springs (Caldwell and Carr 1957; Carr 1984), Florida Bay and the Florida Keys (Schroeder and Foley 1995), the Indian River Lagoon system in Florida (Ehrhart 1983), and the Atlantic Ocean off Florida from Brevard through Broward Counties (Guseman and Ehrhart 1992; Wershoven and Wershoven 1992). The summer developmental habitat for green sea turtles also encompasses estuarine and coastal waters from North Carolina to as far north as Long Island Sound (Musick and Limpus 1997). Additional important foraging areas in the western Atlantic include the Culebra archipelago and other Puerto Rico coastal waters, the south coast of Cuba, the Mosquito Coast of Nicaragua, the Caribbean coast of Panama, scattered areas along Colombia and Brazil (Hirth 1971), and the northwestern coast of the Yucatán Peninsula.

The complete nesting range of green sea turtles within the southeastern United States includes sandy beaches between Texas and North Carolina, as well as the U.S. Virgin Islands and Puerto

Rico (Dow et al. 2007; NMFS and USFWS 1991b). Still, the vast majority of green sea turtle nesting within the southeastern United States occurs in Florida (Johnson and Ehrhart 1994; Meylan et al. 1995). Principal U.S. nesting areas for green sea turtles are in eastern Florida, predominantly Brevard south through Broward counties. For more information on green sea turtle nesting in other ocean basins, refer to the 1991 publication, *Recovery Plan for the Atlantic Green Turtle* (NMFS and USFWS 1991b) or the 2007 publication, *Green Sea Turtle 5-Year Status Review* (NMFS and USFWS 2007a).

*Life History Informati*on

Green sea turtles reproduce sexually, and mating occurs in the waters off nesting beaches. Mature females return to their natal beaches (i.e., the same beaches where they were born) to lay eggs (Balazs 1982; Frazer and Ehrhart 1985) every 2-4 years while males are known to reproduce every year (Balazs 1983). In the southeastern United States, females generally nest between June and September, and peak nesting occurs in June and July (Witherington and Ehrhart 1989). During the nesting season, females nest at approximately 2-week intervals, laying an average of 3-4 clutches (Johnson and Ehrhart 1996). Clutch size often varies among subpopulations, but mean clutch size is approximately 110-115 eggs. In Florida, green sea turtle nests contain an average of 136 eggs (Witherington and Ehrhart 1989). Eggs incubate for approximately 2 months before hatching. Hatchling green sea turtles are approximately 2 in (5 cm) in length and weigh approximately 0.9 ounces (25 grams). Survivorship at any particular nesting site is greatly influenced by the level of anthropogenic stressors, with the more pristine and less disturbed nesting sites (e.g., along the Great Barrier Reef in Australia) showing higher survivorship values than nesting sites known to be highly disturbed (e.g., Nicaragua) (Campbell and Lagueux 2005; Chaloupka and Limpus 2005).

After emerging from the nest, hatchlings swim to offshore areas and go through a post-hatchling pelagic stage where they are believed to live for several years. During this life stage, green sea turtles feed close to the surface on a variety of marine algae and other life associated with drift lines and debris. This early oceanic phase remains one of the most poorly understood aspects of green sea turtle life history (NMFS and USFWS 2007m). Green sea turtles exhibit particularly slow growth rates of about 0.4-2 in (1-5 cm) per year (Green 1993; McDonald-Dutton and Dutton 1998), which may be attributed to their largely herbivorous, low-net energy diet (Bjorndal 1982). At approximately 8-10 in (20-25 cm) carapace length, juveniles leave the pelagic environment and enter nearshore developmental habitats such as protected lagoons and open coastal areas rich in sea grass and marine algae. Growth studies using skeletochronology indicate that green sea turtles in the western Atlantic shift from the oceanic phase to nearshore developmental habitats after approximately 5-6 years (Bresette et al. 2006; Zug and Glor 1998). Within the developmental habitats, juveniles begin the switch to a more herbivorous diet, and by adulthood feed almost exclusively on seagrasses and algae (Rebel 1974), although some populations are known to also feed heavily on invertebrates (Carballo et al. 2002). Green sea turtles mature slowly, requiring 20-50 years to reach sexual maturity (Chaloupka and Musick 1997; Hirth and USFWS 1997).

While in coastal habitats, green sea turtles exhibit site fidelity to specific foraging and nesting grounds, and it is clear they are capable of “homing in” on these sites if displaced (McMichael et al. 2003). Reproductive migrations of Florida green sea turtles have been identified through

flipper tagging and/or satellite telemetry. Based on these studies, the majority of adult female Florida green sea turtles are believed to reside in nearshore foraging areas throughout the Florida Keys and in the waters southwest of Cape Sable, with some post-nesting turtles also residing in Bahamian waters as well (NMFS and USFWS 2007m).

Status and Population Dynamics

Population estimates for marine turtles do not exist because of the difficulty in sampling turtles over their geographic ranges and within their marine environments. Nonetheless, researchers have used nesting data to study trends in reproducing sea turtles over time. A summary of nesting trends is provided in the most recent 5-year status review for the species (NMFS and USFWS 2007m) organized by ocean region (i.e., Western Atlantic Ocean, Central Atlantic Ocean, Eastern Atlantic Ocean, Mediterranean Sea, Western Indian Ocean, Northern Indian Ocean, Eastern Indian Ocean, Southeast Asia, Western Pacific Ocean, Central Pacific Ocean, and Eastern Pacific Ocean). It shows trends at 23 of the 46 nesting sites: 10 appeared to be increasing, 9 appeared to be stable, and 4 appeared to be decreasing. With respect to regional trends, the Pacific, the Western Atlantic, and the Central Atlantic regions appeared to show more positive trends (i.e., more nesting sites increasing than decreasing) while the Southeast Asia, the Eastern Indian Ocean, and possibly the Mediterranean Sea regions appeared to show more negative trends (i.e., more nesting sites decreasing than increasing). These regional determinations should be viewed with caution, because trend data were only available for about half of the total nesting concentration sites examined in the review and site specific data availability appeared to vary across all regions.

The Western Atlantic region (i.e., the focus of this Opinion) was one of the best performing in terms of abundance in the entire review, as there were no sites that appeared to decrease. The 5-year status review for the species reviewed the trend in nest count data for each identified 8 geographic areas considered to be primary sites for green sea turtle nesting in the Atlantic/Caribbean (NMFS and USFWS 2007a): (1) Yucatán Peninsula, Mexico; (2) Tortuguero, Costa Rica; (3) Aves Island, Venezuela; (4) Galibi Reserve, Suriname; (5) Isla Trindade, Brazil; (6) Ascension Island, United Kingdom; (7) Bioko Island, Equatorial Guinea; and (8) Bijagos Achipelago, Guinea-Bissau. Nesting at all of these sites was considered to be stable or increasing with the exception of Bioko Island and the Bijagos Archipelago where the lack of sufficient data precluded a meaningful trend assessment for either (NMFS and USFWS 2007a). Seminoff (2004) likewise reviewed green sea turtle nesting data for 8 sites in the western, eastern, and central Atlantic, including all of the above with the exception that nesting in Florida was reviewed in place of Isla Trindade, Brazil. Seminoff (2004) concluded that all sites in the central and western Atlantic showed increased nesting, with the exception of nesting at Aves Island, Venezuela, while both sites in the eastern Atlantic demonstrated decreased nesting. These sites are not inclusive of all green sea turtle nesting in the Atlantic; however, other sites are not believed to support nesting levels high enough that would change the overall status of the species in the Atlantic (NMFS and USFWS 2007a). More information about site-specific trends for the other major ocean regions can be found in the most recent 5-year status review for the species (see NMFS and USFWS (2007a).

By far, the largest known nesting assemblage in the western Atlantic region occurs at Tortuguero, Costa Rica. According to monitoring data on nest counts, as well as documented

emergences (both nesting and non-nesting events), there appears to be an increasing trend in this nesting assemblage since monitoring began in the early 1970s. For instance, from 1971-1975 there were approximately 41,250 average annual emergences documented and this number increased to an average of 72,200 emergences from 1992-1996 (Bjorndal et al. 1999). Troëng and Rankin (2005) collected nest counts from 1999-2003 and also reported increasing trends in the population consistent with the earlier studies, with nest count data suggesting 17,402-37,290 nesting females per year (NMFS and USFWS 2007a). Modeling by Chaloupka et al. (2008) using data sets of 25 years or more resulted in an estimate of the Tortuguero, Costa Rica, population's growing at 4.9% annually.

In the continental United States, green sea turtle nesting occurs along the Atlantic coast, primarily along the central and southeast coast of Florida where an estimated 200-1,100 females nest each year (Meylan et al. 1994; Weishampel et al. 2003). Occasional nesting has also been documented along the Gulf Coast of Florida (Meylan et al. 1995). More recently, green sea turtle nesting has occurred in North Carolina on Bald Head Island, just east of the mouth of the Cape Fear River, on Onslow Island, and on Cape Hatteras National Seashore. In 2010, a total of 18 nests were found in North Carolina, 6 nests in South Carolina, and 6 nests in Georgia (nesting databases maintained on www.seaturtle.org).

In Florida, index beaches were established to standardize data collection methods and effort on key nesting beaches. Since establishment of the index beaches in 1989, the pattern of green sea turtle nesting has generally shown biennial peaks in abundance with a positive trend during the 10 years of regular monitoring (Figure 15). According to data collected from Florida's index nesting beach survey from 1989-2012, green sea turtle nest counts across Florida have increased approximately ten-fold from a low of 267 in the early 1990s to a high of 25,553 in 2013. Two consecutive years of nesting declines in 2008 and 2009 caused some concern, but this was followed by increases in both 2010 and 2011, a decrease in 2012, and another increase in 2013 (Figure 15). Modeling by Chaloupka et al. (2008) using data sets of 25 years or more has resulted in an estimate of the Florida nesting stock at the Archie Carr National Wildlife Refuge growing at an annual rate of 13.9%.

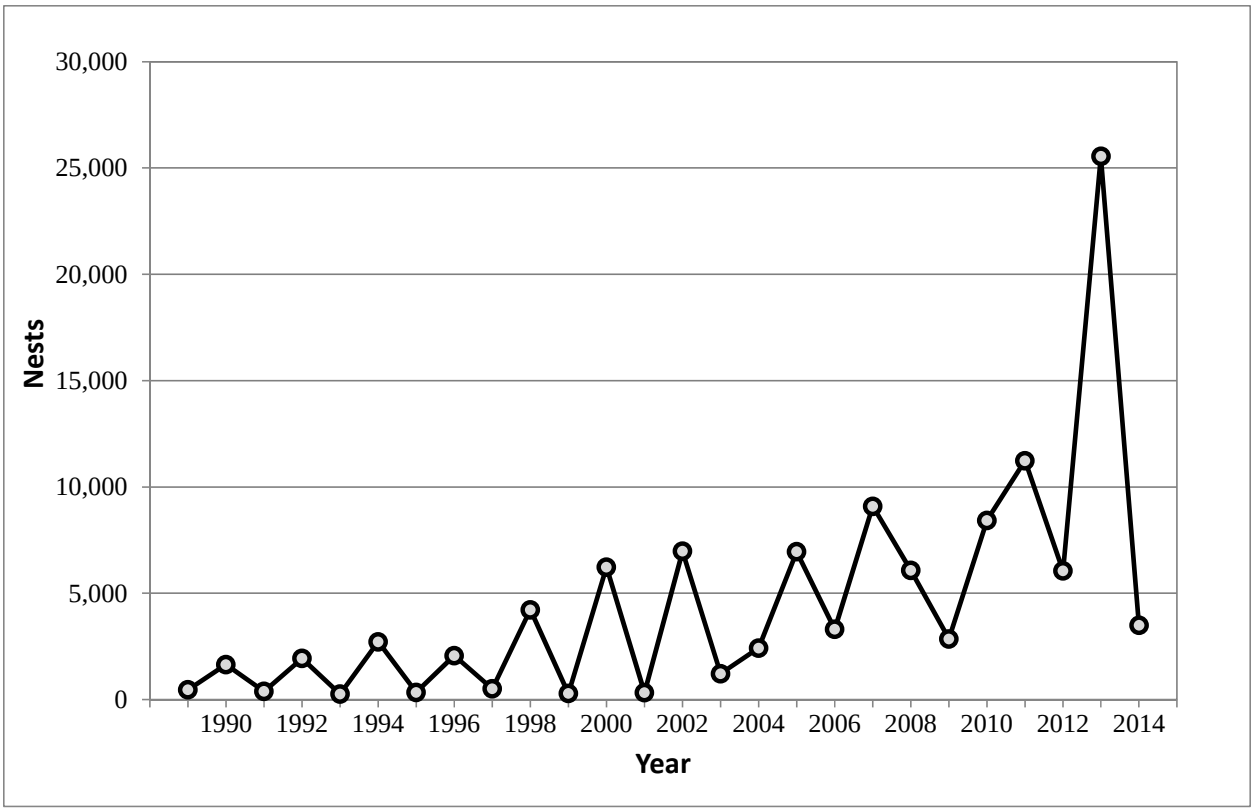


Figure 15. Green sea turtle nesting at Florida index beaches since 1989

Threats

The principal cause of past declines and extirpations of green sea turtle assemblages has been the overexploitation of the species for food and other products. Although intentional take of green sea turtles and their eggs is not extensive within the southeastern United States, green sea turtles that nest and forage in the region may spend large portions of their life history outside the region and outside U.S. jurisdiction, where exploitation is still a threat. Green sea turtles also face many of the same threats as other sea turtle species, including destruction of nesting habitat from storm events, oceanic events such as cold-stunning, pollution (e.g., plastics, petroleum products, petrochemicals), ecosystem alterations (e.g., nesting beach development, beach nourishment and shoreline stabilization, vegetation changes), poaching, global climate change, fisheries interactions, natural predation, and disease. A discussion on general sea turtle threats can be found in Section 3.2.1, with the heading *General Threats Faced by All Sea Turtle Species*

In addition to general threats, green sea turtles are susceptible to natural mortality from Fibropapillomatosis (FP) disease. FP results in the growth of tumors on soft external tissues (flippers, neck, tail, etc.), the carapace, the eyes, the mouth, and internal organs (gastrointestinal tract, heart, lungs, etc.) of turtles (Aguirre et al. 2002; Herbst 1994; Jacobson et al. 1989). These tumors range in size from 0.04 in (0.1 cm) to greater than 11.81 in (30 cm) in diameter and may affect swimming, vision, feeding, and organ function (Aguirre et al. 2002; Herbst 1994; Jacobson et al. 1989). Presently, scientists are unsure of the exact mechanism causing this disease, though it is believed to be related to both an infectious agent, such as a virus (Herbst et al. 1995), and environmental conditions (e.g., habitat degradation, pollution, low wave energy, and shallow water) (Foley et al. 2005). Presently, FP is cosmopolitan, but it has been found to

affect large numbers of animals in specific areas, including Hawaii and Florida (Herbst 1994; Jacobson 1990; Jacobson et al. 1991).

Cold-stunning is another natural threat to green sea turtles. Although it is not considered a major source of mortality in most cases, as temperatures fall below 46.4°F-50°F (8°-10°C) turtles may lose their ability to swim and dive, often floating to the surface. The rate of cooling that precipitates cold-stunning appears to be the primary threat, rather than the water temperature itself (Milton and Lutz 2003). Sea turtles that overwinter in inshore waters are most susceptible to cold-stunning because temperature changes are most rapid in shallow water (Witherington and Ehrhart 1989). During January 2010, an unusually large cold-stunning event in the southeastern United States resulted in around 4,600 sea turtles, mostly greens, found cold-stunned, with hundreds found dead or dying. A large cold-stunning event occurred in the western Gulf of Mexico in February 2011, resulting in approximately 1,650 green sea turtles found cold-stunned in Texas. Of these, approximately 620 were found dead or died after stranding, while approximately 1,030 turtles were rehabilitated and released. Additionally, during this same time frame, approximately 340 green sea turtles were found cold-stunned in Mexico, though approximately 300 of those were subsequently rehabilitated and released.

3.2.3 Kemp's Ridley

The Kemp's ridley sea turtle was listed as endangered on December 2, 1970, under the Endangered Species Conservation Act of 1969, a precursor to the ESA. Internationally, the Kemp's ridley is considered the most endangered sea turtle (Groombridge 1982; TEWG 2000b; Zwinenberg 1977).

Species Description and Distribution

The Kemp's ridley sea turtle is the smallest of all sea turtles. Adults generally weigh less than 100 lb (45 kg) and have a carapace length of around 2.1 ft (65 cm). Adult Kemp's ridley shells are almost as wide as they are long. Coloration changes significantly during development from the grey-black dorsum and plastron of hatchlings, a grey-black dorsum with a yellowish-white plastron as post-pelagic juveniles, and then to the lighter grey-olive carapace and cream-white or yellowish plastron of adults. There are 2 pairs of prefrontal scales on the head, 5 vertebral scutes, usually 5 pairs of costal scutes, and generally 12 pairs of marginal scutes on the carapace. In each bridge adjoining the plastron to the carapace, there are 4 scutes, each of which is perforated by a pore.

Kemp's ridley habitat largely consists of sandy and muddy areas in shallow, nearshore waters less than 120 ft (37 m) deep, although they can also be found in deeper offshore waters. These areas support the primary prey species of the Kemp's ridley sea turtle, which consist of swimming crabs, but may also include fish, jellyfish, and an array of mollusks.

The primary range of Kemp's ridley sea turtles is within the Gulf of Mexico basin, though they also occur in coastal and offshore waters of the U.S. Atlantic Ocean. Juvenile Kemp's ridley sea turtles, possibly carried by oceanic currents, have been recorded as far north as Nova Scotia. Historic records indicate a nesting range from Mustang Island, Texas, in the north to Veracruz, Mexico, in the south. Kemp's ridley sea turtles have recently been nesting along the Atlantic Coast of the United States, with nests recorded from beaches in Florida, Georgia, and the

Carolinas. In 2012, the first Kemp's ridley sea turtle nest was recorded in Virginia. The Kemp's ridley nesting population had been exponentially increasing prior to the recent low nesting years, which may indicate that the population had been experiencing a similar increase. Additional nesting data in the coming years will be required to determine what the recent nesting decline means for the population trajectory.

Life History Information

Kemp's ridley sea turtles share a general life history pattern similar to other sea turtles. Females lay their eggs on coastal beaches where the eggs incubate in sandy nests. After 45-58 days of embryonic development, the hatchlings emerge and swim offshore into deeper, ocean water where they feed and grow until returning at a larger size. Hatchlings generally range from 1.65-1.89 in (42-48 mm) straight carapace length (SCL), 1.26-1.73 in (32-44 mm) in width, and 0.3-0.4 lb (15-20 g) in weight. Their return to nearshore coastal habitats typically occurs around 2 years of age (Ogren 1989), although the time spent in the oceanic zone may vary from 1-4 years or perhaps more (TEWG 2000). Juvenile Kemp's ridley sea turtles use these nearshore coastal habitats from April through November, but move towards more suitable overwintering habitat in deeper offshore waters (or more southern waters along the Atlantic coast) as water temperature drops.

The average rates of growth may vary by location, but generally fall within 2.2-2.9 ± 2.4 in per year (5.5-7.5 ± 6.2 cm/year) (Schmid and Barichivich 2006; Schmid and Woodhead 2000). Age to sexual maturity ranges greatly from 5-16 years, though NMFS et al. (2011) determined the best estimate of age to maturity for Kemp's ridley sea turtles was 12 years. It is unlikely that most adults grow very much after maturity. While some sea turtles nest annually, the weighted mean remigration rate for Kemp's ridley sea turtles is approximately 2 years. Nesting generally occurs from April to July and females lay approximately 2.5 nests per season with each nest containing approximately 100 eggs (Márquez M 1994).

Population Dynamics

Of the 7 species of sea turtles in the world, the Kemp's ridley has declined to the lowest population level. Most of the population of adult females nest on the beaches of Rancho Nuevo, Mexico (Pritchard 1969). When nesting aggregations at Rancho Nuevo were discovered in 1947, adult female populations were estimated to be in excess of 40,000 individuals (Hildebrand 1963). By the mid-1980s, however, nesting numbers from Rancho Nuevo and adjacent Mexican beaches were below 1,000, with a low of 702 nests in 1985. Yet, nesting steadily increased through the 1990s, and then accelerated during the first decade of the twenty-first century (Figure 16), which indicates the species is recovering. It is worth noting that when the Bi-National Kemp's Ridley Sea Turtle Population Restoration Project was initiated in 1978, only Rancho Nuevo nests were recorded. In 1988, nesting data from southern beaches at Playa Dos and Barra del Tordo were added. In 1989, data from the northern beaches of Barra Ostionales and Tepehuajes were added, and most recently in 1996, data from La Pesca and Altamira beaches were recorded. Currently, nesting at Rancho Nuevo accounts for just over 81% of all recorded Kemp's ridley nests in Mexico. Following a significant, unexplained 1-year decline in 2010, Kemp's ridley nests in Mexico reached a record high of 21,797 in 2012 (Gladys Porter Zoo nesting database 2013). In 2013 through 2014, there was a second significant decline, with only 16,385 and 11,279 nests recorded, respectively. A small nesting population is also

emerging in the United States, primarily in Texas, rising from 6 nests in 1996 to 42 in 2004, to a record high of 209 nests in 2012.[20]

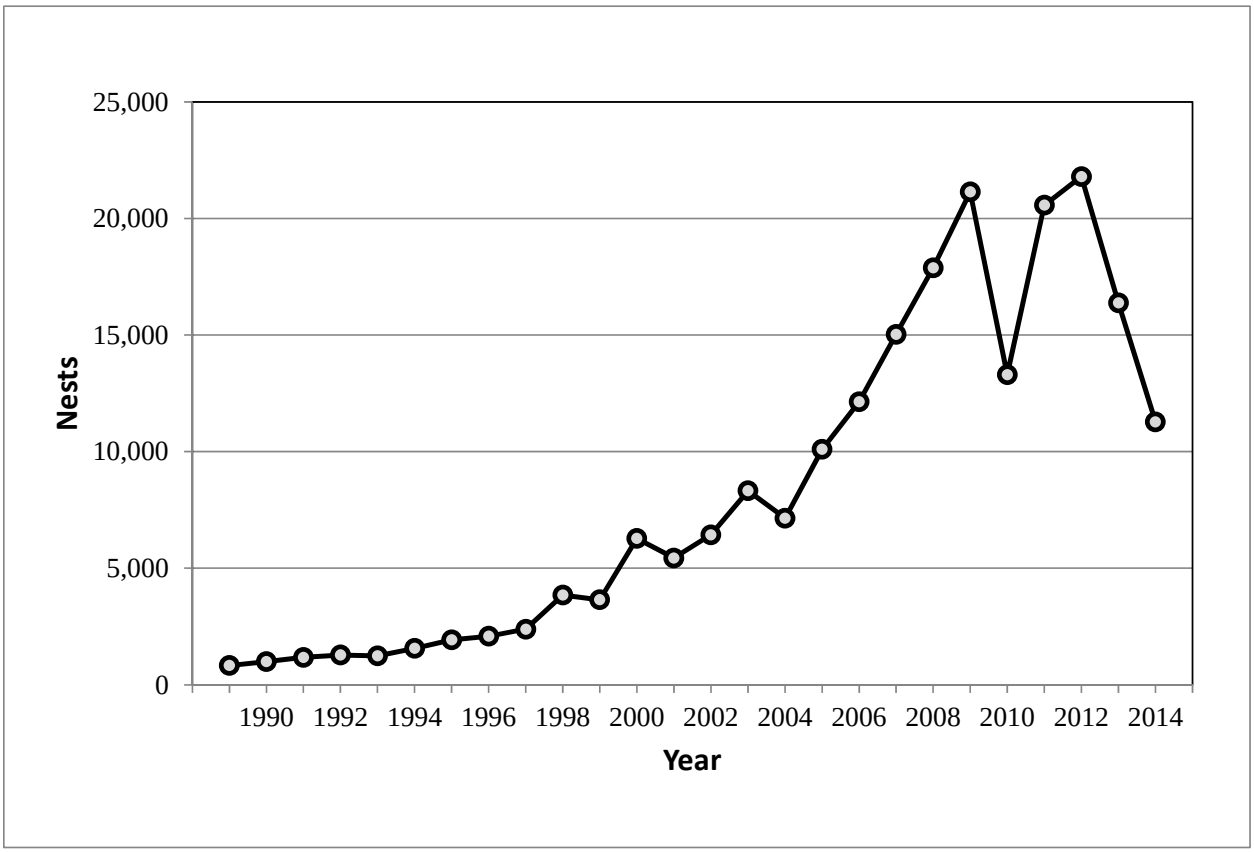


Figure 16. Kemp's ridley nest totals from Mexican beaches (Gladys Porter Zoo nesting database 2014)

Heppell et al. (2005) predicted in a population model that the population is expected to increase at least 12-16% per year and that the population could attain at least 10,000 females nesting on Mexico beaches by 2015. NMFS et al. (2011) produced an updated model that predicted the population to increase 19% per year and attain at least 10,000 females nesting on Mexico beaches by 2011. Approximately 25,000 nests would be needed for an estimate of 10,000 nesters on the beach, based on an average 2.5 nests/nesting female. While counts did not reach 25,000 nests by 2012, it is clear that the population is steadily increasing over the long term. The recent increases in Kemp's ridley sea turtle nesting seen in the last 2 decades is likely due to a combination of management measures including elimination of direct harvest, nest protection, the use of turtle excluder devices (TEDs), reduced trawling effort in Mexico and the United States, and possibly other changes in vital rates (TEWG 1998; TEWG 2000b). While these results are encouraging, the species limited range as well as low global abundance makes it particularly vulnerable to new sources of mortality as well as demographic and environmental randomness, all of which are often difficult to predict with any certainty. Additionally, the significant nesting declines observed in 2010 and 2013-2014 potentially indicate a serious

[20] National Park Service data, http://www.nps.gov/pais/naturescience/strp.htm, http://www.nps.gov/pais/naturescience/current-season.htm

population-level impact, and there is cause for concern regarding the ongoing recovery trajectory.

Threats

Kemp's ridley sea turtles face many of the same threats as other sea turtle species, including destruction of nesting habitat from storm events, oceanic events such as cold-stunning, pollution (plastics, petroleum products, petrochemicals, etc.), ecosystem alterations (nesting beach development, beach nourishment and shoreline stabilization, vegetation changes, etc.), poaching, global climate change, fisheries interactions, natural predation, and disease. A discussion on general sea turtle threats can be found in Section 3.2.1 with the heading *General Threats Faced by All Sea Turtle Species*; the remainder of this section will expand on a few of the aforementioned threats and how they may specifically impact Kemp's ridley sea turtles.

As Kemp's ridley sea turtles continue to recover and nesting arribadas[21] are increasingly established, bacterial and fungal pathogens in nests are also likely to increase. Bacterial and fungal pathogen impacts have been well documented in the large arribadas of the olive ridley at Nancite in Costa Rica (Mo 1988). In some years, and on some sections of the beach, the hatching success can be as low as 5% (Mo 1988). As the Kemp's ridley nest density at Rancho Nuevo and adjacent beaches continues to increase, appropriate monitoring of emergence success will be necessary to determine if there are any density-dependent effects.

Over the past 3 years, NMFS has documented via the Sea Turtle Stranding and Salvage Network (STSSN) data[22] elevated sea turtle strandings in the Northern Gulf of Mexico, particularly throughout the Mississippi Sound area. In the first 3 weeks of June 2010, over 120 sea turtle strandings were reported from Mississippi and Alabama waters, none of which exhibited any signs of external oiling to indicate effects associated with the Deepwater Horizon oil spill event. A total of 644 sea turtle strandings were reported in 2010 from Louisiana, Mississippi, and Alabama waters, 561 (87%) of which were Kemp's ridley sea turtles. During March through May of 2011, 267 sea turtle strandings were reported from Mississippi and Alabama waters alone. A total of 525 sea turtle strandings were reported in 2011 from Louisiana, Mississippi, and Alabama waters, with the majority (455) occurring from March through July, 390 (86%) of which were Kemp's ridley sea turtles. During 2012, a total of 428 sea turtles were reported from Louisiana, Mississippi, and Alabama waters, though the data is incomplete. Of these reported strandings, 301 (70%) were Kemp's ridley sea turtles. These stranding numbers are significantly greater than reported in past years; Louisiana, Mississippi, and Alabama waters reported 42 and 73 sea turtle strandings for 2008 and 2009, respectively. It should be noted that stranding coverage has increased considerably due to the Deepwater Horizon 2010 oil spill event.

Nonetheless, considering that strandings typically represent only a small fraction of actual mortality, these stranding events potentially represent a serious impact to the recovery and survival of the local sea turtle populations. While a definitive cause for these strandings has not been identified, necropsy results indicate a significant number of stranded turtles from these events likely perished due to forced submergence, which is commonly associated with fishery

[21] Arribada is the Spanish word for "arrival" and is the term used for massive synchronized nesting within the genus *Lepidochelys.*

[22] http://www.sefsc.noaa.gov/species/turtles/strandings.htm

interactions (B. Stacy, NMFS, pers. comm. to M. Barnette, NMFS, March 2012). Yet available information indicates fishery effort was extremely limited during the stranding events. The fact that in both 2010 and 2011 approximately 85% of all Louisiana, Mississippi, and Alabama stranded sea turtles were Kemp's ridleys is notable; however, this could simply be a function of the species' preference for shallow, inshore waters coupled with increased population abundance as reflected in recent Kemp's ridleys' nesting increases.

In response to these strandings, and due to speculation that fishery interactions may be the cause, fishery observer effort was shifted to evaluate the inshore skimmer trawl fishery during the summer of 2012. During May-July of that year, observers reported 24 sea turtle interactions in the skimmer trawl fishery, all but one of which were identified as Kemp's ridleys (1 sea turtle was an unidentified hardshell turtle). Encountered sea turtles were all very small, juvenile specimens ranging from 7.6-19.0 in (19.4-48.3 cm) curved carapace length (CCL), and all sea turtles were released alive. The small average size of encountered Kemp's ridleys introduces a potential conservation issue, as over 50% of these reported sea turtles could potentially pass through the maximum 4-in bar spacing of TEDs currently required in the shrimp fishery. Due to this issue, a proposed 2012 rule to require TEDs in the skimmer trawl fishery (77 FR 27411) was not implemented. Based on anecdotal information, these interactions were a relatively new issue for the inshore skimmer trawl fishery. Given the nesting trends and habitat utilization of Kemp's ridley sea turtles, it is likely that fishery interactions in the Northern Gulf of Mexico may continue to be an issue of concern for the species, and one that may potentially slow the rate of recovery for Kemp's ridley sea turtles.

3.2.4 Atlantic Sturgeon

Five separate DPSs of Atlantic sturgeon were listed under the ESA by NMFS effective April 6, 2012 (77 FR 5880 and 5914, February 6, 2012). The New York Bight, Chesapeake Bay, Carolina, and South Atlantic DPSs were listed as endangered. The Gulf of Maine DPS was listed as threatened.

Species Descriptions and Distributions

Atlantic sturgeon are long-lived, late-maturing, estuarine-dependent, anadromous fish distributed along the eastern coast of North America (Waldman and Wirgin 1998). Historically, sightings have been reported from Hamilton Inlet, Labrador, south to the St. Johns River, Florida (Murawski et al. 1977; Smith and Clugston 1997). Atlantic sturgeon may live up to 60 years, reach lengths up to 14 ft, and weigh over 800 lb (ASSRT 2007; Collette and Klein-MacPhee 2002). They are distinguished by armor-like plates (called scutes) and a long protruding snout that has 4 barbels (slender, whisker-like feelers extending from the head used for touch and taste). Atlantic sturgeon spend the majority of their lives in nearshore marine waters, returning to their natal rivers to spawn (Wirgin et al. 2002). Young sturgeon may spend the first few years of life in their natal river estuary before moving out to sea (Wirgin et al. 2002). Sturgeon are omnivorous benthic (bottom) feeders and filter quantities of mud along with their food. Adult sturgeon diets include mollusks, gastropods, amphipods, isopods, and fish. Juvenile sturgeon feed on aquatic insects and other invertebrates (Smith 1985).

Historically, Atlantic sturgeon were present in approximately 38 rivers in the United States from the St. Croix River, Maine to the St. Johns River, Florida, of which 35 rivers have been

confirmed to have had a historical spawning population. Atlantic sturgeon are currently present in approximately 32 of these rivers, and spawning occurs in at least 20 of them. The marine range of Atlantic sturgeon extends from the Hamilton Inlet, Labrador, Canada, to Cape Canaveral, Florida. Because adult Atlantic sturgeon from all DPSs mix extensively in marine waters, we expect fish from all DPSs to be found in the action area.

Life History Information

Atlantic sturgeon populations show clinal variation, with a general trend of faster growth and earlier age at maturity in more southern systems. Atlantic sturgeon mature between the ages of 5-19 years in South Carolina (Smith et al. 1982), between 11-21 years in the Hudson River (Young et al. 1988), and between 22-34 years in the St. Lawrence River (Scott and Crossman 1973). Most Atlantic sturgeon adults likely do not spawn every year. Multiple studies have shown that spawning intervals range from 1-5 years for males (Caron et al. 2002; Collins et al. 2000e; Smith 1985) and 2-5 years for females (Stevenson and Secor 1999; Van Eenennaam et al. 1996; Vladykov and Greely 1963). Fecundity of Atlantic sturgeon has been correlated with age and body size, with egg production ranging from 400,000 to 8,000,000 eggs per year (Dadswell 2006; Smith et al. 1982; Van Eenennaam and Doroshov 1998). The average age at which 50% of maximum lifetime egg production is achieved is estimated to be 29 years, approximately 3-10 times longer than for other bony fish species examined (Boreman 1997).

Spawning adult Atlantic sturgeon generally migrate upriver in spring/early summer, which occurs in February-March in southern systems, April-May in mid-Atlantic systems, and May-July in Canadian systems (Bain 1997; Caron et al. 2002; Murawski et al. 1977; Smith 1985; Smith and Clugston 1997). In some southern rivers, a fall spawning migration may also occur (Moser et al. 1998; Rogers and Weber 1995; Weber and Jennings 1996). In the fall, Hager et al. (2014) captured an Atlantic sturgeon identified as a spawned-out female due to her size and concave stomach and also noted capture of other fish showing signs of wear suggesting males had been engaging in spawning behavior. In Virginia's James River, Balazik et al. (2012) captured 1 fish identified as a female in the fall during the 3-year study with a concave condition of the abdomen consistent with female sturgeon that have spawned recently. In addition, postovulated eggs recovered from the urogenital opening were in an early degradation stage, suggesting the fish had spawned within days (Balazik et al. 2012). Further physiological support for fall spawning is provided by the 9 spermiating males captured along with the female and a grand total of 106 different spermiating males captured during August–October (Balazik et al. 2012). Randall and Sulak (2012) reported similar evidence for fall spawning of the closely related Gulf sturgeon, which included multiple captures of sturgeon in September–November that were ripe or exhibited just-spawned characteristics.

Atlantic sturgeon spawning occurs in fast-flowing water between the salt front and fall line of large rivers (Bain et al. 2000; Borodin 1925; Crance 1987; Leland 1968; Scott and Crossman 1973) over hard substrate, such as cobble, gravel, or boulders, to which the highly adhesive sturgeon eggs adhere (Gilbert 1989; Smith and Clugston 1997). Hatching occurs approximately 94-140 hours after egg deposition and larvae assume a demersal existence (Smith et al. 1980). The yolk sac larval stage is completed in about 8-12 days, during which time the larvae move downstream to rearing grounds (Kynard and Horgan 2002). During the first half of their migration downstream, movement is limited to night. During the day, larvae use benthic

structure (e.g., gravel matrix) as refugia (Kynard and Horgan 2002). During the latter half of migration, when larvae are more fully developed, movement to rearing grounds occurs both day and night. Juvenile sturgeon continue to move further downstream into brackish waters, and eventually become residents in estuarine waters for months or years.

Juvenile and adult Atlantic sturgeon occupy upper estuarine habitat where they frequently congregate around the saltwater/freshwater interface. Estuarine habitats are important for juveniles, serving as nursery areas by providing abundant foraging opportunities, as well as thermal and salinity refuges, for facilitating rapid growth. Some juveniles will take up residency in non-natal rivers that lack active spawning sites (Bain 1997). Residency time of young Atlantic sturgeon in estuarine areas varies between 1-6 years (Schueller and Peterson 2010; Smith 1985), after which Atlantic sturgeon start out-migration to the marine environment. Out-migration of adults from the estuaries to the sea is cued by water temperature and velocity. Adult Atlantic sturgeon will reside in the marine habitat during the non-spawning season and forage extensively. Coastal migrations by adult Atlantic sturgeon are extensive and are known to occur over sand and gravel substrate (Greene et al. 2009). Atlantic sturgeon remain in the marine habitat until the waters begin to warm, at which time ripening adults migrate back to their natal rivers to spawn.

Upstream migration to the spawning grounds is cued primarily by water temperature and velocity. Therefore, fish in the southern portion of the range migrate earlier than those to the north do (Kieffer and Kynard 1993; Smith 1985). In Georgia and South Carolina, migration begins in February or March (Collins et al. 2000a). Males commence upstream migration to the spawning sites when waters reach around 6°C (Dovel and Berggren 1983; Smith 1985; Smith et al. 1982), with females following a few weeks later when water temperatures are closer to 12° or 13°C (Collins et al. 2000a; Dovel and Berggren 1983; Smith 1985). In some rivers, predominantly in the south, a fall spawning migration may also occur (Moser et al. 1998; Rogers and Weber 1995), with running ripe males found August through October and post-spawning females captured in late September and October (Collins et al. 2000e).

Status and Population Dynamics

At the time Atlantic sturgeon were listed, the best available abundance information for each of the 5 DPSs was the estimated number of adult Atlantic sturgeon spawning in each of the rivers on an annual basis. The estimated number of annually spawning adults in each of the river populations is insufficient to quantify the total population numbers for each DPS of Atlantic sturgeon due to the lack of other necessary accompanying life history data. A recently Atlantic sturgeon population estimate was derived from the Northeast Area Monitoring and Assessment Program (NEAMAP). NEAMAP trawl surveys were conducted from Cape Cod, Massachusetts, to Cape Hatteras, North Carolina, in nearshore waters to depths of 60 ft from fall 2007 through spring 2012. The results of these surveys, assuming 50% gear efficiency (i.e., assumption that the gear will capture some, but not all, of the sturgeon in the water column along the tow path, and the survey area is only a portion of Atlantic sturgeon habitat), are presented in Table 6. It is important to note that the NEAMAP surveys were conducted primarily in the Northeast and may underestimate the actual population abundances of the Carolina and South Atlantic DPSs, which are likely more concentrated in the Southeast since they originated from and spawn there.

However, the total ocean population abundance estimates listed in Table 6 currently represent the best available population abundance estimates for the 5 U.S. Atlantic sturgeon DPSs.

Table 6. Summary of Calculated Population Estimates based upon the NEAMAP Survey Swept Area, Assuming 50% Efficiency (NMFS 2013)

DPS	Estimated Ocean Population Abundance	Estimated Ocean Population of Adults	Estimated Ocean Population of Subadults (of size vulnerable to capture in fisheries)
South Atlantic	14,911	3,728	11,183
Carolina	1,356	339	1,017
Chesapeake Bay	8,811	2,203	6,608
New York Bight	34,566	8,642	25,925
Gulf of Maine	7,455	1,864	5,591

South Atlantic DPS

The South Atlantic DPS includes all Atlantic sturgeon that spawn or are spawned in the watersheds (including all rivers and tributaries) of the Ashepoo, Combahee, and Edisto River (ACE) Basins southward along the South Carolina, Georgia, and Florida coastal areas to the St. Johns River, Florida. Rivers known to have current spawning populations within the range of the South Atlantic DPS include the Combahee, Edisto, Savannah, Ogeechee, Altamaha, and Satilla Rivers. We determined spawning was occurring if young-of-the-year (YOY) were observed, or mature adults were present, in freshwater portions of a system. However, in some rivers, spawning by Atlantic sturgeon may not be contributing to population growth because of lack of suitable habitat and the presence of other stressors on juvenile survival and development.

Historically, both the Broad-Coosawatchie and St. Marys Rivers were documented to have spawning populations; there is also evidence that spawning may have occurred in the St. Johns River or one of its tributaries. The spawning population in the St. Marys River, as well as any historical spawning population in the St. Johns, are believed to be extirpated, and the status of the spawning population in the Broad-Coosawatchie is unknown. Both the St. Marys and St. Johns rivers are used as nursery habitat by young Atlantic sturgeon originating from other spawning populations. The use of the Broad-Coosawatchie by sturgeon from other spawning populations is unknown at this time. The presence of historical and current spawning populations in the Ashepoo River has not been documented; however, this river may currently be used for nursery habitat by young Atlantic sturgeon originating from other spawning populations. This represents our current knowledge of the river systems utilized by the South Atlantic DPS for specific life functions, such as spawning, nursery habitat, and foraging. Still, fish from the South Atlantic DPS likely use other river systems than those listed here for their specific life functions.

Prior to the collapse of the fishery in the late 1800s, the sturgeon fishery was the third largest fishery in Georgia. Secor (2002) estimated from U.S. Fish Commission landing reports that approximately 11,000 spawning females were likely present in Georgia and 8,000 adult females were present in South Carolina prior to 1890. The Altamaha River population of the South Atlantic DPS, with an estimated 343 adults spawning annually, is believed to be the largest remaining population in the Southeast, yet is estimated to be only 6% of its historical population size. The abundances of the remaining river populations within the South Atlantic DPS, each

estimated to have fewer than 300 annually spawning adults, are estimated to be less than 1% of what they were historically (ASSRT 2007). The NEAMAP model estimates a minimum ocean population of 14,911 South Atlantic DPS Atlantic sturgeon, of which 3,728 are adults.

Carolina DPS

The Carolina DPS includes all Atlantic sturgeon that are spawned in the watersheds (including all rivers and tributaries) from the Albemarle Sound southward along the southern Virginia, North Carolina, and South Carolina coastal areas to Charleston Harbor. Rivers known to have current spawning populations within the range of the Carolina DPS include the Roanoke, Tar-Pamlico, Cape Fear, Waccamaw, and Yadkin-Pee Dee Rivers. We determined spawning was occurring if YOY were observed, or mature adults were present, in freshwater portions of a system. In some rivers, though, spawning by Atlantic sturgeon may not be contributing to population growth because of lack of suitable habitat and the presence of other stressors on juvenile survival and development. There may also be spawning populations in the Neuse, Santee, and Cooper Rivers, though it is uncertain.

Historically, both the Sampit and Ashley Rivers in South Carolina were documented to have spawning populations at one time, although the spawning population in the Sampit River is believed to be extirpated and the current status of the spawning population in the Ashley River is unknown. Both rivers may be used as nursery habitat by young Atlantic sturgeon originating from other spawning populations. This represents our current knowledge of the river systems utilized by the Carolina DPS for specific life functions, such as spawning, nursery habitat, and foraging. Still, fish from the Carolina DPS likely use other river systems than those listed here for their specific life functions.

Historical landings data indicate that between 7,000 and 10,500 adult female Atlantic sturgeon were present in North Carolina prior to 1890 (Armstrong and Hightower 2002; Secor 2002). Secor (2002) estimates that 8,000 adult females were present in South Carolina during that same time frame. The Atlantic sturgeon spawning population in at least 1 river system (the Sampit River) within the Carolina DPS has been extirpated, and the statuses of 4 additional spawning populations are uncertain. There are believed to be only 5 of 7-10 historical spawning populations remaining in the Carolina DPS. In some rivers, spawning by Atlantic sturgeon may not be contributing to population growth because of lack of suitable habitat and the presence of other stressors on juvenile survival and development. The abundances of the remaining river populations within the DPS, each estimated to have fewer than 300 spawning adults, are estimated to be less than 3% of what they were historically (ASSRT 2007). The NEAMAP model estimates a minimum ocean population of 1,356 Carolina DPS Atlantic sturgeon, of which 339 are adults.

Chesapeake Bay DPS

The Chesapeake Bay DPS includes all anadromous Atlantic sturgeons that are spawned in the watersheds that drain into the Chesapeake Bay and into coastal waters from the Delaware-Maryland border on Fenwick Island to Cape Henry, Virginia. Within this range, Atlantic sturgeon historically spawned in the Susquehanna, Potomac, James, York, Rappahannock, and Nottoway Rivers (ASSRT 2007). Spawning still occurs in the James River, and the presence of juvenile and adult sturgeon in the York River suggests that spawning may occur there as well

(ASSRT 2007; Greene et al. 2009; Musick et al. 1994). However, conclusive evidence of current spawning is available for the James River, only. Atlantic sturgeon that are spawned elsewhere are known to use waters of the Chesapeake Bay for other life functions, such as foraging and as juvenile nursery habitat, before entering the marine system as subadults (ASSRT 2007; Grunwald et al. 2008; Vladykov and Greely 1963; Wirgin et al. 2007).

Historically, the Chesapeake Bay DPS likely supported more than 10,000 spawning adults (ASSRT 2007; KRRMP 1993; Secor 2002). Current estimates of the Chesapeake Bay DPS from the NEAMAP model (Table 6) indicate the current number of spawning adults is likely an order of magnitude lower than historical levels (ASSRT 2007; Kahnle et al. 2007). The NEAMAP model estimates a minimum ocean population of 8,811 Chesapeake Bay DPS Atlantic sturgeon, of which 2,319 are adults.

New York Bight DPS

The New York Bight DPS includes all anadromous Atlantic sturgeon that spawn in the watersheds that drain into coastal waters from Chatham, Massachusetts, to the Delaware-Maryland border on Fenwick Island. Within this range, Atlantic sturgeon historically spawned in the Connecticut, Delaware, Hudson, and Taunton Rivers (ASSRT 2007; Murawski et al. 1977; Secor 2002). Spawning still occurs in the Delaware and Hudson Rivers, but there is no recent evidence (within the last 15 years) of spawning in the Connecticut and Taunton Rivers (ASSRT 2007). Atlantic sturgeon that are spawned elsewhere continue to use habitats within the Connecticut and Taunton Rivers for other life functions (ASSRT 2007; Savoy 2007; Wirgin and King 2011).

Prior to the onset of expanded fisheries exploitation of sturgeon in the 1800s, a conservative historical estimate for the Hudson River Atlantic sturgeon population was 10,000 adult females (Secor 2002). Current population abundance is likely at least one order of magnitude smaller than historical levels (ASSRT 2007; Kahnle et al. 2007; Secor 2002). Based on data collected from 1985-1995, there are 870 spawning adults per year in the Hudson River (Kahnle et al. 2007). Kahnle (2007; 1998) also showed that the level of fishing mortality from the Hudson River Atlantic sturgeon fishery during the period of 1985-1995 exceeded the estimated sustainable level of fishing mortality for the riverine population, and may have led to reduced recruitment. All available data on abundance of juvenile Atlantic sturgeon in the Hudson River Estuary indicate a substantial drop in production of young since the mid-1970s (Kahnle et al. 1998). A decline appeared to occur in the mid- to late 1970s followed by a secondary drop in the late 1980s (ASMFC 2010; Kahnle et al. 1998; Sweka et al. 2007). Catch-per-unit-effort (CPUE) data suggest that recruitment has remained depressed relative to catches of juvenile Atlantic sturgeon in the estuary during the mid- to late 1980s (ASMFC 2010; Sweka et al. 2007). From 1985-2007, there were significant fluctuations in CPUE. The number of juveniles appears to have declined between the late 1980s and early 1990s. While the CPUE is generally higher in the 2000s as compared to the 1990s, significant annual fluctuations make it difficult to discern any trend. The CPUEs from 2000-2007 are generally higher than those from 1990-1999; however, they remain lower than the CPUEs observed in the late 1980s. There is currently not enough information regarding any life stage to establish a trend for the Hudson River population (ASMFC 2010; Sweka et al. 2007).

There is no abundance estimate for the Delaware River population of Atlantic sturgeon. Harvest records from the 1800s indicate that this was historically a large population, with an estimated 180,000 adult females prior to 1890 (Secor 2002; Secor and Waldman 1999). Fisher (2009) sampled the Delaware River in 2009 to target YOY Atlantic sturgeon. The effort captured 34 YOY. Brundage and O'Herron (2003) also collected 32 YOY Atlantic sturgeon from the Delaware River in a separate study. Fisher (2011) reports that genetics information collected from 33 of the 2009 year class YOY indicates that at least 3 females successfully contributed to the 2009 year class. The capture of YOY in 2009 shows that successful spawning is still occurring in the Delaware River, but the relatively low numbers suggest the existing riverine population is limited in size. Similar to the Hudson River, there is currently not enough information to determine a trend for the Delaware River population. The ASSRT (2007) suggested that there may be less than 300 spawning adults per year for the Delaware River portion of the New York Bight DPS. The NEAMAP model estimates a minimum ocean population of 34,566 Atlantic sturgeon, of which 8,642 are adults.

Gulf of Maine DPS

The Gulf of Maine DPS includes all anadromous Atlantic sturgeons that are spawned in the watersheds from the Maine/Canadian border and, extending southward, all watersheds draining into the Gulf of Maine as far south as Chatham, Massachusetts. Within this range, Atlantic sturgeon historically spawned in the Androscoggin, Kennebec, Merrimack, Penobscot, and Sheepscot Rivers (ASSRT 2007). Spawning still occurs in the Kennebec and Androscoggin Rivers, and may still occur in the Penobscot River. Atlantic sturgeon continue to be present in the Kennebec River; in addition, they are captured in directed research projects in the Penobscot River. They are also observed in the Saco, Presumpscot, and Charles rivers where they were unknown to occur before or had not been observed to occur for many years. These observations suggest that the abundance of the Gulf of Maine DPS of Atlantic sturgeon is large enough that recolonization to rivers historically suitable for spawning may be occurring.

Historically, the Gulf of Maine DPS likely supported more than 10,000 spawning adults (ASSRT 2007; KRRMP 1993; Secor 2002), suggesting the recent estimate of spawning adults within the DPS is 1-2 orders of magnitude smaller than historical levels (i.e., hundreds to low thousands) (ASSRT 2007; Kahnle et al. 2007). The CPUE of subadult Atlantic sturgeon in a multifilament gillnet survey conducted on the Kennebec River was considerably greater for the period of 1998-2000 (CPUE = 7.43) compared to the CPUE for the period 1977-1981 (CPUE = 0.30). The CPUE of adult Atlantic sturgeon showed a slight increase over the same time period (1977-1981 CPUE = 0.12 versus 1998-2000 CPUE = 0.21) (Squiers 2004). There is also new evidence of Atlantic sturgeon presence in rivers (e.g., the Saco River) where they have not been observed for many years. Still, there is not enough information to establish a trend for this DPS. The NEAMAP model estimates a minimum ocean population of 7,455 Atlantic sturgeon, of which 1,864 are adults.

Viability of Atlantic Sturgeon DPSs

The concept of a viable population able to adapt to changing environmental conditions is critical to Atlantic sturgeon, and the low population numbers of every river population in the 5 DPSs on the East Coast put them in danger of extinction throughout their range. None of the riverine spawning populations are large or stable enough to provide with any level of certainty for

continued existence of any of the DPSs. Although the largest impact that caused the precipitous decline of the species has been prohibited (directed fishing), the Atlantic sturgeon population sizes within each DPS have remained relatively constant at greatly reduced levels for 100 years. The largest Atlantic sturgeon population in the United States, the Hudson River population within the New York Bight DPS, is estimated to have only 870 spawning adults each year. The Altamaha River population within the South Atlantic DPS is the largest Atlantic sturgeon population in the Southeast and only has an estimated 343 adults spawning annually. All other Atlantic sturgeon river populations in the U.S. are estimated to have less than 300 spawning adults annually.

Small numbers of individuals resulting from drastic reductions in populations, such as occurred with Atlantic sturgeon due to the commercial fishery, can remove the buffer against natural demographic and environmental variability provided by large populations (Berry 1971; Shaffer 1981; Soulé 1980). Recovery of depleted populations is an inherently slow process for a late-maturing species such as Atlantic sturgeon, and they continue to face a variety of other threats that contribute to their risk of extinction. Their late age at maturity provides more opportunities for individual Atlantic sturgeon to be removed from the population before reproducing. While a long life span allows multiple opportunities to contribute to future generations, it also increases the time frame over which exposure to the multitude of threats facing Atlantic sturgeon can occur.

The viability of the Atlantic sturgeon DPSs depends on having multiple self-sustaining riverine spawning populations and maintaining suitable habitat to support the various life functions (spawning, feeding, growth) of Atlantic sturgeon populations. Because a DPS is a group of populations, the stability, viability, and persistence of individual populations affects the persistence and viability of the larger DPS. The loss of any population within a DPS will result in: (1) a long-term gap in the range of the DPS that is unlikely to be recolonized; (2) loss of reproducing individuals; (3) loss of genetic biodiversity; (4) potential loss of unique haplotypes; (5) potential loss of adaptive traits; (6) reduction in total number; and (7) potential for loss of population source of recruits. The loss of a population will negatively impact the persistence and viability of the DPS as a whole, as fewer than 2 individuals per generation spawn outside their natal rivers (King et al. 2001; Waldman et al. 2002; Wirgin et al. 2000). The persistence of individual populations, and in turn the DPS, depends on successful spawning and rearing within the freshwater habitat, the immigration into marine habitats to grow, and then the return of adults to natal rivers to spawn.

Threats

Atlantic sturgeon were once numerous along the East Coast until fisheries for their meat and caviar reduced the populations by over 90% in the late 1800s. Fishing for Atlantic sturgeon became illegal in state waters in 1998 and in remaining U.S. waters in 1999. Dams, dredging, poor water quality, and accidental catch (bycatch) by fishers continue to threaten Atlantic sturgeon. Though Atlantic sturgeon populations appear to be increasing in some rivers, other river populations along the East Coast continue to struggle and some have been eliminated entirely. The 5 DPSs of Atlantic sturgeon were listed as threatened or endangered under the ESA primarily as a result of a combination of habitat restriction and modification, overutilization

(i.e., being taken as bycatch) in commercial fisheries, and the inadequacy of regulatory mechanisms in ameliorating these impacts and threats.

Dams

Dams for hydropower generation, flood control, and navigation adversely affect Atlantic sturgeon by impeding access to spawning, developmental, and foraging habitat, modifying free-flowing rivers to reservoirs, physically damaging fish on upstream and downstream migrations, and altering water quality in the remaining downstream portions of spawning and nursery habitat (ASSRT 2007). Attempts to minimize the impacts of dams using measures such as fish passage have not proven beneficial to Atlantic sturgeon, as they do not regularly use existing fish passage devices, which are generally designed to pass pelagic fish (i.e., those living in the water column) rather than bottom-dwelling species, like sturgeon. Within the range occupied by the Carolina DPS, dams have restricted Atlantic sturgeon spawning and juvenile developmental habitat by blocking over 60% of the historical sturgeon habitat upstream of the dams in the Cape Fear and Santee-Cooper River systems. Water quality (velocity, temperature, and DO downstream of these dams, as well as on the Roanoke River, has been reduced, which modifies and restricts the extent of spawning and nursery habitat for the Carolina DPS.

Within the range of the New York Bight DPS, the Holyoke Dam on the Connecticut River blocks further upstream passage; however, the extent that Atlantic sturgeon historically would have used habitat upstream of Holyoke is unknown. Connectivity may be disrupted by the presence of dams on several smaller rivers in the New York Bight region. Connectivity is disrupted by the presence of dams on several rivers in the range of the Gulf of Maine DPS. Within the Gulf of Maine DPS, access to historical spawning habitat is most severely impacted in the Merrimack River (ASSRT 2007). Construction of the Essex Dam blocked the migration of Atlantic sturgeon to 58% of its historically available habitat (ASSRT 2007). The extent that Atlantic sturgeon are affected by operations of dams in the Gulf of Maine region is currently unknown, although Atlantic sturgeon larvae have been found downstream of the Brunswick Dam in the Androscoggin River. This suggests that Atlantic sturgeon spawning may be occurring in the vicinity of at least 1 hydroelectric project and may be affected by its operations.

Dredging

Riverine, nearshore, and offshore areas are often dredged to support commercial shipping and recreational boating, construction of infrastructure, and marine mining. Environmental impacts of dredging include the direct removal/burial of prey species; turbidity/siltation effects; contaminant resuspension; noise/disturbance; alterations to hydrodynamic regime and physical habitat; and actual loss of riparian habitat (Chytalo 1996; Winger et al. 2000). According to Smith and Clugston (1997), dredging and filling impact important habitat features of Atlantic sturgeon as they disturb benthic fauna, eliminate deep holes, and alter rock substrates.

In the South Atlantic DPS, maintenance dredging is currently modifying Atlantic sturgeon nursery habitat in the Savannah River. Modeling indicates that the proposed deepening of the navigation channel will result in reduced DO and upriver movement of the salt wedge, restricting spawning habitat. Dredging is also modifying nursery and foraging habitat in the St. Johns River. For the Carolina DPS, dredging in spawning and nursery grounds modifies the quality of the habitat and is further restricting the extent of available habitat in the Cape Fear and Cooper

Rivers, where Atlantic sturgeon habitat has already been modified and restricted by the presence of dams. Dredging for navigational purposes is suspected of having reduced available spawning habitat for the Chesapeake Bay DPS in the James River (ASSRT 2007; Bushnoe et al. 2005; Holton and Walsh 1995). Both the Hudson and Delaware rivers have navigation channels that are maintained by dredging. Dredging is also used to maintain channels in the nearshore marine environment. Many rivers in the range of the Gulf of Maine DPS also have navigation channels that are maintained by dredging. Dredging outside of federal channels and in-water construction occurs throughout the range of the New York Bight and Gulf of Maine DPSs.

Water Quality

Atlantic sturgeon rely on a variety of water quality parameters to successfully carry out their life functions. Low DO and the presence of contaminants modify the quality of Atlantic sturgeon habitat and in some cases, restrict the extent of suitable habitat for life functions. Secor (1995) noted a correlation between low abundances of sturgeon during this century and decreasing water quality caused by increased nutrient loading and increased spatial and temporal frequency of hypoxic (low oxygen) conditions. Of particular concern is the high occurrence of low DO coupled with high temperatures in the river systems throughout the range of the Carolina and South Atlantic DPSs in the Southeast. Sturgeon are more highly sensitive to low DO than other fish species (Niklitschek and Secor 2009a; Niklitschek and Secor 2009c) and low DO in combination with high temperature is particularly problematic for Atlantic sturgeon. Studies have shown that juvenile Atlantic sturgeon experience lethal and sublethal (metabolic, growth, feeding) effects as DO drops and temperatures rise (Niklitschek and Secor 2005; Niklitschek and Secor 2009a; Niklitschek and Secor 2009c; Secor and Gunderson 1998).

Reductions in water quality from terrestrial activities have modified habitat utilized by the South Atlantic DPS. Low DO is modifying sturgeon habitat in the Savannah due to dredging, and non-point source inputs are causing low DO in the Ogeechee River and in the St. Marys River, which completely eliminates juvenile nursery habitat in summer. Low DO has also been observed in the St. Johns River in the summer. In the Pamlico and Neuse systems occupied by the Carolina DPS, nutrient-loading and seasonal anoxia are occurring, associated in part with concentrated animal feeding operations (CAFOs). Heavy industrial development and CAFOs have degraded water quality in the Cape Fear River. Water quality in the Waccamaw and Yadkin-Pee Dee Rivers has been affected by industrialization and riverine sediment samples contain high levels of various toxins, including dioxins. Decreased water quality also threatens Atlantic sturgeon of the Chesapeake Bay DPS, especially since the Chesapeake Bay system is vulnerable to the effects of nutrient enrichment due to a relatively low tidal exchange and flushing rate, large surface-to-volume ratio, and strong stratification during the spring and summer months (ASMFC 1998; ASSRT 2007; Pyzik et al. 2004). These conditions contribute to reductions in DO levels throughout the bay. The availability of nursery habitat, in particular, may be limited given the recurrent hypoxia (low DO) conditions within the Bay (Niklitschek and Secor 2005; Niklitschek and Secor 2010). Both the Hudson and Delaware Rivers, as well as other rivers in the New York Bight region, were heavily polluted in the past from industrial and sewer discharges. In the past, many rivers in Maine, including the Androscoggin River, were heavily polluted from industrial discharges from pulp and paper mills. While water quality has improved and most discharges are limited through regulations, many pollutants persist in the benthic environment of the New York Bight and Gulf of Maine DPSs. It is particularly problematic if pollutants are present on

spawning and nursery grounds, as developing eggs and larvae are particularly susceptible to exposure to contaminants.

Water Quantity

Water allocation issues are a growing threat in the Southeast and exacerbate existing water quality problems. Taking water from one basin and transferring it to another fundamentally and irreversibly alters natural water flows in both the originating and receiving basins, which can affect DO levels, temperature, and the ability of the basin of origin to assimilate pollutants (GWC 2006). Water quality within the river systems in the range of the South Atlantic and Carolina DPSs is negatively affected by large water withdrawals. Known water withdrawals of over 240 million gallons per day are permitted from the Savannah River for power generation and municipal uses. However, permits for users withdrawing less than 100,000 gallons per day are not required, so actual water withdrawals from the Savannah and other rivers within the range of the South Atlantic DPS are likely much higher. In the range of the Carolina DPS, 20 interbasin water transfers in existence prior to 1993, averaging 66.5 million gallons per day (mgd), were authorized at their maximum levels without being subjected to an evaluation for certification by the North Carolina Department of Environment and Natural Resources or other resource agencies. Since the 1993 legislation requiring certificates for transfers, almost 170 mgd of interbasin water withdrawals have been authorized, with an additional 60 mgd, pending certification. The removal of large amounts of water from these systems will alter flows, temperature, and DO. Water shortages and "water wars" are already occurring in the rivers occupied by the South Atlantic and Carolina DPSs and will likely be compounded in the future by population growth and potentially by climate change.

Climate Change

The Intergovernmental Panel on Climate Change (IPCC) projects with high confidence that higher water temperatures and changes in extremes, including floods and droughts, will affect water quality and exacerbate many forms of water pollution—from sediments, nutrients, dissolved organic carbon, pathogens, pesticides, and salt, as well as thermal pollution—with possible negative impacts on ecosystems (IPCC 2008). In addition, sea level rise is projected to extend areas of salinization of groundwater and estuaries, resulting in a decrease of freshwater availability for humans and ecosystems in coastal areas. Some of the most heavily populated areas are low-lying, and the threat of salt water entering into its aquifers with projected sea level rise is a concern (USGRG 2004). Existing water allocation issues would be exacerbated, leading to an increase in reliance on interbasin water transfers to meet municipal water needs, further stressing water quality.

Dams, dredging, and poor water quality have already modified and restricted the extent of suitable habitat for Atlantic sturgeon spawning and nursery habitat. Changes in water availability (depth and velocities) and water quality (temperature, salinity, DO, contaminants, etc.) in rivers and coastal waters inhabited by Atlantic sturgeon resulting from climate change will further modify and restrict the extent of suitable habitat for Atlantic sturgeon. Effects could be especially harmful since these populations have already been reduced to low numbers, potentially limiting their capacity for adaptation to changing environmental conditions (Belovsky 1987; Salwasser et al. 1984; Soulé 1987; Thomas 1990).

The effects of changes in water quality (temperature, salinity, DO, contaminants, etc.) in rivers and coastal waters inhabited by Atlantic sturgeon are expected to be more severe for those populations that occur at the southern extreme of the Atlantic sturgeon's range, and in areas that are already subject to poor water quality as a result of eutrophication. The South Atlantic and Carolina DPSs are within a region the IPCC predicts will experience overall climatic drying (IPCC 2008). Atlantic sturgeon from these DPSs are already susceptible to reduced water quality resulting from various factors: inputs of nutrients; contaminants from industrial activities and non-point sources; and interbasin transfers of water. In a simulation of the effects of water temperature on available Atlantic sturgeon habitat in Chesapeake Bay, Niklitschek and Secor (2005) found that a 1°C increase of water temperature in the bay would reduce available sturgeon habitat by 65%.

Vessel Strikes

Vessel strikes are a threat to the Chesapeake Bay and New York Bight DPSs. Eleven Atlantic sturgeon were reported to have been struck by vessels on the James River from 2005 through 2007. Several of these were mature individuals. From 2004-2008, 29 mortalities believed to be the result of vessel strikes were documented in the Delaware River; at least 13 of these fish were large adults. The time of year when these events occurred (predominantly May through July, with 2 in August), indicate the animals were likely adults migrating through the river to the spawning grounds. Because we do not know the percent of total vessel strikes that these observed mortalities represent, we are not able to quantify the number of individuals likely killed as a result of vessel strikes in the Chesapeake and New York Bight DPSs.

Bycatch Mortality

Overutilization of Atlantic sturgeon from directed fishing caused initial severe declines in Atlantic sturgeon populations, from which they have never rebounded. Further, continued overutilization of Atlantic sturgeon as bycatch in commercial fisheries is an ongoing impact to Atlantic sturgeon in all 5 DPSs. Atlantic sturgeon are more sensitive to bycatch mortality because they are a long-lived species, have an older age at maturity, have lower maximum reproductive rates, and a large percentage of egg production occurs later in life. Based on these life history traits, Boreman (1997) calculated that Atlantic sturgeon can only withstand the annual loss of up to 5% of their population to bycatch mortality without suffering population declines. Mortality rates of Atlantic sturgeon taken as bycatch in various types of fishing gear range between 0% and 51%, with the greatest mortality occurring in sturgeon caught by sink gillnets. Currently, there are estimates of the number of Atlantic sturgeon captured and killed in sink gillnet and otter trawl fisheries authorized by Fishery Management Plans (FMPs) in the Northeast Region (Miller and Shepherd 2011). Those estimates indicate from 2006-2010, on average there were 1,548 and 1,569 encounters per year in observed gillnet and trawl fisheries, respectively, with an average of 3,118 encounters combined annually. Mortality rates in gillnet gear were approximately 20%, while mortality rates in otter trawl gear are generally lower, at approximately 5%. Atlantic sturgeon are particularly vulnerable to being caught in sink gillnets; therefore, fisheries using this type of gear account for a high percentage of Atlantic sturgeon bycatch. Atlantic sturgeon are incidentally captured in state and federal fisheries, reducing survivorship of subadult and adult Atlantic sturgeon (ASMFC 2007; Stein et al. 2004). Little data exists on bycatch in the Southeast and high levels of bycatch underreporting are suspected. However, fisheries known to incidentally catch Atlantic sturgeon occur throughout the marine

range of the species and in some riverine waters as well. Because Atlantic sturgeon mix extensively in marine waters and may access multiple river systems, they are subject to being caught in multiple fisheries throughout their range. In addition, stress or injury to Atlantic sturgeon taken as bycatch but released alive may result in increased susceptibility to other threats, such as poor water quality (e.g., exposure to toxins and low DO). This may result in reduced ability to perform major life functions, such as foraging and spawning, or even post-capture mortality.

4 ENVIRONMENTAL BASELINE

This section describes the effects of past and ongoing human and natural factors contributing to the current status of the species, its habitat (including designated critical habitat), and ecosystem within the action area, without the additional effects of the proposed action. In the case of ongoing actions, this section includes the effects that may contribute to the projected future status of the species, its habitat, and ecosystem. The environmental baseline describes a species' and habitat's health based on information available at the time of this consultation.

By regulation (50 CFR 402.02), environmental baselines for Opinions include the past and present impacts of all state, federal, or private actions and other human activities in, or having effects in, the action area. We identify the anticipated impacts of all proposed federal projects in the specific action area of the consultation at issue that have already undergone formal or early Section 7 consultation (as defined in 50 CFR 402.11), as well as the impact of state or private actions, or the impacts of natural phenomena, which are concurrent with the consultation in process (50 CFR 402.02).

Focusing on the impacts of the activities in the action area specifically allows us to assess the prior experience and state (or condition) of the endangered and threatened individuals. This consideration is important because in some states or life history stages, or areas of their ranges, or listed individuals will commonly exhibit, or be more susceptible to, adverse responses to stressors than they will be in other states, stages, or areas within their distributions. These localized stress responses or stressed baseline conditions may increase the severity of the adverse effects expected from the proposed action.

4.1 Sea Turtles

4.1.1 Status and Distribution of Sea Turtles within the Action Area

Sea turtle species occurring in the project area that may be adversely affected by the proposed action are loggerhead, green, and Kemp's ridley. Sea turtles found in the immediate project area may travel widely throughout the Atlantic, Gulf of Mexico, and Caribbean Sea, and individuals found in the action area can potentially be affected by activities anywhere within this wide range. These impacts outside of the action area are discussed and incorporated as part of the overall status of the species as detailed in Section 3 above. The following environmental baseline includes past and ongoing human activities in the action area (Figure 7) that relate to the status of the species. All of these species are highly migratory. The same individuals found in the action area may migrate into offshore waters and thus be impacted by activities occurring there; therefore, the species' statuses in the action area are considered to be the same as their range-wide statuses and supported by the species accounts in Section 3 of this Opinion.

There are approximately 300 kilometers of ocean-facing sandy beaches in South Carolina that provide suitable nesting habitat for sea turtles, including the barrier island beaches to the north and south of the Port of Charleston entrance channel.

4.1.2 Factors Affecting Sea Turtles in the Action Area

4.1.2.1 Federal Actions

In recent years, NMFS has undertaken 5 ESA Section 7 consultations to address the effects of federal actions on sea turtles, and when appropriate, has authorized the incidental taking of these species. Each of those consultations sought to develop ways of reducing the probability of adverse effects of the action on sea turtles. Similarly, NMFS has undertaken recovery actions under the ESA and is addressing the problem of take of sea turtles and sturgeon in the fishing industry and other activities such as USACE dredging operations. The summary below of sources of incidental take of sea turtles and sturgeon includes only those federal actions in the South Atlantic which have already concluded or are currently undergoing formal Section 7 consultation.

4.1.2.1.1 Dredging

The construction and maintenance of federal navigation channels and sand mining ("borrow") areas using hopper dredges has been identified as a source of sea turtle mortality. Hopper dredges move relatively rapidly (compared to sea turtle swimming speeds) and can entrain and kill sea turtles as the drag arm of the moving dredge overtakes the slower-moving or stationary sea turtle. The USACE has Opinions from NMFS covering the use of hopper dredges for maintenance dredging and beach renourishment activities in the Atlantic and Gulf of Mexico. Along the Atlantic coast of the southeastern United States (North Carolina through Florida), the USACE's current Opinion authorizes annual take of up to 35 loggerheads, 7 greens, and 7 Kemp's ridleys sea turtles from hopper dredging activities (NMFS 1997b). Consultation has been reinitiated on this Opinion due to the listing of new species and designation of critical habitat.

NMFS has previously determined in dredging Opinions that while oceangoing hopper-type dredges may lethally entrain protected species, non-hopper type dredging methods (e.g., clamshell or bucket dredging, cutterhead dredging, pipeline dredging, sidecast dredging) are slower and unlikely to overtake or adversely affect them. Incidents of take by mechanical and other non-hopper dredges appears to be exceedingly rare with none observed occurring in the Charleston area.

4.1.2.1.2 ESA Permits

The ESA allows the issuance of permits to take ESA-listed species for the purposes of scientific research (Section 10(a)(1)(A)). In addition, the ESA allows for NMFS to enter into cooperative agreements with states developed under Section 6 of the ESA to assist in recovery actions of listed species. Prior to issuance of these authorizations, the proposal must be reviewed for compliance with Section 7 of the ESA.

Sea turtles are the focus of research activities authorized by a Section 10 permit under the ESA. Authorized activities range from photographing, weighing, and tagging sea turtles incidentally

taken in fisheries, blood sampling, tissue sampling (biopsy), and performing laparoscopy on intentionally captured turtles. The number of authorized takes varies widely depending on the research and species involved but may involve the taking of hundreds of turtles annually. Most takes authorized under these permits are expected to be nonlethal. Before any research permit is issued, the proposal must be reviewed under the permit regulations (i.e., must show a benefit to the species). In addition, since issuance of the permit is a federal activity, issuance of the permit by NMFS must also be reviewed for compliance with Section 7(a)(2) of the ESA to ensure that issuance of the permit does not result in jeopardy to the species. There are currently 7 Section 10(a)(1)(A) scientific research permits issued to study sea turtles in the action area.

4.1.2.2 State or Private Actions

Commercial vessel traffic and recreational pursuits can adversely affect sea turtles through propeller and boat strikes. However, the threat is not constant and is influenced by vessel type, vessel speed, and environmental conditions such as sea state and visibility. Given these variables, it is difficult to definitively evaluate potential risk to sea turtles stemming from specific vessel traffic. This difficulty is compounded by a general lack of information on vessel use trends, particularly in regard to offshore vessel traffic.

The STSSN includes many records of vessel interaction (crush and/or propeller injury) with sea turtles. The STSSN has documented 3,054 South Carolina stranding records (all species and size classes) in their database from 1980 through 2013. The stranding records include all causes of mortality, such as disease, hopper dredge impacts, hypothermic stunning (i.e., cold-stunning), interactions with fisheries, interactions with pollution, and vessel strikes. However, due to the condition of stranded turtles in many cases (i.e., decomposition), it was impossible to definitively determine actual cause of mortality for 70% of the specimens. In addition, it was not possible to determine in many cases whether the vessel strike occurred before or after the turtle's death. Additionally, it should be noted that many turtles killed by anthropogenic causes will not show up in the strandings database, as the mortality event may occur far offshore or the damage to the turtle is so significant the carcass sinks, preventing the turtle from washing ashore. This point is important to remember when considering apparent geographical trends in the data, which may be an artifact of other factors rather than increased mortality risk in one area versus another. For example, turtles injured/killed in one area may potentially be more well-represented in the strandings data due to bathymetric constraints that concentrate both turtles and vessel traffic relatively close to shore when compared to other counties with a broader continental shelf, where turtles may not wash up and be documented in the database. Additionally, stranding information does not indicate where a potential mortality event (e.g., vessel strike) occurred, as a turtle could have been injured/killed at one location and then drifted with currents (i.e., generally northward with the Gulf Stream on the East Coast) for a considerable distance before coming ashore.

Given the variables described above, though there are numerous strandings of turtles indicating vessel strike impacts each year, the exact extent of the vessel traffic impact on sea turtles is not quantifiable at this time.

4.1.2.2.1 Fisheries

Recreational fishing from private vessels and from shore occurs in the area. Observations of recreational fisheries have shown that loggerhead and green sea turtles are known to take baited

hooks, and loggerheads frequently ingest the hooks. Hooked turtles have been reported by the public fishing from boats, piers, and beach, banks, and jetties and from commercial fishers fishing for reef fish and for sharks with both single rigs and bottom longlines (NMFS 2001a). Additionally, lost fishing gear such as line cut after snagging on rocks, or discarded hooks and line, can also pose an entanglement threat to sea turtles in the area. A detailed summary of the known impacts of hook-and-line incidental captures to loggerhead sea turtles can be found in the TEWG reports (TEWG 1998; TEWG 2000b). In August of 2007, NMFS issued a regulation to require any fishing vessels subject to the jurisdiction of the United States to take observers upon NMFS's request (72 FR 43176, August 3, 2007). The purpose of the regulation is to learn more about sea turtle interactions with fishing operations, to evaluate existing measures to reduce sea turtle takes, and to determine whether additional measures to address prohibited sea turtle takes may be necessary.

4.1.2.3 Marine Pollution

Sources of pollutants along the Atlantic coastal regions include atmospheric loading of pollutants such as polychlorinated biphenyls (PCBs), stormwater runoff from coastal towns and cities into rivers and canals emptying into bays and the ocean, and groundwater and other discharges. Nutrient loading from land-based sources such as coastal community discharges is known to stimulate plankton blooms in closed or semi-closed estuarine systems. The effects on larger embayments are unknown. Although pathological effects of oil spills have been documented in laboratory studies of marine mammals and sea turtles (Vargo et al. 1986), the impacts of many other anthropogenic toxins have not been investigated.

4.1.2.4 Conservation and Recovery Actions Shaping the Environmental Baseline

NMFS has promulgated a series of regulations aimed at reducing potential for incidental mortality of sea turtles from commercial fisheries in the action area. These include sea turtle release gear requirements for Atlantic Highly Migratory Species (HMS) fisheries and TED requirements for Southeast shrimp trawl. In addition to regulations, outreach programs have been established and data on sea turtle interactions with recreational fisheries is collected through the Marine Recreational Fishing Statistical Survey (MRFSS). The summaries below discuss all of these measures in more detail.

4.1.2.4.1 Regulations Reducing Threats to Sea Turtles from Fisheries

Bycatch Reduction Measures in the Pelagic Longline Fishery

On July 6, 2004, NMFS published a Final Rule to implement management measures to reduce bycatch and bycatch mortality of Atlantic sea turtles in the Atlantic pelagic longline fishery (69 FR 40734). The management measures include mandatory circle hook and bait requirements, and mandatory possession and use of sea turtle release equipment to reduce bycatch mortality. The rulemaking, based on the results of the 3-year Northeast Distant Closed Area research experiment and other available sea turtle bycatch reduction studies, is expected to have significant benefits to endangered and threatened sea turtles.

Revised Use of Turtle Excluder Devices in Trawl Fisheries

NMFS has also implemented a series of regulations aimed at reducing potential for incidental mortality of sea turtles in commercial shrimp trawl fisheries. In particular, NMFS has required the use of TEDs in southeast United States shrimp trawls since 1989 and in summer flounder

trawls in the mid-Atlantic area (south of Cape Charles, Virginia) since 1992. It has been estimated that TEDs exclude 97% of the sea turtles caught in such trawls. These regulations have been refined over the years to ensure that TED effectiveness is maximized through proper placement and installation, configuration (e.g., width of bar spacing), floatation, and more widespread use.

4.1.2.5 Other Sea Turtle Conservation Efforts

Sea Turtle Handling and Resuscitation Techniques

NMFS published a Final Rule (66 FR 67495, December 31, 2001) detailing handling and resuscitation techniques for sea turtles that are incidentally caught during scientific research or fishing activities. Persons participating in fishing activities or scientific research are required to handle and resuscitate (as necessary) sea turtles as prescribed in the Final Rule. These measures help to prevent mortality of hard-shelled turtles caught in fishing or scientific research gear. In addition, NMFS published NOAA Technical Memorandum SEFSC-524, "Careful Release Protocols for Sea Turtle Release with Minimal Injury," in June 2004.

Outreach and Education, Sea Turtle Entanglements, and Rehabilitation

There is an extensive network of STSSN participants along the Atlantic and Gulf of Mexico coasts who not only collect data on dead sea turtles, but also rescue and rehabilitate any live stranded sea turtles.

Stranding and Salvage Activities by NMFS, USFWS, and the USCG

A Final Rule (70 FR 42508) published on July 25, 2005, allows any agent or employee of NMFS, the USFWS, the U.S. Coast Guard, or any other federal land or water management agency, or any agent or employee of a state agency responsible for fish and wildlife, when acting in the course of his or her official duties, to take endangered sea turtles encountered in the marine environment if such taking is necessary to aid a sick, injured, or entangled endangered sea turtle, or dispose of a dead endangered sea turtle, or salvage a dead endangered sea turtle that may be useful for scientific or educational purposes. NMFS already affords the same protection to sea turtles listed as threatened under the ESA [50 CFR 223.206(b)].

Other Actions

The recovery plans for Kemp's ridley and loggerhead sea turtles are in the process of being updated. Recovery teams comprised of sea turtle experts have been convened and are currently working towards revising these plans based upon the latest and best available information. Five-year status reviews have recently been completed for green, Kemp's ridley, and loggerhead sea turtles. These reviews were conducted to comply with the ESA mandate for periodic status evaluation of listed species to ensure that their threatened or endangered listing status remains accurate. Each review determined that no delisting or reclassification of a species status (i.e., threatened or endangered) was warranted at this time. However, further review of species data for the green, and loggerhead sea turtles was recommended, to evaluate whether DPSs should be established for these species (NMFS and USFWS 2007a-e).

4.2 Atlantic Sturgeon

4.2.1 Status and Distribution of Atlantic Sturgeon within the Action Area

Atlantic sturgeon were likely present in many South Carolina rivers/estuary systems historically, but it is not known where spawning occurred. Secor (2002) estimated that 8,000 spawning females were likely present prior to 1890; since then, populations have declined dramatically (Collins and Smith 1997). During the last 2 decades, Atlantic sturgeon have been observed in most South Carolina coastal rivers, although it is not known if all rivers support a spawning population (Collins and Smith 1997). Atlantic sturgeon are expected to occur throughout the action area both in marine waters and in the rivers associated with the CHP45.

Four Atlantic sturgeon (49-89 cm TL) were captured in 1987 in the Cooper River (Collins and Smith 1997). More recently during winter 2003, 3 juveniles were captured from the Cooper River. Because these sturgeon were not reproducing adults, it is not known if these small fish were residents or migrants as flood waters from the Pee Dee or Waccamaw River could have transported fish to the Santee-Cooper system via Winyah Bay and the Intercoastal Waterway (McCord 2004). Recent research carried out by Post et al. (2014) observed 7 Atlantic sturgeon in the Cooper River during the 3-year project and no Atlantic sturgeon in the Wando River. These fish were more commonly detected in the saltwater tidal zone with the exception of 1 Atlantic sturgeon that made a presumed spawning run to Pinopolis Dam in the fall of 2013. No Atlantic sturgeon spawning was observed. Historically, dredging and relocation trawling activities have both had interactions with Atlantic sturgeon, with 3 adults being observed taken during 22 years of dredging projects and 8 being relocated during relocation trawling efforts during that same time period.

Subadult Atlantic sturgeon form winter aggregations in the shipping channel outside Charleston Harbor (ASSRT 2007). Ongoing work by Arendt et al. (2015) is detecting tagged Atlantic sturgeon in the Charleston shipping channel and surrounding water. Atlantic sturgeon detections were accrued across 70 different animals (plus 1 with a sensor tag), 7 that were detected both in the shipping channel and other coastal waters, 26 that were only detected in the shipping channel, and 38 that were only detected in other coastal waters.

Historically, Atlantic sturgeon were abundant enough in South Carolina to support a commercial fishery with an average catch of 78,864 kg between 1880-1901 (Secor). Current abundance estimates for Atlantic sturgeon are not available for the Cooper River, or the Action Area. While specific abundance estimates are not available, the shad gillnet fishery in the Cooper River reported a 10-year average (2000 to 2009) of ~85 Atlantic sturgeon caught annually. There is no evidence of Atlantic sturgeon spawning in the Cooper River in recent history.

4.2.2 Factors Affecting Atlantic Sturgeon in the Action Area

As stated in Section 2.2 (“Action Area”), the proposed project is located in the Charleston area and includes all areas to be widened and/or dredged from the North Charleston Terminal down the Cooper River and from the Wando Welch Terminal down the Wando River to the confluence with the Cooper River, out the entrance channel to a point approximately 17.3 miles offshore to the sea buoy and from the entrance channel to the hard bottom habitat creation (reef) areas, the SCDNR reef, and the ODMDS location offshore (shown in Figure 1). Additionally, the action

area will include any areas upstream of the project that experience changes in salinity, or approximately 3 miles south of "The Tee" (Figure 7).

4.2.2.1 Federal Actions

In recent years, NMFS has undertaken 9 ESA Section 7 consultations in the waters in or near the Port of Charleston to address the effects of federal actions on listed sturgeon. Of the 9, only 1 of these consultations considered Atlantic sturgeon due to their recent (June 2014) ESA listing.

NMFS is in the process of designating critical habitat for Atlantic sturgeon. The proposed designation is scheduled to publish in November of 2015, with the Final Rule to publish 1 year later.

4.2.2.1.1 Dredging

Maintenance dredging of Federal navigation channels can adversely affect Atlantic sturgeon populations due to their benthic nature. The Cooper River flows into Charleston Harbor, which is one of the busiest ports on the Atlantic Coast and is dredged regularly up to the Naval Weapons Station at about RM 22. No seasonal restrictions are placed on dredging in the Cooper River; however, a restriction is placed on hopper dredging conducted offshore of Charleston Harbor in the shipping channel during the summer months. Hopper dredging is only carried out in the entrance channel in the area (USACE 2014).

Hopper Dredging

Hopper dredges can lethally harm sturgeon directly by entraining sturgeon in dredge drag arms and impeller pumps. Historically, hopper dredging in the entrance channel to the Port of Charleston has taken 1 Atlantic sturgeon (1991 through 2013 data from the Sea Turtle Data Warehouse: http://el.erdc.usace.army.mil/seaturtles/).

Non-Hopper-type Dredging

NMFS has previously determined in dredging Opinions that, while oceangoing hopper-type dredges may lethally entrain protected species, non-hopper type dredging methods (e.g., clamshell or bucket dredging, cutterhead dredging, pipeline dredging,) are slower and unlikely to overtake or adversely affect them. The project may affect Atlantic sturgeon by injury or death as a result of interactions with equipment or materials used during dredging; however, NMFS believes the chance of injury or death from interactions with clamshell and/or hydraulic dredging equipment is discountable as these species are highly mobile and are likely to avoid the areas during construction. Additionally, taking of sturgeon by mechanical and hydraulic cutterhead dredges appears to be exceedingly rare with none observed occurring in the Charleston area (1991 through 2013 data from the Sea Turtle Data Warehouse: http://el.erdc.usace.army.mil/seaturtles/).

Dredging Related Habitat impacts

Riverine, nearshore, and offshore areas are often dredged to support commercial shipping and recreational boating, construction of infrastructure, and marine mining. Dredging activities can pose significant impacts to aquatic ecosystems by removing, disturbing, disposing, and re-suspending bottom sediments, modifying substrate type, and impacting the community structure of benthic macrofauna. Environmental impacts of dredging include the following: (1) direct

removal/burial of organisms; (2) turbidity/siltation effects; (3) contaminant resuspension; (4) noise/disturbance; (5) alterations to hydrodynamic regime and physical habitat; and (6) loss of riparian habitat (Chytalo 1996; Winger et al. 2000). In addition to direct effects, dredging operations may also impact sturgeon by destroying benthic feeding areas, disrupting spawning migrations, altering local hydrology, and resuspending fine sediments in spawning habitat that covers required substrate. Because sturgeon are benthic omnivores, the modification of the benthos could affect the quality, quantity and availability of sturgeon prey species.

4.2.2.1.2 ESA Permits

The ESA allows the issuance of permits to take ESA-listed species for the purposes of scientific research (Section 10(a)(1)(A)). In addition, the ESA allows for NMFS to enter into cooperative agreements with states developed under Section 6 of the ESA, to assist in recovery actions of listed species. Prior to issuance of these authorizations, the proposal must be reviewed for compliance with Section 7 of the ESA.

Authorization of research and enhancement activities on Atlantic sturgeon is established through the issuance of an ESA Section 10(a)(1)(A) permit. There are currently 3 Section 10(a)(1)(A) scientific research permits issued to study sturgeon in the action area. The specific stressors to fish subject to NMFS-issued ESA permit conditions are capture in nets; handling and restraint during examinations; tagging using PIT, internal, and external tags; tissue sampling; anesthetizing; laparoscopy; blood sampling; and gonad biopsy.

4.2.2.2 State or Private Actions

A number of activities that may directly or indirectly affect Atlantic sturgeon include impacts from fisheries, wastewater systems, stormwater systems, and residential or commercial developments adjacent to waterways. The direct and indirect impacts from some of these activities are difficult to quantify. Where possible, conservation actions through the ESA Section 7 processes, ESA Section 10 permitting, ESA Section 6 cooperative agreements, and state permitting programs are being implemented to monitor or study impacts from these sources.

4.2.2.2.1 Fisheries

The ESA listing prohibits the direct harvest of Atlantic sturgeon. Still, sturgeon are taken incidentally in state fisheries that deploy nets. They are also likely targeted by poachers throughout their range (Collins et al. 1996; Dadswell 1979; Dovel et al. 1992). Impacts from poaching are unknown.

The incidental capture of sturgeons in the South Carolina and Georgia gillnet fishery for American shad (*Alosa sapidissima*) and the trawl fishery for penaeid shrimp (*Penaeus* spp.) was summarized by Collins et al. (Collins et al. 1996). Collins et al. (1996) reported the commercial shad fishery was active from approximately mid-January through mid-April along the south Atlantic coast; sturgeons captured in the shad gillnet fishery were primarily adults. Entanglement of sturgeon in gillnets can result in injury and mortality, reduced fecundity, and delayed or aborted spawning migrations (Collins et al. 2000a; Moser 2000; Moser and Ross 1993; Moser and Ross 1995; Weber 1996).

Mandatory reporting of sturgeon bycatch was initiated in 2000 by the Atlantic States Marine Fisheries Commission. According to their data, between 2000 and 2009 the average annual bycatch of Atlantic sturgeon reported by the commercial shad fishery was 84.7 Atlantic sturgeon. Poaching is likely another fishing threat and may be more prevalent where legal markets for sturgeon exist from imports, commercial harvest, or commercial culture; impacts from poaching to individual population segments are unknown.

4.2.2.3 Other Potential Sources of Impacts in the Environmental Baseline

Dams

Dams and their operations are the cause of major instream flow alteration in the Southeast (USFWS et al. 2001). Hill (1996) identified the following impacts of altered flow to anadromous fishes by dams: (1) altered DO concentrations and temperature; (2) artificial destratification; (3) water withdrawal; (4) changed sediment load and channel morphology; (5) accelerated eutrophication and change in nutrient cycling; and (6) contamination of water and sediment. Activities associated with dam maintenance, such as dredging and minor excavations along the shore, can release silt and other fine river sediments which can be deposited in nearby spawning habitat. Dams may reduce the viability of sturgeon populations by removing free-flowing river habitat. Seasonal deterioration of water quality can be severe enough to kill fish in deep storage reservoirs that receive high nutrient loadings from the surrounding watershed (Cochnauer 1986). Important secondary effects of altered flow and temperature regimes include decreases in water quality, particularly in the reservoir part of river segments, and changes in physical habitat suitability, particularly in the free-flowing part of river segments or areas downstream. The most commonly reported factor influencing year-class strength of sturgeon species is flow during the spawning and incubation period (Jager et al. 2002).

The Santee River Basin is geographically segmented by about 50 dams on the mainstem rivers (USFWS et al. 2001). These dams dictate distribution of diadromous fishes throughout the basin as they impede or impair upstream and/or downstream movement. The lowermost Pinopolis Dam blocks access to the basin and historical sturgeon spawning areas and blocks dam-locked sturgeon from accessing foraging areas at the freshwater/saltwater interface.

Between 1943 and 1985, most of the natural flow of the Santee River was diverted into Lake Moultrie and discharged into the Cooper River. This diversion resulted in severe silting in the Cooper River and Charleston Harbor during that period. To alleviate this problem, in 1985 the USACE constructed another canal to redivert water from Lake Moultrie back into the Santee River. The normal operation of Lake Moultrie releases a daily average of 4,500 cubic feet per second (cfs) into the Cooper River—enough to keep the salinity of the river low—and returns the remainder of its discharge—on average about 10,000 cfs—to the Santee River.

Prior to diversion, saline conditions extended ~18 miles up the Cooper River from the mouth of Charleston Harbor and a distinct salt wedge extended upstream ~ 9 miles (Mathews and Shealy 1978; Mathews and Shealy 1982). Following rediversion, saline waters extended approximately 31 miles up the Cooper River, with salinities primarily controlled by tidal stage rather than seasonal freshwater flow. Since rediversion, the lower fresh water discharge rate has eliminated much of the seasonal variability previously reported (Davis et al. 1990). Because of these

reductions in flow and increases in salinity, the quality of habitat below the Pinopolis is less than ideal and does not support viable sturgeon spawning.

In a separate ESA Section 7 consultation[23], passage will be implemented for sturgeon at the Pinopolis Dam. Once that occurs, sturgeon will be able to move into more appropriate waters for spawning and will have access to the adjacent Santee River for foraging and rearing. The Santee River is likely to support foraging activity given its estuarine habitat. While the transition zone moving approximately one-half mile upstream is a measureable change, NMFS believes it will be insignificant for sturgeon living in the waters of the Cooper River and Charleston Harbor due of the availability of habitat upstream and habitat available in the adjacent Santee River once passage is implemented at Pinopolis.

4.2.2.3.1 Water Quality (riverine)

Water quality is influenced by water entering from Charleston Harbor on the rising tide which pushes salt water upriver as far as the Pinopolis Dam. The ongoing presence of the dams' water withdrawals exacerbate these issues. For example, the BP Amoco Cooper River chemicals plant near Charleston has the greatest surface-water use, withdrawing 2,619 million gallons from the Cooper River annually. The Harbor/Cooper River/Wando River portion of the system (consisting of the Tail Race Canal, West Branch Cooper River, East Branch Cooper River, Shipyard Creek, Town Creek, Back River, Goose Creek, Wando River and Charleston Harbor) is not considered to be impaired with respect to dissolved oxygen (with the exception of a Wando River monitoring site); however, available information indicates much of the system does not meet the applicable water quality standard for dissolved oxygen for significant periods of time and is considered water quality limited(SCDHEC 2013).

4.2.2.3.2 Conservation and Recovery Actions Shaping the Environmental Baseline

In 2007, NMFS published a status review report for Atlantic sturgeon. Atlantic Sturgeon was proposed for listing in October 2010 (75 FR 61904 and 61872), and placed on the Endangered Species List (77 FR 5880 and 5419) in February, 2012. The listing was effective April 6, 2012. NMFS has not yet drafted a Recovery Plan nor designated critical habitat for Atlantic Sturgeon. NMFS issued an ESA Section 10(a)(1)(A) permit (#17273) authorizing named federal and state agency personnel to collect, necropsy, sample and salvage dead shortnose and Atlantic sturgeon found beached, sunken, or floating, or those that are euthanized at U.S. facilities authorized to hold captive bred sturgeon. Opportunistic research on salvaged sturgeon may be useful for scientific or educational purposes.

5 EFFECTS OF THE ACTION ON THE SPECIES AND/OR DESIGNATED CRITICAL HABITAT

5.1 Effects of the Action on Sea Turtles and Atlantic Sturgeon

5.1.1 Dredging

The potential for adverse effects of dredging operations on sea turtles and Atlantic sturgeon has been previously assessed by NMFS (NMFS 1991; NMFS 1997b; NMFS 2007b) in the various versions of the regional Biological Opinions (RBO), the 2003 (revised in 2005 and 2007) Gulf of Mexico Regional Biological Opinion on Hopper Dredging (GRBO) completed in 2003 and

[23] Santee Cooper Hydroelectric Project - South Carolina - FERC Project No. 199-205.

revised in 2005 and 2007 (NMFS 2003a; NMFS 2005; NMFS 2007c) and the SARBO (NMFS 1997b). Additionally, the USACE has recently prepared a comprehensive analysis of data from Gulf and Atlantic hopper dredging projects to identify factors affecting sea turtle take rates (Dickerson et al. 2007). Furthermore, the USACE maintains the STDW with historical records of dredging projects and turtle and sturgeon interactions. These are the primary sources, discussed further below, for our analysis of dredging effects on sea turtles and sturgeon.

Non-Hopper Dredging

The project may affect sea turtles or Atlantic sturgeon by injury or death as a result of interactions with equipment or materials used during dredging; however, NMFS believes the chance of injury or death from interactions with clamshell and/or hydraulic dredging equipment is discountable as these species are highly mobile and are likely to avoid the areas during construction. Areas to be dredged using non-hopper dredges include the inner harbor including the turning basin enlargements at the Wando Welch, Navy base, and North Charleston Terminal. These turning basins will all be expanded to accommodate Post Panamax Generation II and Generation III container ships.

Channels maintained at frequent dredging intervals are not expected to be used extensively by sturgeon for feeding or other activities. Dredging activities can impact benthic assemblages either directly or indirectly and may vary in nature, intensity, and duration depending on the project, site location, and time interval between dredging operations. Though initial loss of benthic resources are likely, quick recovery between 6-months (McCauley et al. 1977; Van Dolah et al. 1984; Van Dolah et al. 1979) to two years (Bonsdorff 1980; Ray 1997) is expected; thus, the impacts to sturgeon foraging habitat are expected to be short-term and are therefore insignificant.

NMFS has received very few reported sea turtle takes associated with these dredging methods in the South Atlantic region: only 1 live sea turtle has been taken by a clamshell dredge over the past 20 years. The take occurred at Cape Canaveral, Florida, which routinely has very high local turtle abundance. Cold-stunned turtles have also been taken by cutterhead dredging, but this also rarely happens and has been generally limited to shallow, confined waters (e.g., Laguna Madre, Texas) or bays where turtles get trapped and stunned when the rapid passage of a cold front causes the temperature of the shallow water body to drop abruptly. Due to the infrequency of interactions with these equipment types and the project location and channel depths, NMFS believes that the likelihood of cold stunning is discountable, and also the possibility of a sea turtle being taken by a hydraulic cutterhead or a clamshell dredge is discountable.

Hopper Dredging

Hopper dredging was implicated in the mortality of South Atlantic endangered and threatened sea turtles as early as the late 1970s and in NMFS Opinions issued in 1979, 1980, and others leading to the RBO issued in 1991. This determination was repeated in the 1995 and 1997 SARBOs (NMFS 1995; NMFS 1997b). The measures established in consecutive RBOs (NMFS 1991; NMFS 2007a) to avoid and minimize sea turtle interactions during hopper dredging operations permitted by the USACE in the southeastern United States are included in this project, with the exception of certain project-specific modifications to dredge timing (i.e.,

"dredging window"), to use of the sea turtle deflector dragheads,[24] and conditions of/requirements for relocation trawling. These modifications are discussed in further detail below and in the following sections. Atlantic sturgeon were not included in the previous Opinions due to their recent ESA listing status. The USACE has documented interactions and takes of sturgeon were during both relocation trawling and dredging activities. In the South Atlantic region, only 9 incidental takes have occurred during hopper dredging operations, all of which were Atlantic sturgeon.

Calculation of Sea Turtle Entrainment Rates during Hopper Dredging
To calculate the expected rates of turtle and sturgeon entrainment in hopper dredging for this project, NMFS consulted the STDW to find the most applicable historic dredging information for the Port of Charleston.

From 2000 through 2013, maintenance dredging carried out by hopper dredge in the entrance channel of the Charleston Harbor generated approximately 23,608,818 yd^3 of material (Table 7). Twenty-two sea turtles (16 loggerhead, 4 Kemp's ridley, and 2 green) and 3 Atlantic sturgeon were documented/observed as taken in hopper dredges during these dredging events. To calculated the catch per unit effort (CPUE) we divided the number of animals taken by the quantity of material being dredged (23,6082818yd^3) resulting in CPUE of 0.0000006777 loggerhead (16/23,608,818=0.0000006777), 0.0000000847 green (2/23,608,818=0.0000000847), and 0.0000001694 Kemp's ridley sea turtles (4/23,608,818=0.0000001694), and 0.0000001271 Atlantic sturgeon (3/23,608,818=0.0000001271) per cubic yard dredged. To calculate the number of animals expected to be taken during this project, we then multiply the amount of material to be dredged by hopper during the CHP45 project (11,144,172yd^3) times the CPUE for each species. Our estimated, anticipated *detected* take estimates by species (i.e., those takes witnessed and documented by hopper dredge protected species observers) are 7.5525 loggerhead (11,144,172x0.0000006777=7.5525), 0.9441 green (11,144,172x0.0000000847=0.9441), and 1.8881 Kemp's ridley (11,144,172x 0.0000001694=1.8881)sea turtles, 1.4161 Atlantic sturgeon (11,144,172x 0.0000001271=1.4161). These values were then rounded up because it is not possible to take part of an animal resulting in a final estimated take of 8 loggerhead, 1 green, and 2 Kemp's ridley sea turtles, and 2 Atlantic sturgeon.

[24] The leading edge of the deflector is designed to have a plowing effect of at least 6 in depth when the draghead is being operated so as to deflect, rather than injure or entrain and kill, a sea turtle during dredging operations.

Table 7. Dredged Material Removed and Sea Turtle and Sturgeon Takes during Previous Dredging in the Port of Charleston Entrance Channel, 1991-2013 (STDW)[25]

Year	Quantity Dredged	Loggerhead Sea Turtle (NWA DPS)	Green Sea Turtle	Kemp's Ridley Sea Turtle	Atlantic Sturgeon
2012	1,304,000	0	1	0	1
2010	1,444,703	0	0	0	0
2008	519,537	0	0	0	0
2006	1,178,676	0	0	0	0
2004	1,449,234	3	0	0	0
2003	517,947	0	0	0	0
2001	4,535,537	0	0	0	0
2000	5,627,386	4	1	3	2
1999	1,562,690	1	0	0	0
1997	775,418	5	0	0	0
1995	1,583,677	0	0	0	0
1993	568,350	0	0	0	0
1992	2,165,238	0	0	0	0
1991	376,425	3	0	1	0
Total	3,608,818	16	2	4	3
	CPUE	**0.0000006777**	**0.0000000847**	**0.0000001694**	**0.0000001271**
CHP45	**11,144,172**	**7.5525**	**0.9441**	**1.8881**	**1.4161**
	Rounded up	**8**	**1**	**2**	**2**

Detected vs. Actual Takes

NMFS-approved observers monitor dredged material inflow and overflow screening baskets on many hopper dredging projects, and observers will be required to monitor the proposed action. Dredged material screening, however, is only partially effective, and observed takes likely provide only partial estimates of total sea turtle mortality. NMFS believes that some turtles killed by hopper dredges go undetected because body parts are forced through the sampling screens by water pressure and are buried in the dredged material, or animals are crushed or killed, but their bodies or body parts are not entrained by the suction and so the takes may go unnoticed. The only mortalities that are noticed and documented are those where body parts float, are large enough to be caught in the screens, and can be identified as sea turtle parts. Body parts that are forced through the 4-in (or greater) inflow screens by the suction-pump pressure and that do not float are very unlikely to be observed, since they will sink to the bottom of the hopper and not be detected by the overflow screening. Unobserved takes are not documented, thus, observed takes may under-represent actual lethal takes. It is not known how many turtles are killed but unobserved. Because of this, in the GRBO (NMFS 2003b), in making its jeopardy analysis, NMFS estimated that up to 1 out of 2 impacted turtles may go undetected (i.e., that observed take constituted only about 50% of total take). That estimate was based on region-wide (overall Gulf of Mexico) hopper dredging projects including navigation channel dredging and sand borrow area dredging for beach renourishment projects, year-round, including seasonal

[25] Only years in which hopper dredging occurred are listed in this table. Data was surveyed from 1991 through 2013.

windows when no observers are required, times when 100% coverage is required, and times when only 50% observer coverage is required (i.e., at sand borrow sites).

The proposed dredging of the CHP45 will include 100% observer coverage during hopper dredging operations for the duration of hopper dredging. A significantly greater number of turtles will be detected with 100% observer coverage, but a significant number of turtle parts will still pass through the screens undetected.

NMFS estimates that with 100% observer coverage, protected species observers aboard hopper dredges for the proposed project will detect approximately just 1 of every 2 turtles or sturgeon that are struck by the suction draghead and either crushed and pushed away or entrained during the CHP45. This results in an additional estimated 8 loggerhead, 1 green, and 2 Kemp's ridley sea turtles, and 2 Atlantic sturgeon taken, but not detected, for a total take of 16 loggerhead, 2 green, and 4 Kemp's ridley sea turtles, and 4 Atlantic sturgeon. We will use these totals, by species (estimates rounded up) for our jeopardy analyses because it is not possible to take a fraction of a sea turtle (Table 8).

Table 8. Estimated Sea Turtle Takes and Atlantic Sturgeon (Observed and Unobserved)
(assumed 50% detection rate by onboard protected species observers over the course of the project)

	Loggerhead Sea Turtle	Green Sea Turtle	Kemp's Ridley Sea Turtle	Atl Sturgeon
Observed	8	1	2	2
Unobserved	8	1	2	2
Total species takes for the CHP45	16	2	4	4

As with previous NMFS Opinions on hopper dredging, our subsequent (Section 7 of this Opinion) jeopardy analysis is necessarily based on our knowledge (in this case, our best estimate) of the total number of turtles and Atlantic sturgeon that will be lethally taken, which includes those that are killed but not detected. Our best estimate of turtles and sturgeon lethally taken will be the sum of the observed and unobserved takes, i.e., those observed and documented by onboard protected species observers, plus those unobserved, undocumented lethal takes (because the sea turtle and sturgeon parts were either not entrained, or were entrained but were not seen/counted by onboard protected species observers). For example, the 2003 GRBO on hopper dredging estimated that 80 loggerhead sea turtles would be killed annually by hopper dredges but that only 40 would be detected by onboard observers. Similarly, in this Opinion we have estimated that 16 loggerhead sea turtles, 2 green sea turtles, 4 Kemp's ridley sea turtles, and 4 Atlantic sturgeon will be killed by dredges, but shipboard protected species observers will only detect half of each of these takes by species.

Our ITS is based on observed takes, not only because observed mortality gives us an estimate of unobserved mortality, but because observed, documented take numbers serve as triggers for some of the reasonable and prudent measures, as well as for potential reinitiation of consultation should actual observed takes exceed the anticipated/authorized number of observed takes. Furthermore, our ITS level of anticipated/authorized lethal takes is based on the implementation of relocation trawling, since it is an integral and important part of the proposed action. Without

the implementation of relocation trawling, mortalities resulting from hopper dredge activities are expected to be higher.

A very few turtles (over the years, a fraction of 1%) survive entrainment in hopper dredges, and those that do are usually smaller juveniles that are sucked through the pumps without being dismembered or badly injured. Often they will appear uninjured only to die days later of unknown internal injuries, while in rehabilitation. Experience has shown that the vast majority of hopper-dredge impacted turtles are immediately crushed or dismembered by the violent forces they are subjected to during entrainment. Therefore, we are conservatively predicting that all takes by hopper dredges will be lethal[26].

5.1.1.1 Assigning Interactions to the 5 Atlantic Sturgeon DPSs

Atlantic sturgeon mix extensively in the marine environment, and individuals from all 5 Atlantic sturgeon DPSs could interact with hopper dredging carried out in the entrance channel of the CHP45. In January 2012, the NMFS Northeast Region did a mixed stock analysis (MSA) of the composition of Atlantic sturgeon stocks along the East Coast using tag-recapture data and genetic samples that identify captured fish back to their DPS of origin. Atlantic sturgeon can be assigned to their DPS based on genetic analyses with 92-96% accuracy (ASSRT and NMFS 2007), though some fish used in the MSA could not be assigned to a DPS. Data from the Northeast Fisheries Observer Program (NEFOP) and the At Sea Monitoring (ASM) programs were used in the MSA to determine the percentage of fish from each of the DPSs at the selected locations along the coast.

Marine Mixing Zone 3, which extends from Cape Hatteras to the tip of Florida, corresponds to the South Atlantic portion of the species range where the action area is located. The MSA was updated by the NMFS Northeast Region in February 2013. Since no new data for Marine Mixing Zone 3 were available, NMFS determined that the original data from the NEFOP and ASM programs represent the best available information. According to the MSA, the composition of Atlantic sturgeon in Marine Mixing Zone 3 by DPS is:

- 0-9% Gulf of Maine DPS
- 4-26% New York Bight DPS
- 7-18% Chesapeake Bay DPS
- 10-29% Carolina DPS
- 46-79% South Atlantic DPS

To be conservative, we will assume that the maximum percentage presented for each DPS is representative of the composition of Atlantic sturgeon in the South Atlantic. The numbers of Atlantic sturgeon from of each DPS potentially taken in the hopper dredge were estimated by multiplying the same maximum percentages of each DPS expected to be present in the South

[26] In a recent opinion analyzing a similar action, we adjusted the take estimates to account for sea turtle population growth since the last year of data used in the analysis. We have not taken the same approach in this opinion because of the shorter term of this project. Hopper dredging in the other project was to occur over 6 years, not just two years like this project. As well, data used to calculate take in this opinion included through 2013, which is more recent than the data used in the other opinion. Additionally, inserting population growth data will not change the take values derived in this opinion.

Atlantic by the total number of estimated Atlantic sturgeon captures (4; Table 8) as shown in Table 9. Note that the percentages will add up to more than 100% and the sum of each category of interactions by DPS will be greater than the total number of interactions presented in the previous section due to the usage of the highest percentage calculated by the MSA for each DPS. The total number of estimated and authorized takes from all DPSs will be limited to the actual/total take estimate for Atlantic sturgeon, i.e., 4 animals total (2 observed and 2 unobserved). Using the maximum percentage of each DPS will result in estimating and potentially authorizing up to the specified number from each DPS, but still not in excess of the actual/total take estimate for all DPSs combined.

Table 9. Estimated Number of Atlantic Sturgeon From Each DPS Taken by the Hopper Dredge during the CHP45 by DPS

DPS	Maximum Species Composition	4 Atlantic Sturgeon	Roundup
Gulf of Maine DPS	9%	0.36	1
New York Bight DPS	26%	1.04	2
Chesapeake Bay DPS	18%	0.72	1
Carolina DPS	29%	1.16	2
South Atlantic DPS	79%	3.16	4

5.1.2 Post-Dredging Operations (Bed-leveling Activities)

Bed-leveling is often associated with hopper dredging (and other types of dredging) operations, and may be utilized in this project. Bed-leveling does not use suction but redistributes sediments, rather than removing them. Plows, I-beams, or other seabed-leveling mechanical devices are often used for cleanup operations, i.e., to lower high spots left in channel bottoms and dredged material deposition areas by hopper dredges or other type dredges. Leveling devices typically weigh about 30-50 tons, are fixed with cables to a derrick mounted on a barge pushed or pulled by a tugboat at about 1-2 knots. Some evidence indicates that bed leveling devices may be responsible for occasional sea turtle mortalities (NMFS 2003b). Sea turtles may be crushed as the leveling device passes over a turtle which fails to move or is not pushed out of the way by the sediment wedge “wave” generated by and pushed ahead of the device. Sea turtles in Georgia waters may have been crushed and killed in 2003 by bed-leveling which commenced after the hopper dredge finished its work associated with the Brunswick Harbor Entrance Channel dredging. The local sea turtle stranding network reported stranded crushed sea turtles in the area where the bed-leveler dredge was working, within days after the dredge was in the area. Brunswick Harbor is also one of the sites where sea turtles captured by relocation trawlers sometimes show evidence of brumating (over-wintering) in the muddy channel bottom, which could explain why, if sea turtles were in fact crushed by bed-leveler type dredges (the most likely explanation), they failed to react quickly enough to avoid the bed-leveler. Bed-leveler use at other dredging operations has not resulted in observed or documented sea turtle mortalities; therefore, the best available evidence points to occasional potential interactions to brumating sea turtles at Brunswick. All things considered, the use of bed-levelers is probably preferable (less likely to result in sea turtle interactions) to the use of hopper dredges for cleanup operations, since turtles foraging, resting, or brumating on irregular bottoms are probably more likely to be entrained by suction dragheads than crushed by bed-levelers, because: (1) sea turtle deflector dragheads are less effective on uneven bottoms; (2) hopper dredges move considerably faster

than bed-leveler “dredges”; and (3) bed-levelers do not use suction. Sturgeon mortality has not been linked to any bed leveling activities.

Historically these types of activities have not been required in the project area; however they may occur from Myers Bend and the Wando River downstream to Segment 1 in the entrance channel. NMFS believes it is unlikely that turtles or sturgeon may be adversely affected by potential bed-leveling activities during “high-spot cleanup” during the proposed action. If, however, injurious or lethal bed-leveler interactions appear to have occurred, based on reports of stranded turtles or sturgeon, they shall be immediately reported to NMFS, and reinitiation of consultation will be required.

5.1.3 Water quality

The proposed channel deepening will increase the salinity concentrations in the action area. The EFDC model predicted salinity changes resulting from the project. Figure 11 depicts the overall changes in salinity that are predicted the result from the proposed action. The greatest changes in salinity are projected in the Wando River. However, based on available information, Atlantic Sturgeon rarely utilize the Wando River (B. Post, SCDNR, pers. comm. to K. Reece, NMFS, April 8, 2015). Based on information in the DEIS, all projected salinity increases in the Cooper River will be less than one ppt, with the average change estimated to be less than 0.40 ppt. Due to the very small magnitude of the anticipated changes in salinity throughout portions of the action area, and the salinity tolerance of the life stages of Atlantic sturgeon potentially present in the action area, we anticipate that any direct effects would be insignificant.

The proposed channel deepening may also have an effect on dissolved oxygen (DO). The current average DO level in the Cooper River is 5.77 mg/L. Overall, the current values in the river range from average low values of 4.7 mg/L to average high levels of 7.6 mg/L. None of the data for the current conditions indicates any DO levels in the Cooper River less than 4.4 mg/L. The EFDC model was used to forecast future effect of the proposed project combined with the effects of all anticipated discharges to predict all future changes in DO. The model results indicate very minor DO reductions throughout the action area (average reduction of 0.03mg/L). Additionally, this analysis indicates that the combined effect of the proposed project with on-going and future unrelated discharges would result in Cooper River DO reductions ranging from less than 0.02 mg/L to 0.1256 mg/L. Due to the very small magnitude of the anticipated changes in DO throughout portions of the action area which are used by Atlantic sturgeon, we anticipate that any direct effects would be insignificant.

5.1.4 Dredged Material Disposal

NMFS believes the proposed marine dredged material disposal activities of approximately 40 MCY over the life of the deepening project (Table 1) are not likely to adversely affect sea turtles or Atlantic sturgeon. Sea turtles and sturgeon may be attracted to ODMDS or reef mitigation sites, to forage on the bycatch that may be occasionally found in the dredged material being dumped. As such, these species could be potentially impacted by the sediments being discharged overhead. However, NMFS has never received a report of an injury to a sea turtle or sturgeon resulting from burial in, or impacts from, dredged material disposal, neither from inshore or offshore disposal sites, anywhere the USACE conducts dredged material disposal operations.

Sea turtles and sturgeon are highly mobile and apparently are able to avoid a descending sediment plume discharged at the surface by a hopper dredge opening its hopper doors, or pumping its sediment load over the side. Even if temporarily enveloped in a sediment plume, NMFS believes the possibility of injury, or burial of normal, healthy sea turtles or sturgeon by dredged material (i.e., sand and silt) disposal, is discountable.

NMFS believes that foraging habitat for sea turtles or sturgeon is not likely a limiting factor in the action area due to the expansive amount of similar habitat offshore near the ODMDS and the reef mitigation sites, and thus the loss of potential sand bottom foraging habitat adjacent to, or on the surface of, the disposal areas or in the entrance channel (compared to remaining foraging habitat) from burial by dredged material sediments will have insignificant effects on sea turtles and sturgeon.

Reef mitigation sites include an "L" shaped berm along the south and west perimeter of the ODMDS. This area represents approximately 73 acres of the ODMDS with dimensions roughly 15,000 ft x 16,000 ft x 600 ft wide x 10 ft high. The reef is designed to mitigate for hardbottom impacts resulting from the deepening and would be a two tiered berm created with limestone rock dredged from the entrance channel. Additionally, the USACE will construct 8 new 33-acre reef sites with 4 located along the north side of the channel and 4 located along the south side of the channel (Figure 2). The hard substrate and rugosity of the 8 mitigative reef sites will provide attachment substrate for epifauna and are designed to replace the existing hardbottom that will be dredged as well as provide physical features/vertical structure to provide habitat diversity. Physical features which are believed to be important include material used, shape and landscape, substrate, relationship to currents, and size. While vertical relief is usually highly desirable, the harbottoms being impacted by the entrance channel dredging are not high relief reefs to begin with. The new reef feature will consist of individual low relief mounds separated by existing bottom native sands/sediment. The reef to be constructed will not impair navigation clearances as water depths in the mitigation area are between 35 and 50 feet. Each of the 8 patch reefs will be approximately 33-acres for a total of 264 acres of patch reefs. Combined with the ODMDS "L" shaped berm, a total of approximately 337 acres mitigating for both 186 acres of temporary impacts to hard bottom habitat along the margins of the channel as well as 28.6 acres of hardbottom habitat removed within the entrance channel during deepening activities. Effects to sea turtles and Atlantic sturgeon include the risk of injury from in-water construction (e.g., reef material (consisting of softball and larger basketball size pieces) placement by crane or by being pushed over the side of the barges), but will be discountable due to the species' ability to move away from the project sites if disturbed. As well, sea turtles and Atlantic sturgeon may be adversely affected by being temporarily unable to use the sites for foraging or shelter due to avoidance of construction activities and related noise. These effects will be insignificant because they are located in open water, construction will not restrict movement of species in the area, and there is ample, alternate similar habitat adjacent to the project sites.

The risk of injury to sea turtles from collisions with dredge-related vessels is also considered discountable, considering the species' mobility and the slow speed of the hopper dredge vessels and associated barges and scows. The risk of injury to Atlantic sturgeon from collisions with dredge-related vessels is discountable, considering the species' demersal nature.

5.1.5 Relocation Trawling

While the USACE has proposed relocation trawling activities to reduce the density of sea turtles in the path of hopper dredges, they have not stated when relocation trawling will be carried out or at what effort level. Because no effort level has been stated by the USACE, NMFS will require closed net relocation trawling in RPM #1 (Section 9.3), in specific situations (stated in T&C 12), carried out in specific says (T&C 13). In order to authorize take resulting from relocation trawling, NMFS must require this activity as a RPM and not as a conservation measure. Relocation trawling is required only when it can be done safely, as a means to reduce sea turtle and sturgeon mortalities, because it is a proven method of reducing sea turtle and sturgeon density in front of an advancing hopper dredge and very likely results in reduced sea turtle and sturgeon/hopper dredge interactions.

Nets are dragged on the bottom for 30 minutes or less before each retrieval and re-setting. Its effects are mostly nonlethal and non-injurious to trawl captured sea turtles and sturgeon. Over the course of more than twenty years that relocation trawling has been conducted by the USACE, very few sea turtle mortalities (approximately 7, of which 3 died under unusual circumstances during relocation trawling associated with the Deepwater Horizon oil spill event in the Gulf of Mexico) have occurred, while approximately 3,000 sea turtles have been safely relocated (D. Dickerson, ERDC, Pers. Comm to K. Reece, NMFS, March 27, 2015). There have been no observed sturgeon mortalities during relocation trawling. NMFS has previously estimated in dredging Opinions that the risk of a sea turtle being killed in a capture trawl net (closed net) is less than 0.4%, and NMFS has no new information to alter the basis of that conclusion. NMFS believes that the possibility that a sea turtle or sturgeon will be killed or injured during capture trawling (using modified shrimp trawl nets) is discountable, given the low historic injury/mortality rate.

Relocation trawling conducted at previous Charleston Harbor projects has been somewhat limited; therefore, basing estimates of potential take during relocation trawling for the proposed action is difficult. During previous capture trawling (1991, 1992, 1997, 2000, 2004, and 2006) associated with hopper dredging in the Port of Charleston, a total of 127 sea turtles and 3 Atlantic sturgeon were safely trawled-captured and released over 93 days of relocation trawling[27] (Table 10). Similar capture work carried out by Arendt et al. (2012) between 2004 and 2007 to evaluate loggerhead sea turtle catch rates and demographic distributions in the Charleston entrance channel. Schwenter et al. (Schwenter et al. 2013) documented similar information for kemp's ridley sea turtles. Results from Arendt et al. (2012) and Schwenter et al. (2014) are also included in Table 10 and were used to further refine the CPUE. Results from all data calculate a CPUE for each species by dividing the number of animals previously relocated by the number of trawling days, or 1.5427 loggerhead (263/164=1.5427), 0.0061 green(1/164=0.0061), and 0.0366 Kemp's ridley (6/164=0.0366) sea turtles and 0.0488 Atlantic sturgeons (8/164=0.0488) per relocation trawling day. Hopper dredging in the entrance channel is expected to take 466 days to complete. It is possible for relocation trawling to occur every day during hopper dredging so we calculated potential non-lethal take based on relocation trawling occurring every day during hopper dredging (466). After multiplying the number of potential relocation trawling days times the CPUE and rounding up because you cannot take a fraction of an animal, we calculate that up to 719 (466 days x 1.5427) loggerhead, 3 (466 days x .0061) green, and 18 (466

[27] Data from the STDW

days x .0366) Kemp's ridley sea turtles and 23 (466 days x .0488) Atlantic sturgeon will be relocated during the CHP45.

Table 10. Relocation Trawling Efforts in the Port of Charleston Entrance Channel

Data	Year	Days	Loggerhead Sea Turtle	Green Sea Turtle	Kemp's Sea Turtle	ATL Sturgeon
Arendt et al 2012	2007	2	7	0	0	0
	2007	2	7	0	0	0
	2006	12	43	0	0	0
	2005	12	36	0	0	5
	2005	12	11	0	0	0
	2004	9	49	0	0	0
	2004	12	55	0	2[28]	0
	2004	10	16	0	0	0
STDW	2006	1	0	0	0	1
	2004	3	7	0	0	2
	2000	33	2	0	3	0
	1997	26	2	0	0	0
	1992	4	2	1	0	0
	1991	26	16	0	1	0
	Total	164	253	1	6	8
	CPUE	per day	1.5427	0.0061	0.0366	0.0488
	Potential for all days of hopper	466	718.89	2.84	17.05	22.73
	Rounded up		719.00	3.00	18.00	23.00

5.1.5.1 Assigning Interactions to the 5 Atlantic Sturgeon DPSs

As discussed previously in Section 5.1.1.1, animals relocated during the CHP45 could include sturgeon from all 5 Atlantic sturgeon DPSs. The total numbers of Atlantic sturgeon from of each DPS potentially relocated were estimated by multiplying the same maximum percentages of each DPS expected to be present in the South Atlantic by the total number of estimated Atlantic sturgeon relocations (22.73, Table 10) as shown in Table 11. Note that the percentages will add up to more than 100% and the total of each category of interactions by DPS will be greater than the total number of interactions presented in the previous section due to the usage of the highest percentage calculated by the MSA for each DPS. The total number of estimated and authorized takes from all DPSs will be limited to the actual/total take estimate for Atlantic sturgeon, i.e., 23 animals total. Using the maximum percentage of each DPS will result in estimating and potentially authorizing up to the specified number from each DPS, but still not in excess of the actual/total take estimate for all DPSs combined.

[28] Schwenter, J. A., and coauthors. 2013. Catch Rates and Demographics for Kemp's Ridley (Lepidochelys kempii) Sea Turtles Captured in Near-shore Coastal Waters Between Winyah Bay, SC and St. Augustine, FL. Sea Turlte Biology and Conservation. National Oceanic and Atmospheric Administration, Baltimore, MD.

Table 11. Estimated Number of Atlantic Sturgeon From Each DPS Relocated during the CHP45 by DPS, Rounded Up.

DPS	Maximum Species Composition	22.73 observed Atlantic Sturgeon	Roundup
Gulf of Maine DPS	9%	2.0457	3
New York Bight DPS	26%	5.9098	6
Chesapeake Bay DPS	18%	4.0914	5
Carolina DPS	29%	6.5917	7
South Atlantic DPS	79%	17.9567	18

The effects of this harassment of the turtles or sturgeon during capture and handling during relocation trawling can result in raised levels of stressor hormones, and can cause some discomfort during tagging procedures. Based on past observations obtained during similar research-trawling for turtles, these effects are expected to dissipate within a day (Stabenau et al. 1991). Since turtle or sturgeon recaptures are rare, and recaptures that do occur typically happen several days to weeks after initial capture, cumulative adverse effects of recapture are not expected. We believe that properly conducted and supervised relocation trawling (i.e., observing trawl speed and tow-time limits, and taking adequate precautions to release captured animals) and tagging is unlikely to result in adverse effects to sea turtles or sturgeon. Thus, we believe that the probability that a sea turtle or sturgeon will be injured or killed during capture or non-capture relocation trawling is discountable.

5.2 Project effects on the Cooper River.

The HSI models were informed by the outputs from the hydrodynamic modeling (EFDC) to predict environmental conditions after the project is constructed. This analysis assesses changes in habitat quantity and quality. The HSI model used pre- and post-project salinity, temperature, currents and dissolved oxygen inputs from the EFDC model. Pertinent results of the HSI modeling are summarized below.

5.2.1 Spawning Habitat Impacts

The sturgeon spawning life stage is most affected by salinity in the habitat models. In the portions of the action area where Atlantic sturgeon may occur (throughout the project area but not in the Wando River), the forecast salinity changes occur in areas located approximately 3 miles south of “The Tee” (which is the red line in Figure 7). The proposed action is anticipated to change three “cells” from “suitable” to “non-suitable.” The affected areas are well downriver of historical spawning areas which typically are located in fast-flowing water between the salt front and fall line of large rivers (Bain et al. 2000; Borodin 1925; Crance 1987; Leland 1968; Scott and Crossman 1973) well above the Pinopolis Dam. The following information discusses spawning in the area by both Atlantic and shortnose sturgeon in order to capture the complete sturgeon spawning history in the area. As noted in section in section 3.1.4, shortnose sturgeon are not expected to be adversely affected by this action.

Sturgeon spawning (shortnose only) has been documented in the tailrace area immediately below the Pinopolis Dam since 1997 (Cooke and Leach 1999), but Cooke and Leach (1999) considered this area to be a surrogate spawning site because access upstream is blocked. One Atlantic sturgeon made what was thought to be a spawning run to the Pinolpolis Dam in recent years but no evidence of actual spawning was identified (B. Post, SCDNR, pers. comm. to K. Reece,

NMFS, April 8, 2015). Kynard et al. (1999) as well considered this area atypical of preferred sturgeon spawning habitat, supporting the hypothesis that a migration blockage imposed spawning at a site that was not historically used. Fertilized shortnose sturgeon eggs collected in the Pinopolis Dam tailrace verified spawning despite non-traditional (i.e., barren hard bottom with scattered pockets of clam shell and marl pieces) spawning habitat (Cooke and Leach 2004; Duncan et al. 2004). Recent success of shortnose sturgeon spawning in this area is unknown (B. Post, SCDNR, pers. comm. to K. Reece, NMFS, 2012) but is thought to be unsuccessful due to existing water quality issues (salinity). Previous (1996-1998) population estimate (mark-recapture) of shortnose sturgeon in the Cooper River focused only on adults in the Pinopolis Dam tailrace (Cooke et al. 2004). Ages determined from the pectoral ray of 35 fish sub-sampled from the Pinopolis tailrace averaged 11 years (SD = 3.2), with no fish being less than 5 years old (Cooke et al. 2004). While a single Atlantic sturgeon made what appeared to be a spawning run in recent years (Post et al. 2014), there has been no evidence of any Atlantic sturgeon spawning in the Cooper River. The total area adversely affected by the projected salinity changes in this area is about 2.7% of the approximately 2,154 acres of currently available habitat. Because the habitat impacts of the project occurs in an area not considered spawning habitat, this change is unlikely to impact the spawning of this species and is considered insignificant.

5.2.2 Juvenile Life Stage Habitat Impacts

Juvenile sturgeon have been found to consume primarily benthic infauna while in the estuary (Huff 1975; Mason and Clugston 1993). Thus, any alterations within the estuary that changes either sediment grain size/sorting or chemistry may impact juvenile food resources (Kenny and Rees 1994; Seiderer and Newell 1999). Atlantic sturgeon are considered mature in South Carolina rivers at 5-19 years of age (Smith et al. 1982). Research carried out by Cooke et al. (2005) has shown sturgeon in Pinopolis tailrace averaged 11 years (SD = 3.2), with no fish being less than 5 years old. Juvenile sturgeon move downstream and inhabit brackish waters for a few months; and then move into coastal waters. Dredging modifies the quality of the habitat and further restricts the extent of available habitat in the Cooper River, where sturgeon habitat has already been modified and restricted by the presence of dams. Modeling described in the DEIS (USACE 2014) in several dozen model-grid cells predict substantial decreases in habitat suitability for juvenile Atlantic sturgeon following the CHP45 project (Figure 17) with large areas in the Wando River anticipated to decrease in juvenile habitat quality; however, juvenile Atlantic sturgeon are not known to use the Wando River. Other areas of decreased quality are scattered throughout the project area. Because there is no successful sturgeon spawning in the project area, any juvenile Atlantic sturgeon in the project area are likely migrants from other nearby systems. As migrants, these fish are already acclimated to increased salinity levels. As well, sturgeon are opportunistic feeders and will likely consume whatever types of bottom-dwelling organisms are present and area able to utilize areas with increased salinity with variable prey types and can also move to other more suitable areas. For these reasons, NMFS considers habitat changes in the action area to be insignificant to Atlantic sturgeon.

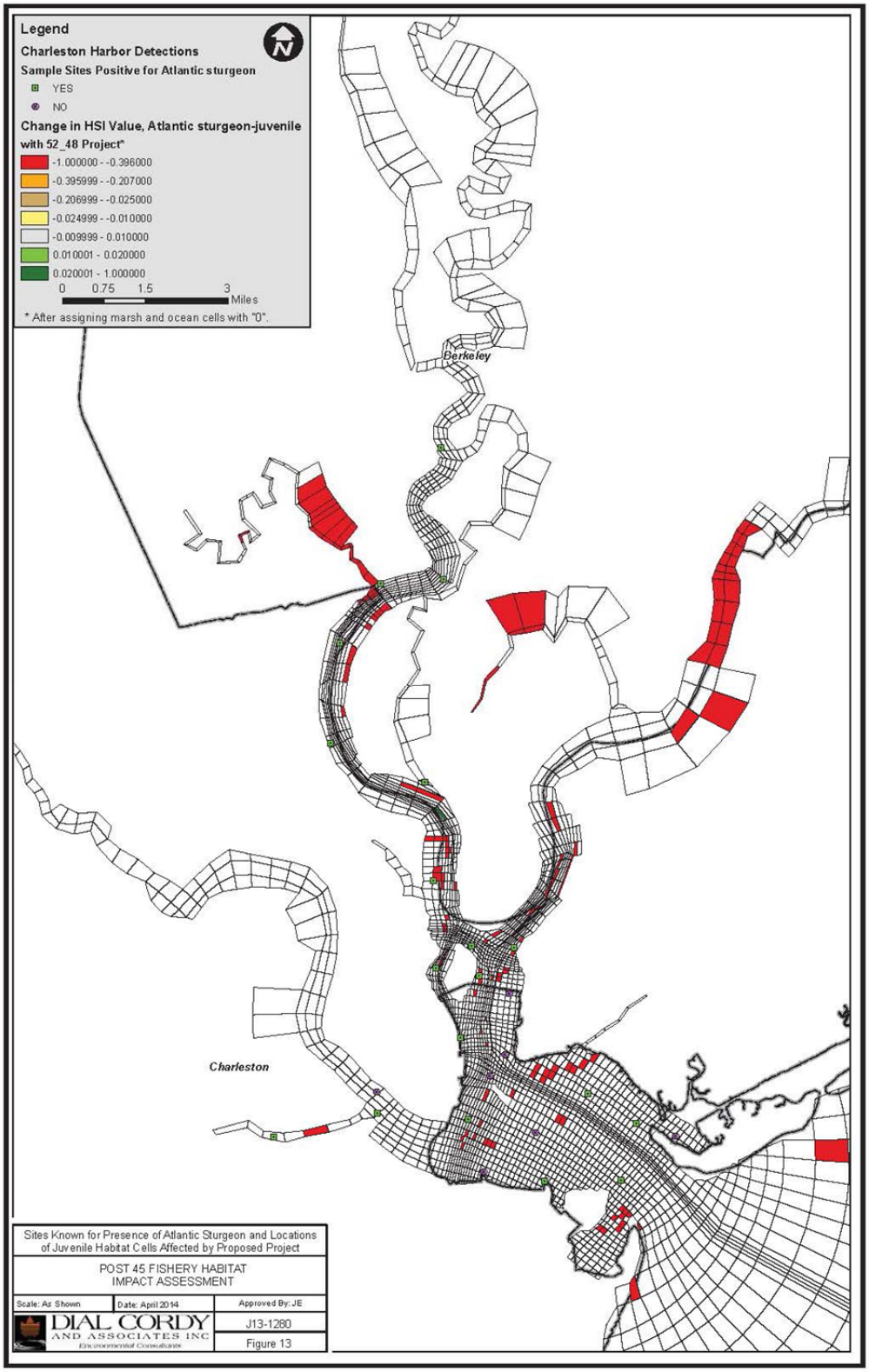


Figure 17. Results of HSI monitoring for juvenile life stage habitats (USACE 2014).

5.2.3 Adult Life Stage Habitat Impacts

The sturgeon adult life stage is most impacted by salinity and temperature. Modeling discussed in the DEIS determined the proposed action would not alter water temperature in a meaningful way to the species (USACE 2014). Salinity appears to be the ultimate driver for the projected impacts to habitats used by adult Atlantic sturgeon. With only a few exceptions at the mouth of the Ashley River, the north shore of James Island, and near Patriots Point heading east past Shem Creek, salinity-triggered habitat changes occur in the navigation channel or along the margins; these changes would result in a 4% reduction of available suitable habitat. (USACE 2014). This is because depths of these areas would increase and would result in a subsequent increase in salinity. These impacts are very small and essentially only take areas that had a salinity of just under the 28.6 ppt threshold to just over 28.6 ppt. SCDNR has documented the occurrence of Atlantic sturgeon within the harbor, and it is unlikely that the small changes to temperature that occur in and along the navigation channel will impact the adult life stage. The modeled results indicate the majority of changes will be within the channel where it is unlikely that sturgeon spend much time foraging. It is unlikely that the adult life stage will be impacted by a change of less than 4% modeled suitable habitat (USACE 2014) within the project area.

6 CUMULATIVE EFFECTS

Cumulative effects are the effects of future state, local, or private activities that are reasonably certain to occur within the action area considered in this Opinion. Federal actions that are unrelated to the proposed action are not considered in this section because they require separate consultation pursuant to Section 7 of the ESA.

Within the action area, major future changes are not anticipated in ongoing human activities described in the environmental baseline. The present human uses of the action area, such as commercial shipping, boating, and fishing, are expected to continue at the present levels of intensity in the near future as are their associated risks of injury or mortality to sea turtles and shortnose sturgeon posed by incidental capture by fishers, vessel collisions, marine debris, chemical discharges, and man-made noises.

6.1 Sea Turtles

Beachfront development, lighting, and beach erosion control are all ongoing activities along the southeastern coast of the United States. These activities potentially reduce or degrade sea turtle nesting habitats or interfere with hatchling movement to sea. Human activities and development along nesting beaches may also discourage sea turtles from nesting sites. The extent to which these activities reduce sea turtle nesting and hatchling production is unknown. But more and more coastal counties have adopted or are adopting more stringent protective measures to protect hatchling sea turtles from the disorienting effects of beach lighting. Some of these measures were drafted in response to lawsuits brought against the counties by concerned citizens who charged the counties with failing to uphold the ESA by allowing unregulated beach lighting—which results in takes of hatchlings.

NMFS presumes that any additional increases in recreational vessel activity in inshore and offshore waters of the Atlantic Ocean will likely increase the risk of turtles taken by injury or mortality in vessel collisions. Recreational hook-and-line fisheries have been known to lethally

take sea turtles. Future cooperation between NMFS and the states on these issues should help decrease take of sea turtles caused by recreational activities. NMFS will continue to work with states to develop ESA Section 6 agreements and Section 10 permits to enhance programs to quantify and mitigate these takes.

6.2 Atlantic Sturgeon

Human activities that affect riverine water quality and quantity such as non-point and point-source discharges are also expected to continue at current rates. Future cooperation between NMFS and the GADNR and SCDNR should help decrease take of sturgeon caused by recreational activities. NMFS will continue to work with states to develop ESA Section 6 agreements and with researchers in Section 10 permits to enhance programs to quantify and mitigate these takes.

Climatically, sea level is expected to continue to rise, as are water temperatures, and levels of precipitation are likely to fluctuate more drastically. Nutrient loading, pollution inputs, lower dissolved oxygen are all expected to be exacerbated. Drought and inter- and intra-state water allocation and their associated impacts will continue and may intensify (IPCC 2008). As well, rise in sea level will likely drive the salt wedge farther upriver, further constricting sturgeon habitat; however, the effect of rising sea level is integrated into the predicted salinity effects of the proposed action (USACE 2014) and is therefore incorporated into the analysis of potential effects due to changes in salinity.

7 JEOPARDY ANALYSIS

The analyses conducted in the previous sections of this Opinion serve to provide a basis to determine whether the proposed action would be likely to jeopardize the continued existence of affected ESA-listed sea turtles and sturgeon. In Section 5, we outlined how the proposed action can affect sea turtles and Atlantic sturgeon and the extent of those effects in terms of estimates of the numbers of each species expected to be killed. Now we turn to an assessment of each species' response to this impact, in terms of overall population effects from the estimated take, and whether those effects of the proposed action, when considered in the context of the status of the species (Section 3), the environmental baseline (Section 4), and the cumulative effects (Section 6), will jeopardize the continued existence of the affected species.

It is the responsibility of the action agency to "insure that any action authorized, funded, or carried out by such agency is not likely to jeopardize the continued existence of any endangered species or threatened species…" (ESA Section 7(a)(2)). Action agencies must consult with and seek assistance from the Services to meet this responsibility. The Services must ultimately determine in an Opinion whether the action jeopardizes listed species. "To jeopardize the continued existence of" means to engage in an action that reasonably would be expected, directly or indirectly, to reduce appreciably the likelihood of both the survival and the recovery of a listed species in the wild by reducing the reproduction, numbers, or distribution of that species (50 CFR 402.02). Thus, in making this determination, NMFS must look at whether the action directly or indirectly reduces the reproduction, numbers, or distribution of a listed species. Then, if there is a reduction in one or more of these elements, we evaluate whether it would be

expected to cause an appreciable reduction in the likelihood of both the survival and the recovery of the species.

In the following section we evaluate the responses of green, Kemp's ridley, and loggerhead (NWA DPS) sea turtles, and Atlantic sturgeon, to the effects of the action.

7.1 Effects of the Action on Green Sea Turtles' Likelihood of Survival and Recovery in the Wild

The nonlethal take of approximately 3 green sea turtles by capture relocation trawling will have no more than temporary, non-injurious effects on them. The lethal take of up to 2 green sea turtles by hopper dredging would reduce the number of green sea turtles as compared to the number that would have been present in the absence of the action assuming all other variables remained the same. The lethal take could also result in the loss of reproductive value as compared to the reproductive value in the absence of the proposed action, if the individual is female, eliminating her contribution to future generations. Greens nest frequently (at approximately 2-week intervals) during a nesting season and nest about every 2-4 years. During each nesting, they can produce an average of 110-115 eggs in each nest. The loss of an adult female could preclude the production of thousands of eggs and hatchlings, of which a small percentage is expected to survive to sexual maturity. Changes in distribution are not expected from lethal takes by hopper dredging during this action. Because the action area is small and sea turtles generally have large ranges in which they disperse, no reduction in the distribution of green sea turtles is expected from the take of 2 individuals.

The 5-year status review for green sea turtles states that of the 7 green sea turtle nesting concentrations in the Atlantic basin for which abundance trend information is available, all were determined to be either stable or increasing (NMFS and USFWS 2007a). That review also states that the annual nesting female population in the Atlantic basin ranges from 29,243-50,539 individuals. Additionally, the pattern of green sea turtle nesting shows biennial peaks in abundance, with a generally positive trend during the 20 years of regular monitoring since establishment of index beaches in Florida in 1989. An average of 5,099 green turtle nests were laid annually in Florida between 2001 and 2006 with a low of 581 in 2001 and a high of 9,644 in 2005 (NMFS and USFWS 2007a).

Although the anticipated mortality of 2 green sea turtles expected from the proposed action would result in an instantaneous reduction in absolute population numbers, it is not likely these small reductions would appreciably reduce the likelihood of survival of the species. If the hatchling survival rate to maturity is greater than the mortality rate of the population, the loss of breeding individuals would be replaced through recruitment of new breeding individuals from successful reproduction of non-taken sea turtles. Capture of sea turtles by relocation trawlers will not affect the adult female nesting population or number of nests per nesting season. Considering that the species' nesting trends are either stable or increasing, we believe the loss of up to 2 green sea turtles associated with the proposed action is not expected to cause an appreciable reduction in the likelihood of survival of this species of sea turtle in the wild.

The Atlantic Recovery Plan for the population of Atlantic green sea turtles (NMFS and USFWS 1991a) lists the following relevant recovery objectives over a period of 25 continuous years:

- The level of nesting in Florida has increased to an average of 5,000 nests per year for at least 6 years.

Green sea turtle nesting in Florida between 2001-2006 was documented as follows: 2001 – 581 nests; 2002 – 9,201 nests; 2003 – 2,622 nests; 2004 – 3,577 nests; 2005 – 9,644 nests; 2006 – 4,970 nests. The average is 5,039 nests annually over those 6 years (2001-2006) (NMFS and USFWS 2007a). Subsequent nesting has shown even higher average numbers (i.e., 2007 – 9,455 nests; 2008 – 6,385 nests; 2009 – 3, 000 nests; 2010 – 8,426 nests; 2011 – 10,701 nests), thus, this recovery criteria continues to be met.

- A reduction in stage class mortality is reflected in higher counts of individuals on foraging grounds.

Several actions are being taken to address this objective; however, there are currently no estimates available specifically addressing changes in abundance of individuals on foraging grounds. Given the clear increases in nesting, however, it is likely that numbers on foraging grounds have increased by at least the same amount. This Opinion's effects analysis assumes that in-water abundance has increased at the same rate as Tortuguero nesting.

The recovery plan includes 3 different recovery actions directly related to the proposed action of this Opinion: (1) Implement and enforce TED regulations (Priority 1), (2) promulgate regulations to reduce fishery related mortality (Priority 2), and (3) provide technology transfer for installation and use of TEDs (Priority). The proposed action does all of these things, thus supports continued implementation of the recovery plan.

The potential injury or mortality of 2 green sea turtles attributed to the proposed action are not likely to reduce population numbers over time due to current population sizes and expected recruitment. Despite the higher level of lethal interactions that occurred in the past, we have still seen positive trends in the status of this species. Capture of sea turtles by relocation trawlers will not affect the adult female nesting population or number of nests per nesting season.

In conclusion, we conclude the proposed action is not likely to impede the recovery objectives above and will not result in an appreciable reduction in the likelihood of green sea turtles' recovery in the wild.

7.2 Effect of the Action on Kemp's Ridley Sea Turtles' Likelihood of Survival and Recovery in the Wild

As demonstrated by nesting increases at the main nesting sites in Mexico, adult Kemp's ridley numbers have increased over the last decade. Recent calculations of nesting females determined from nest counts show that the population trend is increasing towards that recovery goal, with an estimate of 4,047 nesters in 2006 and 5,500 in 2007 (Gladys Porter Zoo 2007; NMFS and USFWS 2007q). Recent nesting data indicated a population of an estimated 8,460 females in 2009 and 5,320 females in 2010 (J. Peña, Gladys Porter Zoo, pers. comm. to S. Heberling, NMFS, March 21, 2011). NMFS et al. (2011) produced an updated model that predicted the

population to increase 19% per year and attain at least 10,000 females nesting on Mexico beaches by 2011. Approximately 25,000 nests would be needed for an estimate of 10,000 nesters on the beach, based on an average 2.5 nests/nesting female. While counts did not reach 25,000 nests by 2012, it is clear that the population is steadily increasing over the long term. Based on this information, the anticipated lethal take of up to 4 Kemp's ridley sea turtles would not be expected to have a detectable effect on the Kemp's ridley sea turtle reproduction or population numbers. Changes in distribution are not expected from lethal takes by hopper dredging during this action. Because the action area is small and sea turtles generally have large ranges in which they disperse, no reduction in the distribution of Kemp's ridley sea turtles is expected from the take of up to 4 individuals.

The nonlethal take of approximately 18 Kemp's ridley sea turtles by capture relocation trawling will have no more than temporary, non-injurious effects on them. Changes in distribution, even short-term, are not expected from nonlethal takes (interactions/releases from relocation trawling, vessel strikes, etc.) during the project. Interactions with vessels and/or relocation trawlers may elicit startle or avoidance responses and the effects of the proposed action may result in temporary changes in behavior of sea turtles (minutes to hours) over small areas, but are not expected to change the distribution of any sea turtles in the action area.

Based on the above analysis, we believe that take of Kemp's ridley sea turtles associated with the proposed action are not reasonably expected to cause, directly or indirectly, an appreciable reduction in the likelihood of survival of these species in the wild.

The following analysis considers the effects of the take on the likelihood of recovery in the wild. We consider the recovery objectives in the recovery plans prepared for each species that relate to population numbers or reproduction that may be affected by the predicted reductions in the numbers or reproduction of sea turtles resulting from the proposed action.

The recovery plan for Kemp's ridley sea turtles (NMFS and USFWS 1992), herein incorporated by reference, lists the following relevant recovery objective:

- Attain a population of at least 10,000 females nesting in a season.

The potential injury or mortality of 4 Kemp's ridleys will result in a reduction in overall population numbers. We already have determined this take is not likely to reduce population numbers over time due to current population sizes and expected recruitment. Capture of sea turtles by relocation trawlers will not affect the adult female nesting population or number of nests per nesting season because Kemp's ridley sea turtles are not known to nest regularly in or near the project area and relocated turtles are not prevented from nesting. Thus, the proposed action will not interfere with achieving the recovery objective and will not result in an appreciable reduction in the likelihood of Kemp's ridley sea turtles' recovery in the wild.

7.3 Effects of the Action on Loggerhead Sea Turtles' (NWA DPS) Likelihood of Survival and Recovery in the Wild

The nonlethal take of approximately 719 loggerhead sea turtles by capture relocation trawling will have no more than temporary, non-injurious effects on them. The lethal take of 16 loggerhead sea turtles by hopper dredges would result in an instantaneous, but temporary reduction in total population numbers. Thus, the proposed action will result in a reduction of sea turtle numbers. Sea turtle mortality resulting from hopper dredges could result in the loss of reproductive value of an adult turtle. For example, an adult female loggerhead sea turtle can lay 3 or 4 clutches of eggs every 2-4 years, with 100-130 eggs per clutch. The annual loss of 1 adult female sea turtle, on average, could preclude the production of thousands of eggs and hatchlings, of which a small percentage is expected to survive to sexual maturity. Thus, the death of an adult female eliminates an individual's contribution to future generations, and the action will result in a reduction in sea turtle reproduction.

Considering the size of the NWA DPS, we believe the loggerhead sea turtle population is sufficiently large enough to persist and recruit new individuals to replace those expected to be lethally taken (i.e., 16 over the course of the project). We use the following estimates to support our determination.

NMFS Southeast Fisheries Science Center (SEFSC) (2009e) estimated the likely minimum adult female population size for the western North Atlantic subpopulation in the 2004-2008 time frame to be between 20,000 and 40,000 (median 30,050) female individuals, with a low likelihood of there being as many as 70,000 individuals. The estimate of western North Atlantic adult loggerhead females was considered conservative for several reasons. The number of nests used for the western North Atlantic was based primarily on U.S. nesting beaches; as such, the results are a slight underestimate of total nests because of the inability to collect complete nest counts for many non-U.S. nesting beaches. In estimating the current population size for adult nesting female loggerhead sea turtles, NMFS SEFSC (2009e) simplified the number of assumptions and reduced uncertainty by using the minimum total annual nest count over the last 5 years (i.e., 48,252 nests). This was a particularly conservative assumption considering how the number of nests and nesting females can vary widely from year to year, (cf., 2008's nest count of 69,668 nests, which would have increased proportionately the adult female estimate to between 30,000 and 60,000). Further, minimal assumptions were made about the distribution of remigration intervals and nests per female parameters, which are fairly robust and well-known parameters.

Although not included in the NMFS SEFSC (2009e) report, in conducting its loggerhead assessment, NMFS SEFSC also produced a much less robust estimate for total benthic females in the western North Atlantic, with a likely range of approximately 60,000-700,000, up to less than 1 million. The estimate of overall benthic females is considered less robust because it is model-derived, assumes a stable age/stage distribution, and is highly dependent upon the life history input parameters. Relative to the more robust estimate of adult females, this estimate of total benthic female population is consistent with our knowledge of loggerhead life history and the relative abundance of adults and benthic juveniles: the benthic juvenile population is an order of magnitude larger than adults. Therefore, we believe female benthic loggerheads number in the hundreds of thousands.

Based on the total numbers of adult females and benthic juvenile females estimated by NMFS SEFSC for the western North Atlantic population of loggerhead sea turtles (now designated as the NWA DPS), the anticipated lethal take resulting from the proposed action (i.e., worst case, up to 16 loggerhead) represents the removal of, at most, approximately 0.043% of the estimated adult loggerhead female population. This level of lethal take of sea turtles also represents the removal of, at most, 0.0019% of the estimated female benthic loggerhead population. These removals are very small and contribute only minimally to the overall mortality on the population. Further, these percentages are likely an overestimation of the impact of the anticipated lethal take resulting from the proposed project on loggerhead sea turtles because of the following reasons. These percentages represent impacts to adult and benthic juvenile female loggerhead sea turtles only, and not to the population as a whole. Because this estimated contribution to mortality is a tiny part of our range of uncertainty across what total mortality might be for loggerhead sea turtles, we do not believe that the small effect posed by the lethal take resulting from the proposed project will be detectable or appreciable.

The potential lethal take of up to 16 loggerheads over the project will result in reduction in numbers when takes occur and possibly by lost future reproduction, but, given the magnitude of these trends and likely large absolute population size, it is unlikely to have any detectable influence on the population objectives and trends noted above. Although the effects of the proposed action will have an instantaneous effect on the overall size of the population, the action will not measurably reduce the size of the population, and will not result in an appreciable reduction in the likelihood of loggerhead sea turtles' survival in the wild.

The recovery plan for the Northwest Atlantic population of the loggerhead turtle (NMFS and USFWS 2008b) which is the same as the NWA DPS, provides additional explanation of the goals and vision for recovery for this population. The following objectives of the recovery plan are most pertinent to the threats posed by the proposed action:

- Ensure that the number of nests in each recovery unit is increasing and that this increase corresponds to an increase in the number of nesting females

The recovery plan anticipates that, with implementation of the plan, the western North Atlantic population will recover within 50-150 years, although it notes that reaching recovery in only 50 years would require a rapid reversal of the then-declining trends of the NRU, PFRU, and NGMRU.

Recovery is the process of removing threats so self-sustaining populations persist in the wild. The proposed action would not impede progress on carrying out any aspect of the recovery program or achieving the overall recovery strategy. The recovery plan estimates that the population will reach recovery in 50-150 years following implementation of recovery actions. The minimum end of the range assumes a rapid reversal of the current declining trends; the higher end assumes that additional time will be needed for recovery actions to bring about population growth.

Recovery Objective No. 1, "Ensure that the number of nests in each recovery unit is increasing…," is the plan's overarching objective and has associated demographic criteria.

Currently, none of the plan's criteria are being met, but the plan acknowledges that it will take 50-150 years to do so. Further reduction of multiple threats throughout the North Atlantic, GOM, and Greater Caribbean will be needed for strong, positive population growth, following implementation of more of the plan's actions. Although any continuing mortality in what might be an already declining population can affect the potential for population growth, we believe the effects of the proposed action would not appreciably reduce the likelihood of a recovery. The potential lethal take of up to 16 loggerheads over the CHP45 project will result in reduction in numbers when takes occur and possibly by lost future reproduction, but, given the magnitude of these trends and likely large absolute population size, it is unlikely to have any detectable influence on the population objectives and trends noted above. Capture of sea turtles by relocation trawlers will not affect the adult female nesting population or number of nests per nesting season.

In conclusion, we conclude that the effects associated with the CHP45 are not expected to cause an appreciable reduction in the likelihood of the recovery of loggerhead sea turtles' (NWA DPS) recovery in the wild.

7.4 Effects of the Action on Atlantic Sturgeons' Likelihood of Survival and Recovery in the Wild

The nonlethal take of approximately 23 Atlantic sturgeon by capture relocation trawling will have no more than temporary, non-injurious effects on them. The expected lethal capture of up to 4 Atlantic sturgeon by hopper dredging, with 1-4 lethal captures of Atlantic sturgeon originating from each of the 5 DPSs (Table 9), would result in a very small reduction in numbers within each DPS, ranging from 0.00579% to 0.14749% (Table 12). These lethal interactions would also result in a reduction in their future reproduction, if some of the individuals taken would be female and would have survived other threats and reproduced in the future. With that exception, the proposed action is not likely to cause a reduction in reproduction. Atlantic sturgeon spawn in the far upstream portions of rivers which are not accessible in this area due to dams blocking access, while the CHP45 occurs in harbor and nearshore waters. Changes in the distribution of Atlantic sturgeon are also not expected from lethal takes attributed to the proposed action. Because all of the potential interactions are expected to occur at random throughout the proposed action area and Atlantic sturgeon are known to disperse widely in the marine environment, the distribution of Atlantic sturgeon in the action area is expected to be unaffected.

Table 12. Estimated percentage of Atlantic Sturgeon taken from each DPS.

DPS	Lethal Take/DPS	Population	% of population
Gulf of Maine DPS	1	7455	0.01341%
New York Bight DPS	2	34566	0.00579%
Chesapeake Bay DPS	1	8811	0.01135%
Carolina DPS	2	1356	0.14749%
South Atlantic DPS	4	14911	0.02683%

We do not believe the reductions in numbers resulting from the proposed action are likely to reduce the population's ability to persist into the future. The loss of such small numbers of individuals will not significantly decrease the overall populations of the DPSs. Based on this

information, the proposed action will not appreciably reduce the likelihood of the 5 Atlantic sturgeon DPSs' survival within their ranges.

Because of the recent listing of the 5 DPSs of Atlantic sturgeon, a recovery plan for the species has not yet been developed. Recovery is the process by which listed species and their ecosystems are restored, and their future is safeguarded to the point that protections under the ESA are no longer needed. The first step in recovering a species is to reduce identified threats; only by alleviating threats can lasting recovery be achieved. An increase in the population to a size that maintains a steady recruitment of individuals representing all life stages would provide population stability and enable the population to sustain itself even in the event of unforeseen and unavoidable impacts. Major threats affecting the 5 Atlantic sturgeon DPSs were summarized in the final listing and include:

1) Dredging that can displace sturgeon while it is occurring and affect the quality of the habitat afterwards by changing the depth, sediment characteristics, and prey availability

The CHP45 project may temporarily displace sturgeon or affect the quality of habitat in the project area. However, the availability of similar available habitat in the marine environment is extensive and will provide sufficient habitat for individuals from any DPS that may be in the project area. Additionally, only adult or subadult sturgeon are expected to be in any areas affected by the CHP45 and as such are able to move throughout all habitat types without adverse impacts. While relocations of Atlantic sturgeon from each of the DPSs is expected to occur during the CHP45 project, mortality associated with the CHP45 is expected to be very low. The potential injury or mortality of 4 Atlantic sturgeon attributed to the proposed action are not likely to reduce population numbers over time due to current population sizes and expected recruitment. The use of relocation trawling will reduce the mortality of Atlantic sturgeon that occurs and increase the survival of Atlantic sturgeon that may be found in the action area for the CHP45. We therefore conclude the proposed action will not appreciably diminish the likelihood of recovery for any of the 5 DPSs of Atlantic sturgeon.

8 CONCLUSION

We have analyzed the best available data, the current status of the species, environmental baseline, effects of the proposed action, and cumulative effects to determine whether the proposed action is likely to jeopardize the continued existence of green, Kemp's ridley or the Northwest Atlantic (NWA) DPS of loggerhead sea turtles, or any DPS of Atlantic sturgeon.

8.1 Green, Kemp's Ridley, or Loggerhead (NWA DPS) Sea Turtles

Because the proposed action is not reasonably expected to reduce appreciably the likelihood of survival and recovery of loggerhead (NWA DPS), green, or Kemp's ridley sea turtles, it is our Opinion that the CHP45 is not likely to jeopardize their continued existence.

8.2 Atlantic Sturgeon

Because the proposed action is not reasonably expected to reduce appreciably the likelihood of survival and recovery of any DPS of Atlantic sturgeon, it is our Opinion that the CHP45 is not likely to jeopardize their continued existence.

9 INCIDENTAL TAKE STATEMENT

Section 9 of the ESA and federal regulation pursuant to Section 4(d) of the ESA prohibit take of endangered and threatened species, respectively, without special exemption. "Take" is defined as *to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture or collect, or to attempt to engage in any such conduct*. "Harm" is further defined by NMFS to include *significant habitat modification or degradation that results in death or injury to listed species by significantly impairing essential behavioral patterns, including breeding, feeding, or sheltering*. "Incidental take" is defined as *take that is incidental to, and not the purpose of, the carrying out of an otherwise lawful activity*. Under the terms of ESA Section 7(b)(4) and Section 7(o)(2), taking that is incidental to and not intended as part of the agency action is not considered to be prohibited taking provided that such taking is in compliance with the terms and conditions of this Incidental Take Statement.

9.1 Anticipated Amount or Extent of Incidental Take

Based on historical and recent distribution data of sea turtles and Atlantic sturgeon, hopper dredge observer reports, observations of past strandings, and increasing populations of green, Kemp's ridley, and loggerhead sea turtles in the action area, we estimate that these species may occur in the action area and may be taken by the hopper dredging operations of this project, by crushing and/or entrainment in suction dragheads. NMFS anticipates incidental observed take will consist of a total up to 8 loggerhead, 1 green, and 2 Kemp's ridley sea turtles and 2 Atlantic sturgeon with unobserved take consisting of the same. NMFS also anticipates that capture trawling may result in up to 235 non-injurious captures and relocations of an estimated (up to) 719 loggerheads, 3 greens, 18 Kemp's ridley sea turtles, and 23 Atlantic sturgeon.

9.2 Effect of the Take

NMFS has determined the anticipated level of incidental take specified in Section 9.1 is not likely to jeopardize the continued existence of loggerhead (NWA DPS), green, or Kemp's ridley sea turtles, or Atlantic sturgeon.

9.3 Reasonable and Prudent Measures

Section 7(b)(4) of the ESA requires NMFS to issue a statement specifying the impact of any incidental take on listed species, which results from an agency action otherwise found to comply with Section 7(a)(2) of the ESA. It also states the RPMs necessary to minimize the impacts of take and the terms and conditions to implement those measures, must be provided and must be followed to minimize those impacts. Only incidental taking by the federal agency that complies with the specified terms and conditions is authorized. 50 CFR 402.14(i)(2) also states that "[r]easonable and prudent measures, along with the terms and conditions that implement them, cannot alter the basic design, scope, duration, or timing of the action and may involve only minor changes."

The RPMs and terms and conditions are specified as required, by 50 CFR 402.14(i), to document the incidental take of ESA-listed species by the proposed action, to minimize the impact of that take, and to specify the procedures to be used to handle any individuals taken. These measures and terms and conditions are non-discretionary and must be implemented by the USACE in order for the protection of Section 7(o)(2) to apply. The USACE has a continuing duty to regulate the

activity covered by this incidental take statement. If the USACE fails to adhere to the terms and conditions through enforceable terms, and/or fails to retain oversight to ensure compliance with these terms and conditions, the protective coverage of Section 7(o)(2) may lapse.

Current regional Opinions for hopper dredging require observers to document takes, deflector draghead usage, and conditions and guidelines for relocation trawling, which NMFS believes are necessary to minimize effects dredging activities on listed sea turtle species that occur in the action area. NMFS has determined that the following RPMs, patterned after long-standing hopper dredging requirements, are necessary and appropriate to minimize impacts of the incidental take of sea turtles during the proposed action. The RPMs that NMFS believes are necessary to minimize and monitor the impacts of the proposed hopper dredging have been discussed with the USACE in the past and are standard operating procedures, including use of sea turtle deflector dragheads, intake and overflow screening, observer and reporting requirements, and relocation trawling. The following RPMs and associated terms and conditions are established to implement these measures, document incidental takes, and specify procedures for handling individuals taken. Only incidental takes that occur while these measures are in full implementation are authorized.

1. The USACE shall implement best management measures, including the use of date-based dredging windows, sea turtle deflector dragheads, intake and overflow screening, and relocation trawling to reduce the risk of injury or mortality of listed species and lessen the number of sea turtles killed by the proposed action.[29]

   Rationale: Date-based dredging windows appear to be very effective in reducing sea turtle entrainments, by avoiding times and places either where turtle densities are high or their behaviors may make them less susceptible to entrainment. Draghead deflectors provide a last line of defense, by acting as physical barriers, reducing the likelihood that turtles that are close to the draghead are actually entrained. When the suction dragheads are not firmly placed on the bottom during dredging operations, sea turtles encountered by the dragheads can be crushed underneath them and/or impinged or sucked into the suction pipes by the powerful suction, almost always resulting in death. Seasonally limiting dredge lights will help reduce potential disorientation effects on female sea turtles approaching the nesting beaches and sea turtle hatchlings making their way seaward from their natal beaches. Relocation (i.e., capture) trawling reduces the risk of turtle entrainment even when turtle densities are high, possibly by either temporarily reducing the local density of turtles in the channel where the dredge is working or by modifying the turtles' behavior temporarily and making them less susceptible to entrainment. In addition, the use of relocation trawling provides the USACE with valuable real-time estimates of sea turtle abundance, takes, and distribution which have been helpful to USACE project planning efforts to reduce sea turtle impacts, for example

[29] While the USACE has proposed relocation trawling activities to reduce the density of sea turtles in the path of hopper dredges, they have not stated when relocation trawling will be carried out or at what effort level. This necessitated NMFS making the decision for USACE via requirements of this biological opinion. In order to authorize take resulting from relocation trawling, NMFS must require this activity as a RPM and not as a conservation measure.

by delaying or changing the location of hopper dredge deployment in response to sea turtle density information in the channel.

2. The USACE shall have measures in place to detect and report all interactions with any protected species (ESA or Marine Mammal Protection Act) resulting from the proposed action. These measures include endangered species observers aboard the hopper dredge and relocation trawlers, screening of dredged material to allow discovery of any entrained turtles and sturgeon, and handling procedures for incidentally taken animals.

   Rationale: NMFS-approved observers monitor dredged material inflow and overflow screening baskets and relocation trawling efforts to detect and report incidental take. Gathering basic biological information (e.g., size, which will help determine the age class) will enable monitoring of the impact of the take on the species taken. Passive Integrated Transponder (PIT) tagging, external flipper tagging, and tissue and genetic sampling of dredge- and trawl-captured turtles and sturgeon will provide important information about the animals taken during project activities. Tagging will provide information about the fate of the turtles and Atlantic sturgeon relocated should they be recaptured or strand subsequent to being relocated. Tissue sampling will identify which sea turtle and Atlantic sturgeon stocks are being impacted and their geographic origin.

9.3.1 Terms and Conditions

"Reasonable and prudent measures" are nondiscretionary measures to minimize the amount or extent of incidental take (50 CFR 402.02). "Terms and conditions" (T&Cs) implement the reasonable and prudent measures (50 CFR 402.14). These terms and conditions must be carried out for the exemption in Section 7(o)(2) to apply.

In order to be exempt from the prohibitions of Section 9 of the ESA, the USACE must comply with the following terms and conditions, which implement the reasonable and prudent measures described above and outline required reporting and monitoring requirements. These T&Cs are non-discretionary.

1) Hopper Dredging (RPM 1): Hopper dredging is allowed in accordance with the SARBO dredging window, November 1 through May 31 or outside of this period only if water temperatures are below 11°C.
2) Non-hopper Type Dredging (RPM 1): Mechanical, pipeline or hydraulic dredges, because they are not known to take turtles, must be used whenever possible.
3) Observers (RPM 2): The USACE shall arrange for NMFS-approved protected species observers to be aboard the hopper dredges to monitor the hopper bin, screening, and dragheads for sea turtles, sturgeon, and their remains. Observer coverage sufficient for 100 percent monitoring (i.e., 2 observers) of hopper dredging operations is required during all hopper dredging.
4) Operational Procedures (RPM 1): During periods in which hopper dredges are operating and NMFS-approved protected species observers are *not* required (December 1 through March 31), the USACE must:

a) Advise inspectors, operators, and vessel captains about the prohibitions on taking, harming, or harassing sea turtles and sturgeon.

b) Instruct the captain of the hopper dredge to avoid any turtles or sturgeon encountered while traveling between the dredge site and offshore disposal area, and to immediately contact the USACE if they are seen in the vicinity.

c) Notify NMFS immediately by email (takereport.nmfsser@noaa.gov) if a sea turtle, sturgeon, or other threatened or protected species is taken by the dredge, and reference this Opinion (F/SER/2015/15433).

5) Dredging Pumps (RPM 1): Standard operating procedure shall be that dredging pumps shall be disengaged by the operator when the dragheads are not firmly on the bottom, to prevent impingement or entrainment of sea turtles within the water column. This precaution is especially important during the cleanup phase of dredging operations when the draghead frequently comes off the bottom and can suck in turtles resting in the shallow depressions between the high spots the draghead is trimming off.

6) Dredge Lighting (RPM 1): From May 1 through October 31, sea turtle nesting and emergence season, all lighting aboard hopper dredges and hopper dredge pumpout barges operating within 3 nmi of sea turtle nesting beaches shall be limited to the minimal lighting necessary to comply with U.S. Coast Guard and/or Occupational Safety and Health Administration requirements. All non-essential lighting on the dredge and pumpout barge shall be minimized through reduction, shielding, lowering, and appropriate placement of lights to minimize illumination of the water to reduce potential disorientation effects on female sea turtles approaching the nesting beaches and sea turtle hatchlings making their way seaward from their natal beaches.

7) Sea Turtle-Deflecting Draghead (RPM 1): A state-of-the-art solid-faced deflector that is attached to the draghead with chains and an adjustable leading chain at the apex of the deflector must be used on all hopper dredges at all times. The use of alternative, experimental dragheads is not authorized without prior written approval from NMFS, in consultation with the USACE Engineering Research and Development Center (ERDC). Slotted draghead deflectors are currently not authorized.

8) Training Personnel on Hopper Dredges (RPM 1): The USACE must ensure that all contracted personnel involved in operating hopper dredges (whether privately-funded or federally-funded projects) receive thorough training on measures of dredge operation that will minimize takes of sea turtles. It shall be the goal of the hopper dredging operation to establish operating procedures that are consistent with those that have been used successfully during hopper dredging in other regions of the coastal United States, and which have proven effective in reducing turtle/dredge interactions. Therefore, USACE ERDC experts or other persons with expertise in this matter shall be involved both in dredge operation training, and installation, adjustment, and monitoring of the rigid deflector draghead assembly.

9) Screening (RPM 2): When sea turtle or sturgeon observers are required on hopper dredges, 100% inflow screening of dredged material is required and 100% overflow screening is recommended. If conditions prevent 100% inflow screening, inflow

screening may be reduced gradually, as further detailed in the following, but 100% overflow screening is then required.

a) Screen Size: The hopper's inflow screens should have 4-in by 4-in screening. If the USACE, in consultation with observers and the draghead operator, determines that the draghead is clogging and reducing production substantially (other than in sand borrow areas), the screens may be modified sequentially. Mesh size may be increased to 8-in by 8-in; if that fails to solve the clogging problem, then 16-in by 16-in openings may be used. Clogging should be greatly reduced or eliminated with these options; however, further clogging may compel removal of the screening altogether, in which case effective 100% overflow monitoring and screening is mandatory. The USACE shall notify NMFS beforehand if inflow screening is going to be reduced or eliminated, what attempts were made to reduce the clogging problem, and provide details of how effective overflow screening will be achieved.

b) Need for Flexible, Graduated Screens: NMFS believes that this flexible, graduated-screen option is necessary, since the need to constantly clear the inflow screens will increase the time it takes to complete the project and therefore increase the exposure of sea turtles to the risk of impingement or entrainment. Additionally, there are increased risks to sea turtles in the water column when the inflow is halted to clear screens, because this results in clogged intake pipes, which may have to be lifted from the bottom to discharge the clay by applying suction.

10) Dredge Take Reporting and Final Report (RPM 2): Observer reports of incidental take by hopper dredges must be emailed to the Southeast Regional Office (takereport.nmfsser@noaa.gov with reference to this Opinion - F/SER/2015/15433) by onboard NMFS-approved protected species observers, the dredging company, or the USACE within 24 hours of any sea turtle, sturgeon, or other listed species take observed.

A final report summarizing the results of the hopper dredging and any documented sea turtle, sturgeon, or other listed species takes must be submitted to NMFS (takereport.nmfsser@noaa.gov with reference to this Opinion) within 60 working days of completion of the dredging project. The reports shall contain information on project location (specific channel/area dredged), start-up and completion dates, cubic yards of material dredged, problems encountered, incidental takes and sightings of protected species, mitigative actions taken (if relocation trawling, the number and species of turtles relocated), screening type (inflow, overflow) utilized, daily water temperatures, name of dredge, names of endangered species observers, percent observer coverage, and any other information the USACE deems relevant.

11) Sea Turtle Strandings (RPM 2): The USACE Project Manager or designated representative shall notify the STSSN state representative (contact information available at: http://www.sefsc.noaa.gov/seaturtleSTSSN.jsp) of the start-up and completion of hopper dredging operations and bed-leveler dredging operations and ask to be notified of any sea turtle strandings in the project area that, in the estimation of STSSN personnel, bear signs of potential draghead impingement or entrainment, or interaction with a bed-leveling type dredge.

    Information on any such strandings shall be reported in writing within 30 days of project end to NMFS's Southeast Regional Office (takereport.nmfsser@noaa.gov with reference

to this Opinion) with a report detailing incidents, with photographs when available, of stranded sea turtles that bear indications of draghead impingement or entrainment. Because the deaths of turtles, if hopper dredge related, have already been accounted for in NMFS's jeopardy analysis as turtles not observed being taken during hopper dredging operations, these strandings will not be counted against the USACE's take limit. NMFS and the USACE will use these stranding reports to assess whether they suggest a greater extent of effects than predicted in this Opinion.

12) Conditions Requiring Relocation Trawling (RPM 1): The USACE shall require relocation trawling to start as soon as possible within 72 hours if either:

    a) 2 or more turtles are taken by hopper dredges in a 24-hour period, or

    b) Total dredge takes in the project approach 75% (rounded-down) of any of the incidental take limits from Section 9.1 of this Opinion.

    Relocation trawling may be suspended if no relocation or dredge takes occur within 14 days unless take limits for any species have been reached.

13) Closed-net Relocation Trawling (RPM 1): Any relocation trawling conducted or contracted by the USACE to temporarily reduce abundance of these listed species during hopper dredging in order to reduce the possibility of lethal hopper dredge interactions, is subject to the following conditions:

    a) The net must be closed at all times during trawling.

    b) Trawl Time: Trawl tow-time duration shall not exceed 42 minutes (measured from the time the trawl doors enter the water until the time the trawl doors are out of the water) and trawl speeds shall not exceed 3.5 knots.

    c) Protected Species Handling During Trawling: Handling of sea turtles and sturgeon captured during relocation trawling in association with the dredging project shall be conducted by NMFS-approved protected species observers. Sea turtles and sturgeon captured pursuant to relocation trawling shall be handled in a manner designed to ensure their safety and viability, and shall be released over the side of the vessel, away from the propeller, and only after ensuring that the vessel's propeller is in the neutral, or disengaged, position (i.e., not rotating). Sea Turtle Research Techniques Manual is attached (Appendix A) http://www.sefsc.noaa.gov/turtles/TM_579_SEFSC_STRTM.pdf. Any handling of Atlantic sturgeon captured in the relocation trawling will comply with the attached NMFS's *Protocol for Use of Shortnose, Atlantic, Gulf, and Green Sturgeons* (Attachment B) http://www.nmfs.noaa.gov/pr/pdfs/species/kahn_mohead_2010.pdf.

    d) Captured Sea Turtle Holding Conditions: Sea turtles may be held briefly for the collection of important biological information, prior to their release. Captured sea turtles shall be kept moist, and shaded whenever possible, until they are released, according to the requirements of T&C No. 13-e, below.

    e) Biological Data Collection: When safely possible, all sea turtles and Atlantic sturgeon shall be measured, tagged, weighed, and a tissue sample taken prior to release. Any external tags shall be noted and data recorded into the observers' log and take forms. Only NMFS-approved protected species observers or observer candidates in training

under the direct supervision of a NMFS-approved protected species observer shall conduct the tagging/measuring/weighing/tissues sampling operations. Tissue samples will be sent to NMFS for processing and analysis.

For sea turtles, collect tissue samples following the protocols above, the remaining specimen(s) or body parts of dead sea turtles must be preserved (preferably iced or refrigerated or frozen if necessary) until sampling and disposal procedures are discussed with the NMFS contact identified below. If it is not possible to retain the carcass, please scan the carcass for PIT tags and flipper tags, collect a tissue sample, and photograph the animal. Mark the carcass, if possible, and dispose of carcass near original site of capture.

Dr. Brian Stacy
NOAA/NMFS and University of Florida
2187 Mowry Road, Building 471
Gainesville, FL 32611
Brian.Stacy@noaa.gov
PH: 352-283-3370

For sturgeon, mark the carcass (in order to identify in the case of recapture), if possible, and dispose of carcass near original site of capture. Send samples, copy of Protected Species Incidental Take Form and supporting data within 1 month of the date the sample is taken.

Tim King in West Virginia
USGS Leetown Science Center, Aquatic Ecology Branch
11649 Leetown Road
Kearneysville, WV 25430
PH: 304-724-4450

f) Take and Release Time During Trawling – Turtles: Turtles shall be kept no longer than 3 hours prior to release and shall be released not less than 3 nmi from the dredge site. If 2 or more released turtles are later recaptured, subsequent turtle captures shall be released not less than 5 nmi away. If it can be done safely, turtles may be transferred onto another vessel for transport to the release area to enable the relocation trawler to keep sweeping the dredge site without interruption. The 3 hour holding time may be extended up to 24 hours only for sea turtles that require monitoring after resuscitation.

g) Injuries: Injured sea turtles shall be immediately transported to the nearest sea turtle rehabilitation facility. Minor skin abrasions resulting from trawl capture are considered non-injurious. The USACE shall ensure that logistical arrangements and support to accomplish this transport are pre-planned and ready. The USACE shall bear the financial cost of any subsequent treatment, rehabilitation, and release if the observer or State Sea Turtle Coordinator determines that the injuries were caused by the project.

h) Flipper Tagging: All sea turtles captured by relocation trawling shall be flipper-tagged prior to release with external tags which shall be obtained prior to the project from the University of Florida's Archie Carr Center for Sea Turtle Research. This Opinion serves as the permitting authority for any NMFS-approved protected species observer aboard these relocation trawlers to flipper-tag with external tags (e.g., Inconel tags) captured sea turtles. Columbus crabs or other organisms living on external sea turtle surfaces may also be sampled and removed under this Opinion's authority.

i) PIT-Tag: This Opinion serves as the permitting authority for any NMFS-approved protected species observer aboard a relocation trawler to PIT-tag captured sea turtles and sturgeon. Tagging of sea turtles and sturgeon is not required to be done if the NMFS-approved protected species observer does not have prior training or experience in said activity; however, if the observer has received prior training in PIT tagging procedures, then the observer shall tag the animal prior to release (in addition to the standard external tagging):

   i) Sea turtle PIT tagging must be performed in accordance with the protocol detailed in Appendix A.

   ii) PIT tags used must be sterile, individually-wrapped tags to prevent disease transmission. PIT tags should be 125-kHz, glass-encapsulated tags–the smallest ones made. Note: If scanning reveals a PIT tag and it was not difficult to find, then do not insert another PIT tag; simply record the tag number and location, and frequency, if known. If for some reason the tag is difficult to detect (e.g., tag is embedded deep in muscle, or is a 400-kHz tag), then insert one in the other shoulder.

   iii) All sturgeon handled shall be scanned for a PIT tag; codes shall be included in the take report submitted to NMFS. The PIT tag reader shall be able to read both 125 kHz and 134 kHz tags. Sturgeon without PIT tags will have one installed per guidance in Attachment B. Previously PIT-tagged fish must not be re-tagged.

   iv) All unmarked sturgeon less than 300 mm in total length would be tagged using 11.9 mm x 2.1 mm PIT tags injected using a 12-gauge needle at an angle of 60º to 80º in the dorsal musculature (left and just anterior to the dorsal fin) with the copper antenna oriented up for maximum signal strength. No fish would be double-tagged with PIT tags. The last step after injecting PIT tags would be to verify and record the PIT tag code with a tag reader. PIT tags may also be inserted under scutes after discussing with NMFS.

j) Sea Turtle PIT-Tag Scanning and Data Submission Requirements: All sea turtles captured by relocation trawling or dredges shall be thoroughly scanned for the presence of PIT tags prior to release using a multi-frequency scanner powerful enough to read multiple frequencies (including 125-, 128-, 134-, and 400-kHz tags) and read tags deeply embedded in muscle tissue (e.g., manufactured by Trovan, Biomark, or Avid). Turtles whose scans show they have been previously PIT tagged shall nevertheless be externally flipper tagged. Sea turtle data collected (PIT tag scan data and external tagging data) shall be submitted to NOAA, National Marine Fisheries Service, Southeast Fisheries Science Center, Attn: Lisa Belskis, 75 Virginia

Beach Drive, Miami, Florida 33149. All sea turtle data collected shall be submitted in electronic format within 60 days of project completion to Lisa.Belskis@noaa.gov. Sea turtle external flipper tag and PIT tag data generated and collected by relocation trawlers shall also be submitted to the Cooperative Marine Turtle Tagging Program (CMTTP), on the appropriate CMTTP form, at the University of Florida's Archie Carr Center for Sea Turtle Research.

k) Handling Fibropapillomatose Turtles: NMFS-approved protected species observers are not required to handle viral fibropapilloma tumors if they believe there is a health hazard to themselves and choose not to. When handling sea turtles infected with fibropapilloma tumors, observers must maintain a separate set of sampling equipment for handling animals displaying fibropapilloma tumors or lesions.

l) Additional Data Collection Allowed During the Handling of Sea Turtles, Sturgeon, and Other Incidentally Caught ESA-listed Species: The USACE shall allow NMFS-approved protected species observers to conduct additional investigations that may include more invasive procedures (e.g., blood-letting, laparoscopies, external tumor removals, anal and gastric lavages, mounting satellite or radio transmitters) and partake in or assist in research projects but only if (1) the additional work does not interfere with any project operations (e.g., dredging activities, relocation trawling); (2) the observer holds a valid federal research permit (and any required state permits) authorizing the activities, either as the permit holder, or as designated agent of the permit holder; (3) the additional work does not incur any additional expenses to the USACE or the USACE approves of the expense; and (4) the observer has first coordinated with USACE Charleston District and notified NMFS's Southeast Regional Office, Protected Resources Division. Limitations are as follows:

i) Leatherback sea turtles cannot be retained and should be returned to the water as soon as possible.

ii) In instances of hardshell sea turtle capture, observers or may retain incidentally captured animals for species research projects if the person(s) conducting the research, is on board or nearby, and holds a valid federal research permit (and any required state permits) authorizing the activities, either as the permit holder, or as designated agent of the permit holder. All additional procedures performed on retained animals must be authorized through the research permit(s). Collaborative research activities must begin within 1 hour of capture and the animal should be returned to the water within 5 hours (of time of capture). If required, animals may be held on board for up 12 hours provided that conditions during holding meet all research permit requirements and safe handling practices are followed. If research does not commence within 1 hour, the animal must be returned to the water. The intent of this provision is to minimize impacts to sea turtles by allowing, where appropriate, incidentally captured sea turtles to be used as research subjects. This reduces the need for additional animals to undergo the stress of capture associated with permitted scientific research.

14) Relocation Trawling Report (RPM 2): The USACE shall provide NMFS's Southeast Regional Office (takereport.nmfsser@noaa.gov with reference to this Opinion) with an end-of-project report within 30 days of completion of any relocation trawling. This

report may be incorporated into the final report summarizing the results of the hopper dredging project.

10 CONSERVATION RECOMMENDATIONS

Section 7(a)(1) of the ESA directs federal agencies to, in consultation with the Services, use their authorities to further the purposes of the ESA by carrying out conservation programs for the benefit of the threatened and endangered species. Conservation recommendations identified in Opinions can assist action agencies in implementing their responsibilities under Section 7(a)(1). Conservation recommendations are discretionary activities designed to minimize or avoid adverse effects of a proposed action on listed species or critical habitat, to help implement recovery plans, or to develop information. The following conservation recommendation(s) is (are) (a) discretionary measure(s) that NMFS believes is (are) consistent with this obligation and therefore should be carried out by the federal action agency:

Please notify NMFS if the federal action agency carries out any of these recommendations so that we will be kept informed of actions that are intended to improve the conservation of listed species or their designated critical habitats.

1. Draghead Modifications and Bed-Leveling Studies: The USACE should supplement other efforts to develop modifications to existing dredges to reduce or eliminate take of sea turtles and Atlantic sturgeon, and develop methods to minimize take of these species during "cleanup" operations when the draghead maintains only intermittent contact with the bottom. Some method to level the "peaks and valleys" created by dredging would reduce the amount of time dragheads are off the bottom. NMFS is ready to assist the USACE in conducting studies to evaluate bed-leveling devices and their potential for interaction with sea turtles and sturgeon, and develop modifications if needed.

2. Draghead Evaluation Studies and Protocol: Additional research, development, and improved performance is needed before the V-shaped rigid deflector draghead can replace seasonal restrictions as a method of reducing sea turtle and sturgeon captures during hopper dredging activities. Development of a more effective deflector draghead or other entrainment-deterring device (or combination of devices, including use of acoustic deterrents) could potentially reduce the need for sea turtle and sturgeon relocation or result in expansion of the preferred winter dredging window. NMFS should be consulted regarding the development of a protocol for draghead evaluation tests. NMFS recommends that USACE coordinate with ERDC, the Association of Dredge Contractors of America, and dredge operators (Manson, Bean-Stuyvesant, Great Lakes, Natco, etc.) regarding additional reasonable measures they may take to further reduce the likelihood of sea turtle and sturgeon takes.

3. Continuous Improvements in Monitoring and Detecting Takes: The USACE should seek continuous improvements in detecting takes and should determine, through research and development, a better method for monitoring and estimating sea turtle and Atlantic sturgeon takes by hopper dredge. Observation of overflow and inflow screening is only partially effective and provides only partial estimates of total sea turtle and sturgeon mortality.

4. Overflow Screening: The USACE should encourage dredging companies to develop or modify existing overflow screening methods on their company's dredge vessels for maximum effectiveness of screening and monitoring. Horizontal overflow screening is preferable to vertical overflow screening because NMFS considers that horizontal overflow screening is significantly more effective at detecting evidence of protected species entrainment than vertical overflow screening.

5. Preferential Consideration for Horizontal Overflow Screening: The USACE should give preferential consideration to hopper dredges with horizontal overflow screening when awarding hopper dredging contracts for areas where new materials, large amounts of debris, or clay may be encountered, or have historically been encountered. Excessive inflow screen clogging may, in some instances, necessitate removal of inflow screening, at which point, effective overflow screening becomes more important.

6. Section 10 Research Permits, Relocation Trawling, Piggy-Back Research, and 50 CFR Part 223 Authority to Conduct Research on Salvaged, Dead Specimens: NMFS recommends that USACE ERDC apply to NMFS for an ESA Section 10 research permit to conduct additional endangered species research on species incidentally captured during traditional relocation trawling. SERO shall assist the USACE with the permit application process.

   NMFS also encourages the USACE to cooperate with NMFS scientists, other federal agencies' scientists, and university scientists holding appropriate research permits to make more use of sea turtles and Atlantic sturgeon taken or captured by hopper dredges and relocation trawlers pursuant to the authority conferred by this Opinion. NMFS encourages "piggy-back" research projects by duly-permitted or authorized individuals or their authorized designees.

   Important research can be conducted without a Section 10 permit on salvaged dead specimens. Under current federal regulations (see 50 CFR 223.206 (b): Exception for injured, dead, or stranded [threatened sea turtle] specimens), "Agents…of a Federal land or water management agency may…salvage a dead specimen which may be useful for scientific study." Similar regulations at 50 CFR 222.310 provide "salvaging" authority for endangered sea turtles.

7. Draghead Improvements - Water Ports: NMFS recommends that the USACE require, or at least recommend, that dredge operators have all dragheads on hopper dredges contracted by the USACE for dredging projects outfitted (eventually) with water ports located in the top of the dragheads to help prevent the dragheads from becoming plugged with sediments. When the dragheads become plugged with sediments, the dragheads are often raised off the bottom by the dredge operator with the suction pumps on in order to take in enough water to help clear clogs in the dragarm pipeline, which increases the likelihood that sea turtles in the vicinity of the draghead will be taken by the dredge. Water ports located in the top of the dragheads would relieve the necessity of raising the draghead off the bottom to perform such an action, and reduce the chance of incidental take of sea turtles.

NMFS supports and recommends the implementation of proposals by ERDC and USACE personnel for various draghead modifications to address scenarios where turtles may be entrained during hopper dredging (Dickerson and Clausner 2003). These proposals include: (1) an adjustable visor; (2) water jets for flaps to prevent plugging and thus reduce the requirement to lift the draghead off the bottom; and (3) a valve arrangement (which mimics the function of a "Hoffer" valve used on cutterhead type dredges to allow additional water to be brought in when the suction line is plugging) that will provide a very large amount of water into the suction pipe, and thereby significantly reduce flow through the visor when the draghead is lifted off the bottom, reducing the potential to take a turtle.

8. Economic Incentives for No Protected Species Takes: The USACE should consider devising and implementing some method of significant economic incentives to hopper dredge operators such as financial reimbursement based on their satisfactory completion of dredging operations, or X number of cubic yards of material moved, or hours of dredging performed, without taking sea turtles or sturgeon. This may encourage dredging companies to research and develop "species friendly" dredging methods, such as more effective deflector dragheads; pre-deflectors; top-located water ports on drag arms, etc.

9. Sodium Vapor Lights on Offshore Equipment: On offshore equipment (i.e., hopper dredges, pumpout barges), shielded low-pressure sodium vapor lights are highly recommended for lights that cannot be eliminated when the vessels are operating with 10 mi of sea turtle nesting beaches.

11 REINITIATION OF CONSULTATION

As provided in 50 CFR 402.16, reinitiation of formal consultation is required where discretionary federal action agency involvement or control over the action has been retained, or is authorized by law, and if (1) the amount or extent of incidental take is exceeded, (2) new information reveals effects of the agency action on listed species or designated critical habitat in a manner or to an extent not considered in this Opinion, (3) the agency action is subsequently modified in a manner that causes an effect on the listed species or critical habitat not considered in this Opinion (e.g., modifications to vessel speed restrictions, using explosives for blasting or using disposal areas not considered in this Opinion), or (4) a new species is listed or critical habitat designated that may be affected by the action.

12 LITERATURE CITED

Ackerman, R. A. 1997. The nest environment and the embryonic development of sea turtles. Pages 83-106 *in* P. L. Lutz, and J. A. Musick, editors. The Biology of Sea Turtles. CRC Press, Boca Raton.

Addison, D. S. 1997. Sea turtle nesting on Cay Sal, Bahamas, recorded June 2-4, 1996. Bahamas Journal of Science 5:34-35.

Addison, D. S., and B. Morford. 1996. Sea turtle nesting activity on the Cay Sal Bank, Bahamas. Bahamas Journal of Science 3:31-36.

Aguilar, R., J. Mas, and X. Pastor. 1994. Impact of Spanish swordfish longline fisheries on the loggerhead sea turtle *Caretta caretta* population in the western Mediterranean. Pages 91-96 *in* J. I. Richardson, and T. H. Richardson, editors. Proceedings of the 12th Annual Workshop on Sea Turtle Biology and Conservation. U.S. Department of Commerce, Jekyll Island, Georgia.

Aguirre, A. A., G. H. Balazs, T. R. Spraker, S. K. K. Murakawa, and B. Zimmerman. 2002. Pathology of Oropharyngeal Fibropapillomatosis in Green Turtles *Chelonia mydas*. Journal of Aquatic Animal Health 14(4):298-304.

Antonelis, G. A., J. D. Baker, T. C. Johanos, R. C. Braun, and A. L. Harting. 2006. Hawaiian monk seal (*Monachus schauinslandi*): status and conservation issues. Atoll Research Bulletin 543:75-101.

Arendt, M., and coauthors. 2009. Examination of local movement and migratory behavior of sea turtles during spring and summer along the Atlantic Coast off the Southeastern United States. South Carolina Department of Natural Resources.

Arendt, M., and coauthors. 2015. Temporal and spatial distribution of adult Atlantic sturgeon (Acipenser oxyrinchus) in U.S. Territory waters off South Carolina and Georgia. Grant Performance Semiannual Report: 1 July 2014 to 31 December 2014. Gant Number: NA13NMF4720045. South Carolina Department of Natural Resources.

Arendt, M. D., and coauthors. 2012. Catch rates and demographics of loggerhead sea turtles (*Caretta caretta*) captured from the Charleston, South Carolina, shipping channel during the period of mandatory use of turtle excluder devices (TEDs). Fisheries Bulletin 110:98-109.

Armstrong, J. L., and J. E. Hightower. 2002. Potential for restoration of the Roanoke River population of Atlantic sturgeon. Journal of Applied Ichthyology 18(4-6):475-480.

ASMFC. 1998. American shad and Atlantic sturgeon stock assessment peer review: Terms of reference and advisory report. Atlantic States Marine Fisheries Commission, Washington D.C.

ASMFC. 2007. Special Report to the Atlantic Sturgeon Management Board: Estimation of Atlantic sturgeon bycatch in coastal Atlantic commercial fisheries of New England and the Mid-Atlantic.

ASMFC. 2010. Atlantic States Marine Fisheries Commission Annual Report.

ASSRT. 2007. Status Review of Atlantic Sturgeon (*Acipenser oxyrinchus oxyrinchus*). National Marine Fisheries Service, Northeast Regional Office by Atlantic Sturgeon Status Review Team.

ASSRT, and NMFS. 2007. Status Review of Atlantic Sturgeon (Acipenser oxyrinchus oxyrinchus).

Bain, M., N. Haley, D. Peterson, J. R. Waldman, and K. Arend. 2000. Harvest and habitats of Atlantic sturgeon *Acipenser oxyrinchus* Mitchill, 1815 in the Hudson River estuary: lessons for sturgeon conservation. Boletín. Instituto Español de Oceanografía 16:43-53.

Bain, M. B. 1997. Atlantic and shortnose sturgeons of the Hudson River: common and divergent life history attributes. Environmental Biology of Fishes 48(1):347-358.

Baker, J. D., C. L. Littnan, and D. W. Johnston. 2006. Potential effects of sea level rise on the terrestrial habitats of endangered and endemic megafauna in the Northwestern Hawaiian Islands. Endangered Species Research 2:21-30.

Balazik, M. T., G. C. Garman, J. P. Van Eenennaam, J. Mohler, and L. C. Woods. 2012. Empirical Evidence of Fall Spawning by Atlantic Sturgeon in the James River, Virginia. Transactions of the American Fisheries Society 141(6):1465-1471.

Balazs, G. 1982. Growth rates of immature green turtles in the Hawaiian Archipelago. Pages 117-125 *in* K. A. Bjorndal, editor. Biology and Conservation of Sea Turtles. Smithsonian Institution Press, Washington D.C.

Balazs, G. H. 1983. Recovery records of adult green turtles observed or originally tagged at French Frigate Shoals, northwestern Hawaiian Islands. NMFS, Washington, D.C.; Springfield, VA.

Belovsky, G. E. 1987. Extinction models and mammalian persistence. Chapter 3 In: Soulé, M.E. (ed), Viable Populations for Conservation. Cambridge University Press, pp.35-57.

Berry, R. J. 1971. Conservation aspects of the genetical constitution of populations. Pages 177-206 *in* E. D. Duffey, and A. S. Watt, editors. The Scientific Management of Animal and Plant Communities for Conservation, Blackwell, Oxford.

Bjorndal, K. A. 1982. The consequences of herbivory for the life history pattern of the Caribbean green turtle, *Chelonia mydas*. Pages 111-116 *in* K. A. Bjorndal, editor. Biology and Conservation of Sea Turtles. Smithsonian Institution Press, Washington, D.C.

Bjorndal, K. A., A. B. Bolten, and M. Y. Chaloupka. 2005. Evaluating trends in abundance of immature green turtles, *Chelonia mydas*, in the Greater Caribbean. Ecological Applications 15(1):304-314.

Bjorndal, K. A., A. B. Bolten, T. Dellinger, C. Delgado, and H. R. Martins. 2003. Compensatory growth in oceanic loggerhead sea turtles: Response to a stochastic environment. Ecology 84(5):1237-1249.

Bjorndal, K. A., J. A. Wetherall, A. B. Bolten, and J. A. Mortimer. 1999. Twenty-Six Years of Green Turtle Nesting at Tortuguero, Costa Rica: An Encouraging Trend. Conservation Biology 13(1):126-134.

Bolten, A. B., K. A. Bjorndal, and H. R. Martins. 1994. Life history model for the loggerhead sea turtle (Caretta caretta) populations in the Atlantic: Potential impacts of a longline fishery. U.S. Department of Commerce.

Bolten, A. B., and coauthors. 1998. Transatlantic developmental migrations of loggerhead sea turtles demonstrated by mtDNA sequence analysis. Ecological Applications 8:1-7.

Bolten, A. B., and B. E. Witherington. 2003. Loggerhead sea turtles. Smithsonian Books, Washington, D.C.

Bonsdorff, E. 1980. Macrozoobenthic recolonization of dredged brackish water bay in SW Finland. Ophelia Supplement 1:145-155.

Boreman, J. 1997. Sensitivity of North American sturgeons and paddlefish to fishing mortality. Environmental Biology of Fishes 48(1):399-405.

Borodin, N. 1925. Biological Observations on the Atlantic Sturgeon (Acipenser sturio). Transactions of the American Fisheries Society 55(1):184-190.

Bouchard, S., and coauthors. 1998. Effects of Exposed Pilings on Sea Turtle Nesting Activity at Melbourne Beach, Florida. Journal of Coastal Research 14:1343-1347.

Bowen, B. W., and coauthors. 1992. Global Population Structure and Natural History of the Green Turtle (*Chelonia mydas*) in Terms of Matriarchal Phylogeny. Evolution 46:865-881.

Bresette, M., D. Singewald, and E. De Maye. 2006. Recruitment of post-pelagic green turtles (*Chelonia mydas*) to nearshore reefs on Florida's east coast. Pages 288 *in* M. Frick, A. Panagopoulou, A. F. Rees, and K. Williams, editors. Twenty-sixth Annual Symposium on Sea Turtle Biology and Conservation. International Sea Turtle Society, Athens, Greece.

Brundage, H. M., and J. C. O. Herron. 2003. Population estimate for shortnose sturgeon in the Delaware River. Presented at the 2003 Shortnose Sturgeon Conference, 7-9 July 2003.

Bushnoe, T., J. Musick, and D. Ha. 2005. Essential spawning and nursery habitat of Atlantic sturgeon (*Acipenser oxyrinchus*) in Virginia. Virginia Institute of Marine Science, Gloucester Point, Virigina.

Caldwell, D. K., and A. Carr. 1957. Status of the sea turtle fishery in Florida. Pages 457-463 *in* Transactions of the 22nd North American Wildlife Conference.

Campbell, C. L., and C. J. Lagueux. 2005. Survival Probability Estimates for Large Juvenile and Adult Green Turtles (*Chelonia mydas*) Exposed to an Artisanal Marine Turtle Fishery in the Western Caribbean. Herpetologica 61(2):91-103.

Carballo, A. Y., C. Olabarria, and T. Garza Osuna. 2002. Analysis of four macroalgal assemblages along the Pacific Mexican coast during and after the 1997-98 El Niño. Ecosystems 5(8):749-760.

Caron, F., D. Hatin, and R. Fortin. 2002. Biological characteristics of adult Atlantic sturgeon (*Acipenser oxyrinchus*) in the St Lawrence River estuary and the effectiveness of management rules. Journal of Applied Ichthyology 18(4-6):580-585.

Carr, A. 1984. So Excellent a Fishe. Charles Scribner's Sons, New York.

Carr, A. 1986. New perspectives on the pelagic stage of sea turtle development. National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southeast Fisheries Center, Panama City Laboratory, Panama City, FL.

Chaloupka, M., and C. Limpus. 2005. Estimates of sex- and age-class-specific survival probabilities for a southern Great Barrier Reef green sea turtle population. Marine Biology 146(6):1251-1261.

Chaloupka, M., T. M. Work, G. H. Balazs, S. K. K. Murakawa, and R. Morris. 2008. Cause-specific temporal and spatial trends in green sea turtle strandings in the Hawaiian Archipelago (1982-2003). Marine Biology 154:887-898.

Chaloupka, M. Y., and J. A. Musick. 1997. Age, growth, and population dynamics. Pages 233-276 *in* P. L. Lutz, and J. A. Musick, editors. The Biology of Sea Turtles. CRC Press, Boca Raton.

Chytalo, K. 1996. Summary of Long Island Sound dredging windows strategy workshop. Management of Atlantic Coastal Marine Fish Habitat: Proceedings of a workshop for habitat managers. ASMFC Habitat Management Series #2.

Cochnauer, T. 1986. Abundance, distribution, growth and management of white sturgeon (Acipenser transmontanus) in the Middle Snake River, Idaho. University of Idaho.

Collette, B., and G. Klein-MacPhee. 2002. Fishes of the Gulf of Maine, 3rd edition. Smithsonian Institution Press.

Collins, M. R., S. G. Rogers, and T. I. J. Smith. 1996. Bycatch of Sturgeons along the Southern Atlantic Coast of the USA. North American Journal of Fisheries Management 16:24-29.

Collins, M. R., S. G. Rogers, T. I. J. Smith, and M. L. Moser. 2000a. Primary factors affecting sturgeon populations in the southeastern United States: fishing mortality and degradation of essential habitats. Bulletin of Marine Science 66(3):917-928.

Collins, M. R., and T. I. J. Smith. 1997. Management Briefs: Distributions of Shortnose and Atlantic Sturgeons in South Carolina. North American Journal of Fisheries Management 17(4):995-1000.

Collins, M. R., T. I. J. Smith, W. C. Post, and O. Pashuk. 2000e. Habitat Utilization and Biological Characteristics of Adult Atlantic Sturgeon in Two South Carolina Rivers. Transactions of the American Fisheries Society 129(4):982-988.

Conant, T. A., and coauthors. 2009. Loggerhead sea turtle (*Caretta caretta*) 2009 status review under the U.S. Endangered Species Act. Report of the Loggerhead Biological Review Team to the National Marine Fisheries Service, August 2009.

Cooke, D. W., J. P. Kirk, J. V. Morrow Jr, and S. D. Leach. 2004. Population dynamics of a migration limited shortnose sturgeon population. Pages 82-91 *in* Proceedings of Annual Conference of Southeastern Association for Fish and Wildlife Agencies.

Cooke, D. W., and S. D. Leach. 1999. Santee-Cooper blueback herring studies. Annual Report, SCR 1-22. South Carolina Department of Natural Resources, Columbia, South Carolina. .

Cooke, D. W., and S. D. Leach. 2004. Implications of a migration impediment on shortnose sturgeon spawning. North American Journal of Fisheries Management 24(4):1460-1468.

Crance, J. H. 1987. Habitat suitability index curves for anadromous fishes. In: Common strategies of anadromous and catadromous fishes: proceedings of an International Symposium held in Boston, Massachusetts, USA, March 9-13, 1986. Pages 554 *in* M. J. Dadswell, editor. American Fisheries Society, Bethesda, Maryland.

Crouse, D. T. 1999. Population Modeling and Implications for Caribbean Hawksbill Sea Turtle Management Chelonian Conservation and Biology 3(2):185-188.

D'Ilio, S., D. Mattei, M. F. Blasi, A. Alimonti, and S. Bogialli. 2011. The occurrence of chemical elements and POPs in loggerhead turtles (*Caretta caretta*): an overview. Marine Pollution Bulletin 62(8):1606-1615.

Dadswell, M. J. 1979. Biology and population characteristics of the shortnose sturgeon Acipenser brevirostrum LeSueur 1818 (Osteichthyes:Acipenseridae), in the Saint John River Estuary, New Brunswick, Canada. Can J Zool 57(11):2186-2210.

Dadswell, M. J. 2006. A review of the status of Atlantic sturgeon in Canada, with comparisons to populations in the United States and Europe. Fisheries 31(5):218-229.

Daniels, R., T. White, and K. Chapman. 1993. Sea-level rise: Destruction of threatened and endangered species habitat in South Carolina. Environmental Management 17(3):373-385.

Davis, K. B., G. S. Anderson, and A. M. Durel. 1990. A physical and ecological characterization of the Charleston Harbor Estuarine System. Final Report to South Carolina Coastal Council, Charleston, SC. R. Van Dolah, P. H. Wendt, and E. L. Wenner, editors. Hydrography, Charleston, SC.

Dickerson, D., M. Wolters, and C. Theriot. 2007. Commitments of the Corps of Engineers: Navigation, dredging, and sea turtles. Pages 191 *in* Twenty-Fourth Annual Symposium on Sea Turtle Biology and Conservation.

Dickerson, D. D., and J. E. Clausner. 2003. Draft: Summary of Sea Turtle/Dredging Issues and Recommended Action Tasks Generated by the Improved Draghead Design Meeting, September 4, 2003, Atlanta, Georgia. U.S. Army Corps of Engineers, Engineering Research and Development Center, Vicksburg, Mississippi.

Dodd, C. K. 1988. Synopsis of the biological data on the loggerhead sea turtle: *Caretta caretta* (Linnaeus, 1758). U.S. Fish and Wildlife Service, U.S. Department of the Interior, Washington, D.C.

Doughty, R. W. 1984. Sea turtles in Texas: a forgotten commerce. Southwestern Historical Quarterly 88:43-70.

Dovel, W. L., and T. J. Berggren. 1983. Atlantic sturgeon of the Hudson River estuary, New York. New York. Fish and Game Journal 30:140-172.

Dovel, W. L., A. W. Pekovitch, and T. J. Berggren. 1992. Biology of the shortnose sturgeon (Acipenser brevirostrum Lesueur, 1818) in the Hudson River estuary, New York. Pages 187-216 *in* C. L. Smith, editor. In: Estuarine Research in the 1980s. State University of New York Press, Albany, New York.

Dow, W., K. Eckert, M. Palmer, and P. Kramer. 2007. An Atlas of Sea Turtle Nesting Habitat for the Wider Caribbean Region. The Wider Caribbean Sea Turtle Conservation Network and The Nature Conservancy, Beaufort, North Carolina.

Duncan, M. S., J. J. Isely, and D. W. Cooke. 2004. Evaluation of shortnose sturgeon spawning in the Pinopolis Dam tailrace, South Carolina. North American Journal of Fisheries Management 24(3):932-938.

Dutton, P. H., and coauthors. 2008. Composition of Hawaiian green turtle foraging aggregations: mtDNA evidence for a distinct regional population. Endangered Species Research 5:37-44.

Ehrhart, L. M. 1983. Marine Turtles of the Indian River Lagoon System. Florida Scientist 46:334-346.

Ehrhart, L. M., W. E. Redfoot, and D. Bagley. 2007. Marine turtles of the central region of the Indian River Lagoon system. Florida Scientist 70(4):415-434.

Ehrhart, L. M., and R. G. Yoder. 1978. Marine turtles of Merritt Island National Wildlife Refuge, Kennedy Space Center, Florida. Pages 25-30 *in* G. E. Henderson, editor Proceedings of the Florida and Interregional Conference on Sea Turtles. Florida Marine Research Publications.

Epperly, S. P., J. Braun-McNeill, and P. M. Richards. 2007. Trends in the catch rates of sea turtles in North Carolina, U.S.A. Endangered Species Research 3:283-293.

Fish, M. R., and coauthors. 2005. Predicting the Impact of Sea-Level Rise on Caribbean Sea Turtle Nesting Habitat. Conservation Biology 19(2):482-491.

Fisher, M. 2009. Atlantic Sturgeon Progress Report. Delaware State Wildlife Grant, Project T 4-1. December 16, 2008 to December 15, 2009.

Fisher, M. 2011. Atlantic Sturgeon Progress Report. Delaware State Wildlife Grant, Project T 4-1, October 1, 2006 to October 15, 2010.

Fitzsimmons, N. N., L. W. Farrington, M. J. McCann, C. J. Limpus, and C. Moritz. 2006. Green turtle populations in the Indo-Pacific: a (genetic) view from microsatellites. Pages 111 *in*

N. Pilcher, editor Proceedings of the Twenty-Third Annual Symposium on Sea Turtle Biology and Conservation. NOAA Technical Memorandum NMFS-SEFSC-536.

Foley, A. M., B. A. Schroeder, and S. L. MacPherson. 2008. Post-nesting migrations and resident areas of Florida loggerhead turtles (Caretta caretta). Pages 75-76 *in* H. J. Kalb, A. Rohde, K. Gayheart, and K. Shanker, editors. Twenty-Fifth Annual Symposium on Sea Turtle Biology and Conservation.

Foley, A. M., B. A. Schroeder, A. E. Redlow, K. J. Fick-Child, and W. G. Teas. 2005. Fibropapillomatosis in stranded green turtles (*Chelonia mydas*) from the eastern United States (1980-98): trends and associations with environmental factors. Journal of Wildlife Diseases 41(1):29-41.

Frazer, N. B., and L. M. Ehrhart. 1985. Preliminary Growth Models for Green, *Chelonia mydas*, and Loggerhead, *Caretta caretta*, Turtles in the Wild. Copeia 1985(1):73-79.

Garrett, C. 2004. Priority Substances of Interest in the Georgia Basin - Profiles and background information on current toxics issues. Technical Supporting Document.

Gavilan, F. M. 2001. Status and distribution of the loggerhead turtle, (Caretta caretta), in the wider Caribbean region. Pages 36-40 *in* K. L. Eckert, and F. A. Abreu Grobois, editors. Marine turtle conservation in the wider Caribbean region: a dialogue for effective regional management, St. Croix, U.S. Virgin Islands.

George, C., and P. Naessig. 2006. Right whale 2425 Vessel Collision Report -NEWS Aerial Survey. Georgia Department of Natural Resources, Wildlife Trust.

Geraci, J. R. 1990. Physiologic and toxic effects on cetaceans. Sea Mammals and Oil: Confronting the Risks. J. R. Geraci & D. J. St. Aubin (eds.). p.167-197. Academic Press, San Diego. ISBN 0-12-280600-X.

Gilbert, C. R. 1989. Species profiles : life histories and environmental requirements of coastal fishes and invertebrates (Mid-Atlantic Bight) : Atlantic and shortnose sturgeons. Coastal Ecology Group, Waterways Experiment Station, U.S. Dept. of the Interior, Fish and Wildlife Service, Research and Development, National Wetlands Research Center, Vicksburg, MS, Washington, DC.

Girard, C., A. D. Tucker, and B. Calmettes. 2009. Post-nesting migrations of loggerhead sea turtles in the Gulf of Mexico: dispersal in highly dynamic conditions. Marine Biology 156(9):1827-1839.

Gladys Porter Zoo. 2007. Report on the Mexico/United States of America Population Restoration Project for the Kemp's Ridley Sea Turtle, Lepidocheyls kempii, on the coasts of Tamaulipas and Veracruz, Mexico – 2007. U.S. Fish and Wildlife Service, Department of Interior.

Good, C. 2008. Spatial Ecology of the North Atlantic Right Whale (*Eubalaena glacialis*). Dissertation. Duke University, Durham, NC.

Grant, S. C. H., and P. S. Ross. 2002. Southern resident killer whales at risk: Toxic chemicals in the British Columbia and Washington environment. Fisheries and Oceans Canada, Institute of Ocean Sciences, Sidney, British Columbia, Canada.

Green, D. 1993. Growth rates of wild immature green turtles in the Galapagos Islands, Ecuador. Journal of Herpetology 27(3):338-341.

Greene, K. E., J. L. Zimmerman, R. W. Laney, and J. C. Thomas-Blate. 2009. Atlantic coast diadromous fish habitat: A review of utilization, threats, recommendations for conservation, and research needs. Atlantic States Marine Fisheries Commission Washington, D.C.

Groombridge, B. 1982. Kemp's Ridley or Atlantic Ridley, *Lepidochelys kempii* (Garman 1880). Pages 201-208 *in* The IUCN Amphibia, Reptilia Red Data Book.

Grunwald, C., L. Maceda, J. Waldman, J. Stabile, and I. Wirgin. 2008. Conservation of Atlantic sturgeon Acipenser oxyrinchus oxyrinchus: delineation of stock structure and distinct population segments. Conservation Genetics 9(5):1111-1124.

Guseman, J. L., and L. M. Ehrhart. 1992. Ecological geography of western Atlantic loggerheads and green turtles: evidence from remote tag recoveries. M. Salmon, and J. Wyneken, editors. 11th Annual Workshop on Sea Turtle Biology and Conservation. NOAA Technical Memorandum NMFS.

GWC. 2006. Georgia Water Coalition. Interbasin Transfer Fact Sheet. http://www.garivers.org/gawater/pdf%20files/IBT%20fact%20sheet02-06.pdf.

Hager, C., J. Kahn, C. Watterson, J. Russo, and K. Hartman. 2014. Evidence of Atlantic Sturgeon Spawning in the York River System. Transactions of the American Fisheries Society 143(5):1217-1219.

Hart, K. M., M. M. Lamont, I. Fujisaki, A. D. Tucker, and R. R. Carthy. 2012. Common coastal foraging areas for loggerheads in the Gulf of Mexico: Opportunities for marine conservation. Biological Conservation 145(1):185-194.

Hartwell, S. I. 2004. Distribution of DDT in sediments off the central California coast. Marine Pollution Bulletin 49:299-305.

Hawkes, L. A., A. C. Broderick, M. H. Godfrey, and B. J. Godley. 2007. Investigating the potential impacts of climate change on a marine turtle population. Global Change Biology 13(5):923-932.

Hays, G. C., and coauthors. 2001. The diving behaviour of green turtles undertaking oceanic migration to and from Ascension Island: dive durations, dive profiles and depth distribution. Journal of Experimental Biology 204:4093-4098.

Hays, G. C., and coauthors. 2002. Water temperature and internesting intervals for loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) sea turtles. Journal of Thermal Biology 27(5):429-432.

Heppell, S. S., and coauthors. 2005. A population model to estimate recovery time, population size, and management impacts on Kemp's ridley sea turtles. Chelonian Conservation and Biology 4(4):767-773.

Heppell, S. S., L. B. Crowder, D. T. Crouse, S. P. Epperly, and N. B. Frazer. 2003. Population models for Atlantic loggerheads: past, present, and future. Pages 255-273 *in* A. B. Bolten, and B. E. Witherington, editors. Loggerhead Sea Turtles. Smithsonian Books, Washington.

Herbst, L. H. 1994. Fibropapillomatosis of marine turtles. Annual Review of Fish Diseases 4:389-425.

Herbst, L. H., and coauthors. 1995. An infectious etiology for green turtle fibropapillomatosis. Proceedings of the American Association for Cancer Research Annual Meeting 36:117.

Hildebrand, H. 1963. Hallazgo del area de anidación de la tortuga "lora" *Lepidochelys kempii* (Garman 1880), en la costa occidental del Golfo de México (Rept. Chel.). Ciencia Mex 22(1):105-112.

Hildebrand, H. H. 1982. A historical review of the status of sea turtle populations in the western Gulf of Mexico. Pages 447-453 *in* K. A. Bjorndal, editor. Biology and Conservation of Sea Turtles. Smithsonian Institution Press. Washington, D.C.

Hill, J. 1996. Environmental considerations in licensing hydropower projects: policies and practices at the Federal Energy Regulatory Commission. American Fisheries Society Symposium. 1996.

Hirth, H. F. 1971. Synopsis of biological data on the green turtle *Chelonia mydas* (Linnaeus) 1758. Food and Agriculture Organization of the United Nations, Rome.

Hirth, H. F., and USFWS. 1997. Synopsis of the biological data on the green turtle Chelonia mydas (Linnaeus 1758). U.S. Fish and Wildlife Service, U.S. Dept. of the Interior, Washington, D.C.

Holton, J. W. J., and J. B. Walsh. 1995. Long-term dredged material management plan for the upper James River, Virginia. Waterway Surveys and Engineering, Ltd, Virginia Beach, VA.

Huff, J. A. 1975. Life history of Gulf of Mexico sturgeon, *Acipenser oxyrhynchus desotoi*, in Suwannee River, Florida. Florida Dept. of Natural Resources, Marine Research Laboratory, St. Petersburg, Fla.

IPCC. 2008. Climate Change and Water, Technical Paper of the Intergovernmental Panel on Climate Change, IPCC Secretariat, Geneva, 210 pp.

Iwata, H., S. Tanabe, N. Sakai, and R. Tatsukawa. 1993. Distribution of persistent organochlorines in the oceanic air and surface seawater and the role of ocean on their global transport and fate Environmental Science and Technology 27:1080- 1098.

Jacobson, E. R. 1990. An update on green turtle fibropapilloma. Marine Turtle Newsletter 49:7-8.

Jacobson, E. R., and coauthors. 1989. Cutaneous fibropapillomas of green turtles (*Chelonia mydas*). Journal of Comparative Pathology 101(1):39-52.

Jacobson, E. R., S. B. Simpson, and J. P. Sundberg. 1991. Fibropapillomas in green turtles. Pages 99-100 *in* G. H. Balazs, and S. G. Pooley, editors. Research Plan for Marine Turtle Fibropapilloma. NOAA.

Jager, H. I., and coauthors. 2002. A Simulation Study of Factors Controlling White Sturgeon Recruitment in the Snake River. Pages 127-150 *in*.

Jenkins, W. E., T. I. J. Smith, L. D. Heyward, and D. M. Knott. 1993. Tolerance of shortnose sturgeon, Acipenser brevirostrum, juveniles to different salinity and dissolved oxygen concentrations. Pages 476-484 *in* E. A.G. Eversole, editor Proceedings of the 47th Annual Conference of the Southeastern Association of Fish and Wildlife Agencies, Atlanta, Georgia.

Jensen, A., and G. Silber. 2003a. Large Whale Ship Strike Database. U.S. Department of Commerce.

Jensen, A., and G. Silber. 2003d. Large Whale Ship Strike Database. U.S. Department of Commerce.

Johnson, S. A., and L. M. Ehrhart. 1994. Nest-site fidelity of the Florida green turtle. B. A. Schroeder, and B. Witherington, editors. Proceedings of the 13th Annual Symposium on Sea Turtle Biology and Conservation.

Johnson, S. A., and L. M. Ehrhart. 1996. Reproductive Ecology of the Florida Green Turtle: Clutch Frequency. Journal of Herpetology 30:407-410.

Kahnle, A. W., K. A. Hattala, and K. A. McKown. 2007. Status of Atlantic Sturgeon of the Hudson River Estuary, New York, USA. American Fisheries Society Symposium 56:347-363.

Kahnle, A. W., and coauthors. 1998. Stock Status of Atlantic sturgeon of Atlantic Coast Estuaries. Atlantic States Marine Fisheries Comission.

Kenny, A. J., and H. L. Rees. 1994. The effects of marine gravel extraction on the macrobenthos: Early post-dredging recolonization. Marine Pollution Bulletin 28(7):442-447.

Kieffer, M. C., and B. Kynard. 1993. Annual Movements of Shortnose and Atlantic Sturgeons in the Merrimack River, Massachusetts. Transactions of the American Fisheries Society 122(6):1088-1103.

King, T. L., B. A. Lubinski, and A. P. Spidle. 2001. Microsatellite DNA variation in Atlantic sturgeon (Acipenser oxyrinchus oxyrinchus) and cross-species amplification in the Acipenseridae. Conservation Genetics 2(2):103-119.

Kite-Powell, H. K., A. R. Knowlton, and M. Brown. 2007. Modeling the effect of vessel speed on right whale ship strike risk. Project report for NOAA/NMFS Project NA04NMF47202394.

Knowlton, A. R., and S. D. Kraus. 2001. Mortality and serious injury of northern right whales (Eubalaena glacialis) in the western North Atlantic Ocean. Journal of Cetacean Research and Management (Special Issue) 2:193-208.

Kraus, S. D. 1990. Rates and Potential Causes of Mortality in North Atlantic Right Whales (*Eubalaena glacialis*). Marine Mammal Science 6(4):278-291.

Kraus, S. D., and coauthors. 2005. Ecology. North Atlantic right whales in crisis. Science 309(5734):561-2.

KRRMP. 1993. Kennebec River Resource Management Plan: Balancing Hydropower Generation and Other Uses. Final Report to the Maine State Planning Office, Augusta, ME.

Kynard, B. 1997. Life history, latitudinal patterns, and status of the shortnose sturgeon, Acipenser brevirostrum. Environmental Biology of Fishes 48(1):319-334.

Kynard, B., and M. Horgan. 2002. Ontogenetic behavior and migration of Atlantic sturgeon, Acipenser oxyrinchus oxyrinchus, and shortnose sturgeon, A. brevirostrum, with notes on social behavior. Environmental Behavior of Fishes 63:137-150.

Kynard, B., M. Kieffer, M. Burlingame, and M. Horgan. 1999. Studies on shortnose sturgeon. Final Report to Northeast Utilities Service Company, Berlin CT and the City of Holyoke, MA.

Lagueux, C. 2001. Status and distribution of the green turtle, Chelonia mydas, in the Wider Caribbean Region, pp. 32-35. In: K. L. Eckert and F. A. Abreu Grobois (eds.). 2001 Proceedings of the Regional Meeting: Marine Turtle Conservation in the Wider Caribbean Region: A Dialogue for Effective Regional Management. Santo Domingo, 16-18 November 1999. WIDECAST, IUCN-MTSG, WWF, UNEP-CEP.

Laist, D. W., A. R. Knowlton, J. G. Mead, A. S. Collet, and M. Podesta. 2001. Collisions Between Ships and Whales. Marine Mammal Science 17(1):35-75.

Laist, D. W., and C. Shaw. 2006. PRELIMINARY EVIDENCE THAT BOAT SPEED RESTRICTIONS REDUCE DEATHS OF FLORIDA MANATEES. Marine Mammal Science 22(2):472-479.

Laurent, L., and coauthors. 1998. Molecular resolution of marine turtle stock composition in fishery bycatch: a case study in the Mediterranean. Molecular Ecology 7:1529-1542.

Law, R. J., and coauthors. 1991. Concentrations of trace metals in the livers of marine mammals (seals, porpoises and dolphins) from waters around the British Isles. Marine Pollution Bulletin 22:183-191.

Leland, J. G. 1968. A survey of the sturgeon fishery of South Carolina. Bears Bluff Laboratories, Wadmalaw Island, S.C.

Lutcavage, M. E., P. Plotkin, B. Witherington, and P. L. Lutz. 1997. Human impacts on sea turtle survival. Pages 432 *in* P. L. Lutz, and J. A. Musick, editors. The Biology of Sea Turtles. CRC Press.

Márquez M, R. 1990. Sea turtles of the world: an annotated and illustrated catalogue of sea turtle species known to date. Food and Agriculture Organization of the United Nations, Rome.

Márquez M, R. 1994. Synopsis of biological data on the Kemp's ridley turtle, *Lepidochelys kempii* (Garman 1880). NOAA Technical Memorandum NMFS-SEFSC-343. U. S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southeast Fisheries Science Center, Miami, FL.

Mason, W. T., and J. P. Clugston. 1993. Foods of the Gulf sturgeon (*Acipenser oxyrhynchus desotoi*) in the Suwannee River, Florida. Transactions of the American Fisheries Society 122(3):378-385.

Mathews, T. D., and M. H. Shealy. 1978. Hydrography of South Carolina estuaries, with emphasis on the North and South Edisto and Cooper Rivers. SC Marine Resource Center, Charleston, SC. Technical Report No. 19.

Mathews, T. D., and M. H. Shealy. 1982. A description of the salinity regimes of major South Carolina estuaries. SC Marine Resource Center, Charleston, SC. Technical Report No. 54.

Matkin, C. O., and E. Saulitis. 1997. Restoration notebook: killer whale (Orcinus orca). Exxon Valdez Oil Spill Trustee Council, Anchorage, Alaska.

McCauley, J. E., R. A. Parr, and D. R. Hancock. 1977. Benthic infauna and maintenance dredging: a case study. Wat. Res. 11:233-242.

McCord, J. W. 2004. ASMFC Atlantic Sturgeon Plan – amendment 1 South Carolina annual report for calendar-year 2003. Compliance report submitted to Atlantic States Marine Fisheries Commission, October 19, 2004. Washington, DC.

McDonald-Dutton, D., and P. H. Dutton. 1998. Accelerated growth in San Diego Bay green turtles? Pages 175-176 *in* S. P. Epperly, and J. Braun, editors. Proceedings of the seventeenth annual symposium on sea turtle biology and conservation. NOAA Technical Memorandum NMFS-SEFSC-415. National Marine Fisheries Service, Southeast Fisheries Science Center, Orlando, FL.

McMichael, E., R. R. Carthy, and J. A. Seminoff. 2003. Evidence of Homing Behavior in Juvenile Green Turtles in the Northeastern Gulf of Mexico. Pages 223-224 *in* J. A. Seminoff, editor Proceedings of the Twenty-Second Annual Symposium on Sea Turtle Biology and Conservation. NOAA Technical Memorandum NMFS-SEFSC-503. National Marine Fisheries Service, Southeast Fisheries Science Center, Miami, FL.

Meylan, A. 1999. Status of the hawksbill turtle (Eretmochelys imbricata) in the Caribbean region. . Chelonian Conservation and Biology 3(2):177-184.

Meylan, A. B., B. A. Schroeder, and A. Mosier. 1995. Sea Turtle Nesting Activity in the State of Florida, 1979-1992. Florida Department of Environmental Protection, Florida Marine Research Institute, St. Petersburg, FL.

Meylan, A. M., B. Schroeder, and A. Mosier. 1994. Marine Turtle Nesting Activity in the State of Florida, 1979-1992. Pages 83 *in* K. A. Bjorndal, A. B. Bolten, D. A. Johnson, and P. J. Eliazar, editors. Proceedings of the Fourteenth Annual Symposium on Sea Turtle Biology

and Conservation. NOAA Technical Memorandum NMFS-SEFSC-351. National Marine Fisheries Service, Southeast Fisheries Science Center, Hilton Head, SC.
Miller, T., and G. Shepherd. 2011. Summary of Discard Estimates for Atlantic Sturgeon. Population Dynamics Branch, Northeast Fisheries Science Center.
Milton, S. L., and P. L. Lutz. 2003. Physiological and Genetic Responses to Environmental Stress. Pages 163-197 *in* P. L. Lutz, J. A. Musick, and J. Wyneken, editors. The Biology of Sea Turtles, volume 2. CRC Press, Boca Raton, FL.
Mo, C. L. 1988. Effect of bacterial and fungal infection on hatching success of olive ridley sea turtle eggs. U. S. World Wildlife Fund.
Moncada, F., and coauthors. 2010. Movement patterns of loggerhead turtles Caretta caretta in Cuban waters inferred from flipper tag recaptures. Endangered Species Research 11(1):61-68.
Moon, D., D. S. Mackenzie, and D. W. Owens. 1997. Simulated Hibernation of Sea Turtles in the Laboratory: I. Feeding, Breathing Frequency, Blood PH, and Blood Gases. Journal of Experimental Zoology 278: 372-380.
Moser, M. L. 2000. A protocol for use of shortnose and Atlantic sturgeons. Pages 1 online resource (18 p.) *in* NOAA technical memorandum NMFS-OPR ; 18. U.S. Dept. of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, [Silver Spring, Md.].
Moser, M. L., J. B. Bichy, and S. B. Roberts. 1998. Sturgeon Distibution in North Carolina. Center for Marine Science Research, Wilmington, North Carolina.
Moser, M. L., and S. W. Ross. 1993. Distribution and movements of shortnose sturgeon (Acipenser brevirostrum) and other anadromous fishes of the lower Cape Fear River, North Carolina. Final Report to the U.S. Army Corps of Engineers, Wilmington, North Carolina.
Moser, M. L., and S. W. Ross. 1995. Habitat Use and Movements of Shortnose and Atlantic Sturgeons in the Lower Cape Fear River, North Carolina. Transactions of the American Fisheries Society 124(2):225-234.
Murawski, S. A., A. L. Pacheco, and United States. National Marine Fisheries Service. 1977. Biological and fisheries data on Atlantic sturgeon, Acipenser oxyrhynchus (Mitchill). Sandy Hook Laboratory, Northeast Fisheries Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, U.S. Dept. of Commerce, Highlands, N.J.
Murphy, T. M., and S. R. Hopkins. 1984. Aerial and ground surveys of marine turtle nesting beaches in the southeast region. NMFS-SEFSC.
Musick, J. A., R. E. Jenkins, and N. B. Burkhead. 1994. Sturgeons, Family Acipenseridae. R. E. Jenkins, and N. B. Burkhead, editors. Freshwater Fishes of Virginia. American Fisheries Society, Bethesda, MD.
Musick, J. A., and C. J. Limpus. 1997. Habitat utilization and migration in juvenile sea turtles. Pages 432 *in* P. L. Lutz, and J. A. Musick, editors. The Biology of Sea Turtles. CRC Press.
Niklitschek, E. J., and D. H. Secor. 2005. Modeling spatial and temporal variation of suitable nursery habitats for Atlantic sturgeon in the Chesapeake Bay. Estuarine, Coastal and Shelf Science 64(1):135-148.
Niklitschek, E. J., and D. H. Secor. 2009a. Dissolved oxygen, temperature and salinity effects on the ecophysiology and survival of juvenile Atlantic sturgeon in estuarine waters: I.

Laboratory results. Journal of Experimental Marine Biology and Ecology 381(Supplement 1):S150-S160.
Niklitschek, E. J., and D. H. Secor. 2009c. Dissolved oxygen, temperature and salinity effects on the ecophysiology and survival of juvenile Atlantic sturgeon in estuarine waters: II. Model development and testing. Journal of Experimental Marine Biology and Ecology 381(Supplement 1):S161-S172.
Niklitschek, E. J., and D. H. Secor. 2010. Experimental and field evidence of behavioural habitat selection by juvenile Atlantic Acipenser oxyrinchus oxyrinchus and shortnose Acipenser brevirostrum sturgeons. Journal of Fish Biology 77(6):1293-1308.
NMFS-SEFSC. 2001. Stock assessments of loggerhead and leatherback sea turtles: and, an assessment of the impact of the pelagic longline fishery on the loggerhead and leatherback sea turtles of the western North Atlantic. U.S. Dept. of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southeast Fisheries Science Center, Miami, FL.
NMFS-SEFSC. 2009a. An assessment of loggerhead sea turtles to estimate impacts of mortality reductions on population dynamics. NMFS Southeast Fisheries Science Center.
NMFS-SEFSC. 2009e. Estimated impacts of mortality reductions on loggerhead sea turtle population dynamics, preliminary results. Presented at the meeting of the Reef Fish Management Committee of the Gulf of Mexico Fishery Management Council. Gulf of Mexico Fishery Management Council, Tamps, FL.
NMFS. 1991. Biological Opinion for the Dredging of channels in the Southeastern United States from North Carolina through Cape Canaveral, Florida. .
NMFS. 1995. Endangered Species Act section 7 consultation on United States Coast Guard vessel and aircraft activities along the Atlantic coast. Biological Opinion. September 15.
NMFS. 1997a. ESA Section 7 consultation on Navy activities off the southeastern United States along the Atlantic Coast. Biological Opinion.
NMFS. 1997b. ESA Section 7 consultation on the continued hopper dredging of channels and borrow areas in the southeastern United States. Biological Opinion.
NMFS. 2001a. Biological Opinion: Endangered Species Act section 7 consultation on the reinitiation of consultation on the Atlantic highly migratory species fishery management plan and its associated fisheries.
NMFS. 2001b. Stock assessments of loggerhead and leatherback sea turtles and an assessment of the impact of the pelagic longline fishery on the loggerhead and leatherback sea turtles of the western North Atlantic.
NMFS. 2003a. Biological Opinion on Dredging of Gulf of Mexico Navigation Channels and Sand Mining ("Borrow") Areas Using Hopper Dredges by COE Galveston, New Orleans, Mobile, and Jacksonville Districts (Consultation Number F/SER/2000/01287). National Marine Fisheries Service, Southeast Regional Office, Protected Resources Division, St. Petersburg, Florida [Plus Revisions].
NMFS. 2003b. Endangered Species Act (ESA) Section 7 Consultation on the U.S. Army Corps of Engineers on the Use of Bed-Leveling Equipment During the Brunswick Harbor Deepening Project. Biological Opinion (Consultation Number I/SER/2003/01048). September 11, 2003. NOAA National Marine Fisheries Service, Southeast Regional Office.
NMFS. 2005. Revision No. 1 to November 19, 2003, Gulf of Mexico Regional Biological Opinion (GOM RBO) on Hopper Dredging of Navigation Channels and Borrow Areas in

the U.S. Gulf of Mexico. National Marine Fisheries Service, Southeast Regional Office, Protected Resources Division, St. Petersburg, Florida. 22p.

NMFS. 2007a. Endangered Species Act section 7 consultation on the dredging of Gulf of Mexico navigation channels and sand mining ("borrow") areas using hopper dredges by USACE Galveston, New Orleans, Mobile, and Jacksonville Districts. Revised Biological Opinion (November 2003). January 2007.

NMFS. 2007b. ESA Section 7 consultation on Gulfport Harbor Navigation Project maintenance dredging and disposal. Biological Opinion.

NMFS. 2007c. Revision 2 to the National Marine Fisheries Service (NMFS) November 19, 2003, Gulf of Mexico Regional Biological Opinion (GRBO) to the U.S. Army Corps of Engineers (COE) on Hopper Dredging of Navigation Channels and Borrow Areas in the U.S. Gulf of Mexico. National Marine Fisheries Service, Southeast Regional Office, Protected Resources Division, St. Petersburg, Florida. 15p.

NMFS. 2015. Endangered and Threatened Species; Critical Habitat for Endangered North Atlantic Right Whale; Proposed Rule. Federal Register 80(34):9313 -9345.

NMFS, and USFWS. 1991a. Recovery Plan for U.S. Population of Atlantic Green Turtle (*Chelonia mydas*) National Marine Fisheries Service, Washington, D.C.

NMFS, and USFWS. 1991b. Recovery plan for U.S. population of Atlantic green turtle (Chelonia mydas).

NMFS, and USFWS. 1992. Recovery Plan for the Kemp's Ridley Sea Turtle (*Lepidochelys kempii*). Pages 47 *in* U.S. Department of Interior, and U.S. Department of Commerce, editors. U.S. Fish and Wildlife Service, National Marine Fisheries Service.

NMFS, and USFWS. 1993. Recovery plan for hawksbill turtles in the U.S. Caribbean Sea, Atlantic Ocean, and Gulf of Mexico (Eretmochelys imbricata). U.S. Dept. of Commerce, National Oceanic and Atmopsheric Administration U.S. Dept. of the Interior, U.S. Fish and Wildlife Service, [Washington, D.C].

NMFS, and USFWS. 1995. Status reviews for sea turtles listed under the Endangered Species Act of 1973. National Marine Fisheries Service, Silver Spring, MD.

NMFS, and USFWS. 2007a. Green Sea Turtle (*Chelonia mydas*) 5-year review: Summary and Evaluation. National Marine Fisheries Service, Silver Spring, MD.

NMFS, and USFWS. 2007m. Hawksbill Sea Turtle (*Eretmochelys imbricata*) 5-year review: Summary and Evaluation. National Marine Fisheries Service, Silver Spring, MD.

NMFS, and USFWS. 2007q. Kemp's ridley Sea Turtle (*Lepidochelys kempii*) 5-year review: Summary and Evaluation. National Marine Fisheries Service, Silver Spring, MD.

NMFS, and USFWS. 2007v. Leatherback Sea Turtle (*Dermochelys coriacea*) 5-year review: Summary and Evaluation. National Marine Fisheries Service, Silver Spring, MD.

NMFS, and USFWS. 2007w. Loggerhead Sea Turtle (*Caretta caretta*) 5-year review: Summary and Evaluation. National Marine Fisheries Service, Silver Spring, MD.

NMFS, and USFWS. 2008a. Draft recovery plan for the northwest Atlantic population of the loggerhead sea turtle (*Caretta caretta*): Second revision. National Marine Fisheries Service and U.S. Fish and Wildlife Service, Silver Spring, Maryland.

NMFS, and USFWS. 2008b. Recovery Plan for the Northwest Atlantic Population of the Loggerhead Sea Turtle (*Caretta caretta*), Second Revision National Marine Fisheries Service, Silver Spring, MD.

NMFS, USFWS, and SEMARNAT. 2011. Bi-National Recovery Plan for the Kemp's Ridley Sea Turtle (*Lepidochelys kempii*), Second Revision. National Marine Fisheries Service, Silver Spring, Maryland.

NRC. 1990. Decline of the Sea Turtles: Causes and Prevention. National Academy Press, 030904247X, Washington, D.C.

NSWNPS. 2009. December 9, 2009 Marine Wildlife Situation Report -Single Animal. Southern/South Coast/Ulladulla. New South Wales National Park Service.

Ogren, L. H. 1989. Distribution of juvenile and sub-adult Kemp's ridley sea turtle: Preliminary results from 1984-1987 surveys. C. W. Caillouet, and A. M. Landry, editors. First Intl. Symp. on Kemp's Ridley Sea Turtle Biol, Conserv. and Management, Galvaston, TX.

Pace, R. M., and G. Silber. 2005. Abstract. Simple analyses of ship and large whale collisions: Does speed kill? . Sixteenth Biennial Conference on the Biology of Marine Mammals, San Diego, CA.

Palmer, A. G. 2001. Seasonal, Diel, and Tidal Movements of Shortnose Sturgeon (Acipenser Brevirostrum) in the Cooper River, South Carolina. University of Charleston, South Carolina.

Panigada, S., and coauthors. 2006. Mediterranean fin whales at risk from fatal ship strikes. Marine Pollution Bulletin 52(10):1287-1298.

Pike, D. A., R. L. Antworth, and J. C. Stiner. 2006. Earlier Nesting Contributes to Shorter Nesting Seasons for the Loggerhead Seaturtle, Caretta caretta. Journal of Herpetology 40(1):91-94.

Post, B., T. Darden, D. Peterson, M. Loeffler, and C. Collier. 2014. Research and Management of Endangered and Threatened Species in the Southeast: Riverine Movements of Shortnose and Atlantic sturgeon. S. C. Department of Natural Resources.

Pritchard, P. C. H. 1969. The survival status of ridley sea-turtles in American waters. Biological Conservation 2(1):13-17.

Pyzik, L., J. Caddick, and P. Marx. 2004. Chesapeake Bay: Introduction to an ecosystem. EPA 903-R-04-003, CBP/TRS 232100.

Randall, M. T., and K. J. Sulak. 2012. Evidence of autumn spawning in Suwannee River gulf sturgeon, *Acipenser oxyrinchus desotoi* (Vladykov, 1955). Journal of Applied Ichthyology 28(4):489-495.

Ray, G. 1997. Benthic Characterization of Wilmington Harbor and Cape Fear Estuary, Wilmington, North Carolina. Final Report Prepared for the U.S. Army Corps of Engineers - Wilmington District. U.S. Army Engineers Waterways Experiment Station, Vicksburg, MS.

Rebel, T. P. 1974. Sea turtles and the turtle industry of the West Indies, Florida, and the Gulf of Mexico, Revised edition. University of Miami Press, Coral Gables, FL.

Rogers, S. G., and W. Weber. 1995. Status and restoration of Atlantic and shortnose sturgeons in Georgia, Final Report. National Marine Fisheries Service, Southeast Regional Office, St. Petersburg, Florida.

Salwasser, H., S. P. Mealey, and K. Johnson. 1984. Wildlife population viability: a question of risk. Pages 421-439 *in* Transactions of the North American Wildlife and Natural Resources Conference.

Savoy, T. 2007. Prey Eaten by Atlantic Sturgeon in Connecticut Waters. American Fisheries Society Symposium 56:157.

SCDHEC. 2013. Watershed Water Quality Assessment: Santee River Basin. Technical Report No. 0620-13. Bureau of Water, Columbia, S.C. .

Schmid, J. R., and J. A. Barichivich. 2006. *Lepidochelys kempii*–Kemp's ridley. Pages 128-141 *in* P. A. Meylan, editor. Biology and conservation of Florida turtles. Chelonian Research Monographs, volume 3.

Schmid, J. R., and A. Woodhead. 2000. Von Bertalanffy growth models for wild Kemp's ridley turtles: analysis of the NMFS Miami Laboratory tagging database. U. S. Dept. of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southeast Fisheries Science Center, Miami, Florida.

Schroeder, B. A., and A. M. Foley. 1995. Population studies of marine turtles in Florida Bay. Pages 117 *in* J. I. Richardson, and T. H. Richardson, editors. Proceedings of the Twelfth Annual Workshop on Sea Turtle Biology and Conservation. NOAA.

Schueller, P., and D. L. Peterson. 2010. Abundance and Recruitment of Juvenile Atlantic Sturgeon in the Altamaha River, Georgia. Transactions of the American Fisheries Society 139(5):1526-1535.

Schwenter, J. A., and coauthors. 2013. Catch Rates and Demographics for Kemp's Ridley (Lepidochelys kempii) Sea Turtles Captured in Near-shore Coastal Waters Between Winyah Bay, SC and St. Augustine, FL. Sea Turlte Biology and Conservation. National Oceanic and Atmospheric Administration, Baltimore, MD.

Scott, W. B., and E. J. Crossman. 1973. Freshwater fishes of Canada., Fisheries Research Board of Canada Bulletin.

Secor, D. 1995. Chesapeake Bay Atlantic sturgeon: current status and future recovery. Summary of findings and recommendations from a workshop convened 8 November 1994 at Chesapeake Biological Laboratory. Chesapeake Bay Biological Laboratory, Center for Estuarine and Environmental Studies, University of Maryland System, Solomons, MD.

Secor, D. H. 2002. Atlantic sturgeon fisheries and stock abundances during the late nineteenth century. Pages 89-98 *in* American Fisheries Society Symposium.

Secor, D. H., and T. E. Gunderson. 1998. Effects of hypoxia and temperature on survival, growth, and respiration of juvenile Atlantic sturgeon (*Acipenser oxyrinchus*). Fishery Bulletin U.S. 96:603-613.

Secor, D. H., and J. R. Waldman. 1999. Historical Abundance of Delaware Bay Atlantic Sturgeon and Potential Rate of Recovery. Pages 203-216 *in* American Fisheries Society Symposium.

Seiderer, L. J., and R. C. Newell. 1999. Analysis of the relationship between sediment composition and benthic community structure in coastal deposits: Implications for marine aggregate dredging. ICES Journal of Marine Science: Journal du Conseil 56(5):757-765.

Seminoff, J. A. 2004. 2004 global status assessment: Green turtle (*Chelonia mydas*). IUCN Marine Turtle Specialist Group Review.

Shaffer, M. L. 1981. Minimum Population Sizes for Species Conservation. BioScience 31(2):131-134.

Shaver, D. J. 1994. Relative Abundance, Temporal Patterns, and Growth of Sea Turtles at the Mansfield Channel, Texas. Journal of Herpetology 28(4):491-497.

Smith, T. 1985. The fishery, biology, and management of Atlantic sturgeon, Acipenser oxyrhynchus, in North America. Environmental Biology of Fishes 14(1):61-72.

Smith, T. I. J., and J. P. Clugston. 1997. Status and management of Atlantic sturgeon, Acipenser oxyrinchus, in North America. Environmental Biology of Fishes 48(1-4):335-346.

Smith, T. I. J., E. K. Dingley, and E. E. Marchette. 1980. Induced spawning and culture of Atlantic sturgeon Progressive Fish Culturist 42:147-151.

Smith, T. I. J., D. E. Marchette, and R. A. Smiley. 1982. Life history, ecology, culture and management of Atlantic sturgeon, Acipenser oxyrhynchus, Mitchill, in South Carolina. Final Report to U.S. Fish and Wildlife Service Resources Department.

Snover, M. L. 2002. Growth and ontogeny of sea turtles using skeletochronology: Methods, validation and application to conservation. Duke University.

Soulé, M. E. 1980. Thresholds for survival: maintaining fitness and evolutionary potential. Pages 151-170 *in* M. E. Soulé, and B. A. Wilcox, editors. Conservation Biology: An Evolutionary-Ecological Perspective. Sinauer Associates, Sunderland, MA.

Soulé, M. E. 1987. Where do we go from here? Chapter 10 In: Soulé, M.E. (ed), Viable Populations for Conservation. Cambridge University Press, pp.175-183.

Squiers, T. 2004. State of Maine 2004 Atlantic sturgeon compliance report to the Atlantic States Marine Fisheries Commission. Report submitted to Atlantic States Marine Fisheries Commission, December 22, 2004, Washington, D.C.

Stabenau, E. K., T. A. Heming, and J. F. Mitchell. 1991. Respiratory, acid-base and ionic status of Kemp's ridley sea turtles (Lepidochelys kempii) subjected to trawling. . Comparative Biochemistry and Physiology 99A(1/ 2):107-111.

STAC. 2006. Sea Turtle Interactions With North Carolina Fisheries - Review and Recommendations. North Carolina Division of Marine Fisheries Morehead City, North Carolina.

Stein, A. B., K. D. Friedland, and M. Sutherland. 2004. Atlantic Sturgeon Marine Bycatch and Mortality on the Continental Shelf of the Northeast United States. North American Journal of Fisheries Management 24(1):171-183.

Stevenson, J. C., and D. H. Secor. 1999. Age determination and growth of Hudson River Atlantic sturgeon (*Acipenser oxyrinchus*). Fishery Bulletin 97:153-166.

Storelli, M. M., G. Barone, A. Storelli, and G. O. Marcotrigiano. 2008. Total and subcellular distribution of trace elements (Cd, Cu and Zn) in the liver and kidney of green turtles (Chelonia mydas) from the Mediterranean Sea. Chemosphere 70:908-913.

Sweka, J., and coauthors. 2007. Juvenile Atlantic sturgeon habitat use in Newburgh and Haverstraw Bays of the Hudson River: Implications for Population Monitoring. North American Journal of Fisheries Management 27:1058-1067.

TEWG. 1998. An assessment of the Kemp's ridley (*Lepidochelys kempii*) and loggerhead (*Caretta caretta*) sea turtle populations in the western North Atlantic. U. S. Dept. Commerce.

TEWG. 2000a. Assessment update for the kemp's ridley and loggerhead sea turtle populations in the western North Atlantic : a report of the Turtle Expert Working Group. U.S. Dept. of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southeast Fisheries Science Center, Miami, Fla.

TEWG. 2000b. Assessment update for the Kemp's ridley and loggerhead sea turtle populations in the western North Atlantic: a report of the Turtle Expert Working Group. U. S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southeast Fisheries Science Center, Miami, FL.

TEWG. 2007. An Assessment of the Leatherback Turtle Population in the Atlantic Ocean. NOAA.

TEWG. 2009. An Assessment of the Loggerhead Turtle Population in the Western North Atlantic Ocean. NOAA.

Thomas, C. D. 1990. What Do Real Population Dynamics Tell Us About Minimum Viable Population Sizes? Conservation Biology 4(3):324-327.

Troëng, S., and E. Rankin. 2005. Long-term conservation efforts contribute to positive green turtle Chelonia mydas nesting trend at Tortuguero, Costa Rica. Biological Conservation 121(1):111-116.

Tucker, A. D. 2010. Nest site fidelity and clutch frequency of loggerhead turtles are better elucidated by satellite telemetry than by nocturnal tagging efforts: Implications for stock estimation. Journal of Experimental Marine Biology and Ecology 383(1):48-55.

USACE. 2014. CHARLESTON HARBOR POST 45: Draft Integrated Feasibility Report and Environmental Impact Statement, Charleston, South Carolina.

USFWS, NMFS, and SCDNR. 2001. Santee-Cooper Basin Diadromous Fish Passage Restoration Plan. U.S. Fish and Wildlife Service, National Marine Fisheries Service, South Carolina Department of Natural Resources, Charleston, South Carolina.

USGRG. 2004. U.S. National Assessment of the Potential Consequences of Climate Variability and Change, Regional Paper: The Southeast. U.S. Global Research Group. Washington, D.C., August 20, 2004.

Van Dolah, R. F., D. R. Calder, and D. M. Knott. 1984. Effects of dredging and open water disposal in a South Carolina estuary. Estuaries 7:28-37.

Van Dolah, R. F., D. R. Calder, D. M. Knott, and M. S. Maclin. 1979. Effects of dredging and unconfined disposal on macrobenthic communities in Sewee Bay, South Carolina. Tech. Rep. 39. South Carolina Marine Resources Center, Charleston, SC.

Van Eenennaam, J. P., and S. I. Doroshov. 1998. Effects of age and body size on gonadal development of Atlantic sturgeon. Journal of Fish Biology 53(3):624-637.

Van Eenennaam, J. P., and coauthors. 1996. Reproductive Conditions of the Atlantic Sturgeon (Acipenser oxyrinchus) in the Hudson River. Estuaries 19(4):769-777.

Vanderlaan, A. S. M., and C. T. Taggart. 2007. Vessel Collisions with Whales: The Probability of Lethal Injury Based on Vessel Speed. Marine Mammal Science 23(1):144-156.

Vargo, S., P. Lutz, D. Odell, E. V. Vleet, and G. Bossart. 1986. Effects of oil on marine turtles, Florida Institute of Oceanography.

Vladykov, V. D., and J. R. Greely. 1963. Order Acipenseroidei. Pages 1630 pp *in* Fishes of Western North Atlantic, Sears Foundation. Marine Research, Yale University.

Waldman, J. R., C. Grunwald, J. Stabile, and I. Wirgin. 2002. Impacts of life history and biogeography on the genetic stock structure of Atlantic sturgeon Acipenser oxyrinchus oxyrinchus, Gulf sturgeon A-oxyrinchus desotoi, and shortnose sturgeon A-brevirostrum. Journal of Applied Ichthyology 18(4-6):509-518.

Waldman, J. R., and I. I. Wirgin. 1998. Status and Restoration Options for Atlantic Sturgeon in North America. Conservation Biology 12(3):631-638.

Waring, G. T., E. Josephson, K. Maze-Foley, and P. E. Rosel. 2012. US Atlantic and Gulf of Mexico marine mammal stock assessments - 2011. NOAA.

Waring, G. T., E. Josephson, K. Maze-Foley, and P. E. Rosel. 2014. U.S. Atlantic and Gulf of Mexico Marine Mammal Stock Assessments -- 2013. NOAA Technical Memorandum NMFS-NE-228. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Northeast Fisheries Science Center, NMFS-NE-228, Woods Hole, MA.

Weber, W. 1996. Population size and habitat use of shortnose sturgeon, Acipenser brevirostrum, in the Ogeechee River system, Georgia. University of Georgia, Athens, Georgia.

Weber, W., and C. A. Jennings. 1996. Endangered species management plan for the shortnose sturgeon, *Acipenser brevirostrum*. Final Report to Port Stewart Military Reservation, Fort Stewart, GA.

Weishampel, J. F., D. A. Bagley, and L. M. Ehrhart. 2004. Earlier nesting by loggerhead sea turtles following sea surface warming. Global Change Biology 10:1424-1427.

Weishampel, J. F., D. A. Bagley, L. M. Ehrhart, and B. L. Rodenbeck. 2003. Spatiotemporal patterns of annual sea turtle nesting behaviors along an East Central Florida beach. Biological Conservation 110(2):295-303.

Wershoven, J. L., and R. W. Wershoven. 1992. Juvenile green turtles in their nearshore habitat of Broward County, Florida: A five year review. 11th Annual Workshop on Sea Turtle Biology and Conservation. NOAA Technical Memorandum NMFS.

Winger, P. V., P. J. Lasier, D. H. White, and J. T. Seginak. 2000. Effects of Contaminants in Dredge Material from the Lower Savannah River. Archives of Environmental Contamination and Toxicology 38(1):128-136.

Wirgin, I., C. Grunwald, J. Stabile, and J. Waldman. 2007. Genetic evidence for relict Atlantic sturgeon stocks along the mid-Atlantic coast of the USA. North American Journal of Fisheries Management 27(4):1214-1229.

Wirgin, I., and T. King. 2011. Mixed stock analysis of Atlantic sturgeon from coastal locales and a non-spawning river. NMFS Sturgeon Workshop, Alexandria, VA.

Wirgin, I., J. Waldman, J. Stabile, B. Lubinski, and T. King. 2002. Comparison of mitochondrial DNA control region sequence and microsatellite DNA analyses in estimating population structure and gene flow rates in Atlantic sturgeon Acipenser oxyrinchus. Journal of Applied Ichthyology 18(4-6):313-319.

Wirgin, I., and coauthors. 2000. Genetic Structure of Atlantic Sturgeon Populations Based on Mitochondrial DNA Control Region Sequences. Transactions of the American Fisheries Society 129(2):476-486.

Witherington, B., and L. M. Ehrhart. 1989. Hypothermic stunning and mortality of marine turtles in the Indian River Lagoon system, Florida. Copeia 1989:696-703.

Witherington, B., S. Hirama, and A. Mosier. 2003. Effects of beach armoring structures on marine turtle nesting. Florida Fish and Wildlife Conservation Commission.

Witherington, B., S. Hirama, and A. Mosier. 2007. Changes to armoring and other barriers to sea turtle nesting following severe hurricanes striking Florida beaches. Florida Fish and Wildlife Conservation Commission.

Witherington, B. E. 1992. Behavioral responses of nesting sea turtles to artificial lighting. Herpetologica 48(1):31-39.

Witherington, B. E. 2002. Ecology of neonate loggerhead turtles inhabiting lines of downwelling near a Gulf Stream front. Marine Biology 140(4):843-853.

Witherington, B. E., and K. A. Bjorndal. 1991. Influences of artificial lighting on the seaward orientation of hatchling loggerhead turtles, Caretta caretta. Biological Conservation 55(2):139-149.

Witzell, W. N. 2002. Immature Atlantic loggerhead turtles (Caretta caretta): suggested changes to the life history model. Herpetological Review 33(4):266-269.

Young, J. R., T. B. Hoff, W. P. Dey, and J. G. Hoff. 1988. Management recommendations for a Hudson River Atlantic sturgeon fishery based on an age-structured population model.

Fisheries Research in the Hudson River. State of University of New York Press, Albany, New York.
Ziegeweid, J., C. Jennings, and D. Peterson. 2008. Thermal maxima for juvenile shortnose sturgeon acclimated to different temperatures. Environmental Biology of Fishes 82(3):299-307.
Zoodsma, B. 2005. March 10, 2005 Vessel/Right Whale Collision: Incident and Vessel Characteristics. National Oceanic Atmospheric Administration, National Marine Fisheries Service, Fernandina Beach, FL.
Zug, G. R., and R. E. Glor. 1998. Estimates of age and growth in a population of green sea turtles (*Chelonia mydas*) from the Indian River lagoon system, Florida: A skeletochronological analysis. Canadian Journal of Zoology 76(8):1497-1506.
Zurita, J. C., and coauthors. 2003. Nesting loggerhead and green sea turtles in Quintana Roo, Mexico. Pages 125-126 *in* Twenty-Second Annual Symposium on Sea Turtle Biology and Conservation, Miami, FL.
Zwinenberg, A. J. 1977. Kemp's ridley, *Lepidochelys kempii* (Garman 1880), undoubtedly the most endangered marine turtle today (with notes on the current status of *Lepidochelys olivacea*). Bulletin of the Maryland Herpetological Society 13(3):378-384.
Zydlewski, G. B., M. T. Kinnison, P. E. Dionne, J. Zydlewski, and G. S. Wippelhauser. 2011. Shortnose sturgeon use small coastal rivers: the importance of habitat connectivity. Journal of Applied Ichthyology 27:41-44.

13 Appendix A: Sea Turtle Research Techniques Manual

http://www.sefsc.noaa.gov/turtles/TM_579_SEFSC_STRTM.pdf

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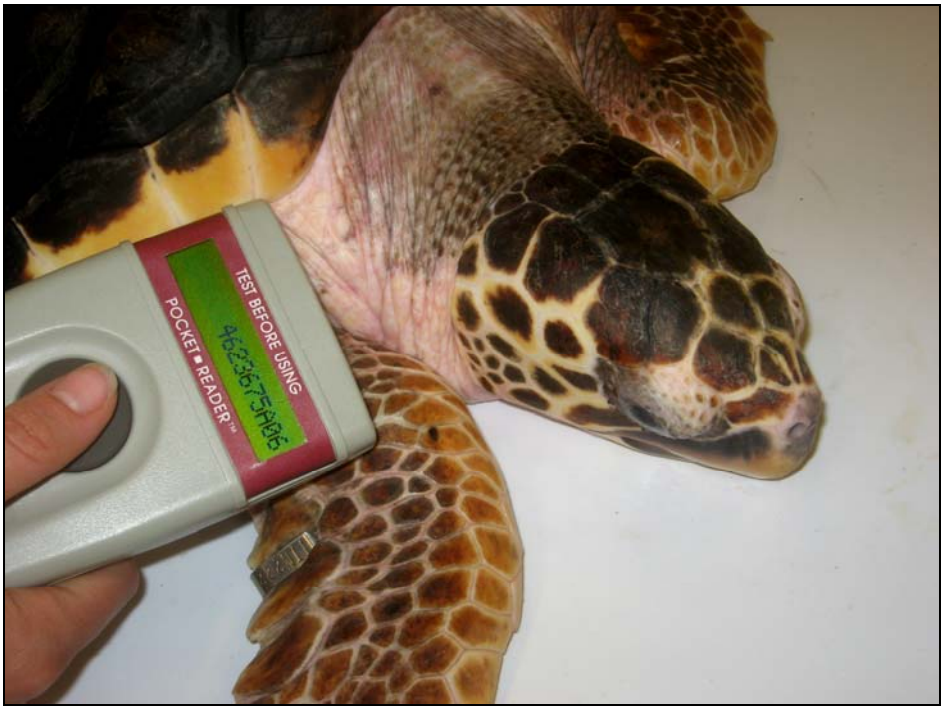

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Preface

This document is a compilation of the current research techniques and protocols of the National Marine Fisheries Service (NMFS) Southeast Fisheries Science Center (SEFSC). This sea turtle research techniques manual was developed in support of NMFS/SEFSC research permit applications and to provide a comprehensive training document for NMFS researchers and fishery observers. Methods vary among researchers, but the techniques described here are accepted by the SEFSC after consultation with research, academic, and veterinary colleagues.

Only authorized personnel may conduct the procedures described in this manual while working with listed threatened or endangered sea turtles. The Endangered Species Act of 1973 prohibits any person from harassing, harming, pursuing, hunting, shooting, wounding, killing, trapping, capturing or collecting any listed threatened or endangered species. Authorization to "take" (as described in the previous sentence) a listed threatened or endangered species must be granted under an ESA Section 10(a)(1)(B) permit or similar authorization. Additional state permits or import permits may be required as well. When conducting research, authorized personnel must carry all relevant permits and authorization letters and follow all terms and conditions, including reporting requirements, as outlined in the permit(s).

While this document represents the best practices currently available, sea turtle research is a dynamic field, and new techniques and technologies may become available in the future. Periodic updates will be made to this document to reflect these changes, and revised documents will be available online at:

http://www.sefsc.noaa.gov/seaturtletechmemos.jsp

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Chapter 1: Species Identification

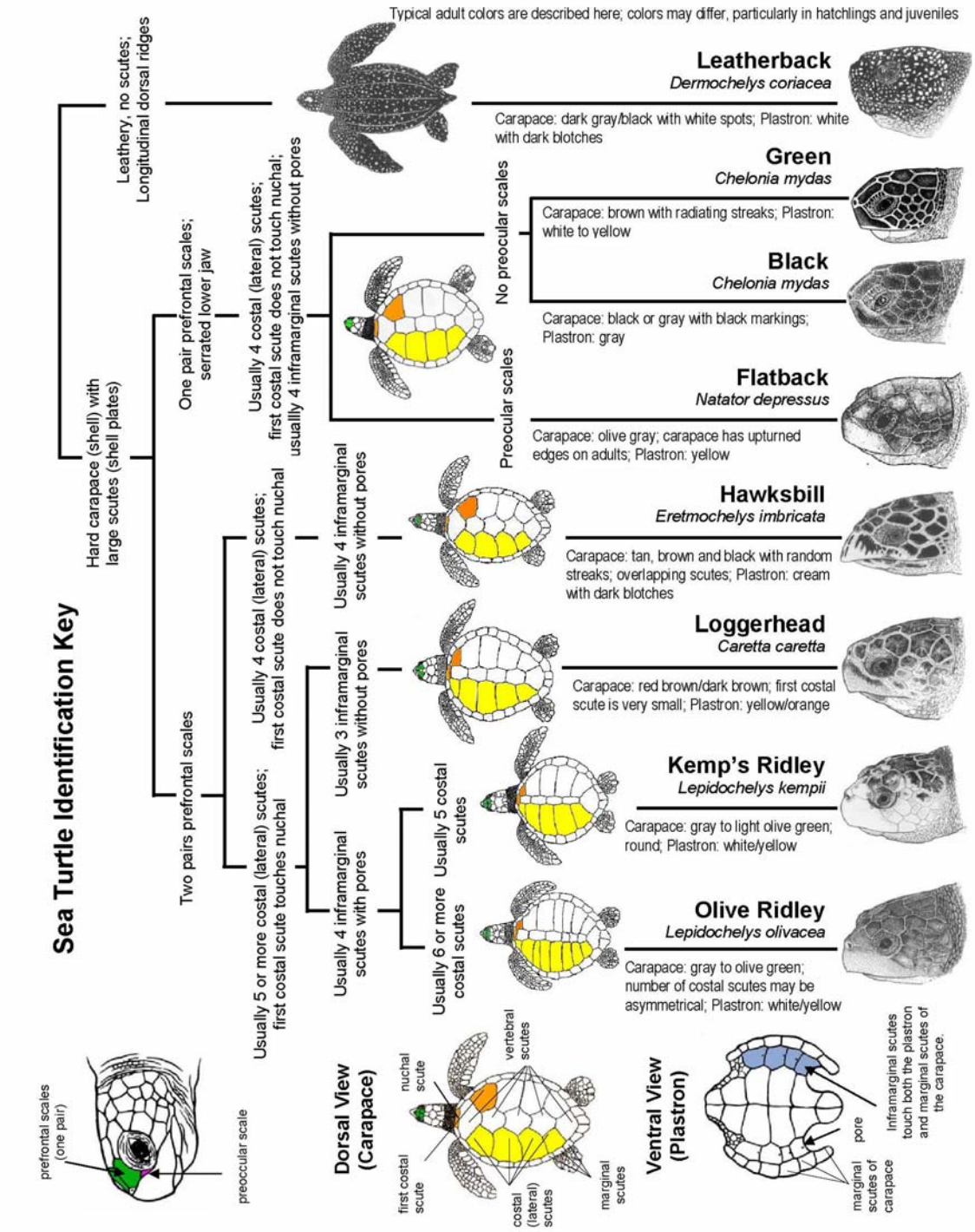

Figure 1-1. Sea turtle identification key (NMFS/SEFSC diagram modified from seaturtle.org).

seaturtle.org

Leatherback, ***Dermochelys coriacea*** (**Spanish:** Baula, Tortuga Laúd, Tora, Cardón, Tinglar; **French:** Tortue Luth; **Portuguese:** Tartaruga Gigante, Tartaruga-de-couro)
Adult Size Range: Length: 165-190+ cm/ 65-75+ in; **Weight:** 400-500 kg females, males to 900 kg/ 885-1985 lb
Range: All oceans, sub-arctic to tropical; mainly pelagic oceanic (surface dwelling in the open ocean) but found in bays and over continental shelves

Green, Black*, ***Chelonia mydas*** (**Spanish:** Tortuga Verde, Tortuga Blanca; Tortuga Negra, Prieta; **French**: Tortue Verte; **Portuguese:** Tartaruga Verde, Aruanã)
Adult Size Range: Length: 90-120 cm/ 35-45 in; **Weight:** 120-230 kg/ 265-510 lb
Range: All subtropical and tropical seas; bays and coastal waters; black form restricted to eastern Pacific Ocean; pelagic oceanic (surface dwelling in the open ocean) small juveniles; benthic neritic (bottom dwelling in coastal waters) large juveniles and adults
*The status of the black turtle or eastern Pacific green turtle as *Chelonia agassizii* or *C. mydas agassizii* as a distinct species or subspecies is not supported, although it is often treated as such.

Flatback, ***Natator depressus*** (**Spanish:** Kikila, Tortuga Aplanada, Tortuga Franca Oriental; **French:** Chelonée à dos Plat; **Portuguese:** Tartaruga de Casco Achatado)
Adult Size Range: Length: to 100 cm/ 40 in; **Weight:** to 90 kg/ 200 lb
Range: Tropical coastal Australia, including the waters up to Irian Jaya, Papua New Guinea and Java; pelagic neritic (surface dwelling in coastal waters)

Hawksbill, ***Eretmochelys imbricata*** (**Spanish:** Tortuga Carey; **French:** Tortue Imbriquée, Tortue Caret; **Portuguese:** Tartaruga-de-pente, Tartaruga de Escamas, Tartaruga Bico de Falcão, Tartaruga Verdadeira)
Adult Size Range: Length: 90-110+ cm/ 35-45+ in; **Weight:** 60-80 kg/ 130-175 lb
Range: All oceans; tropical waters, rarely subtropical; reef areas; pelagic oceanic (surface dwelling in the open ocean) small juveniles; benthic neritic (bottom dwelling in coastal waters) large juveniles and adults

Loggerhead, ***Caretta caretta*** (**Spanish:** Caguama, Amarilla, Cabezona, Tortuga Boba; **French:** Caouanne; **Portuguese:** Tartaruga Boba, Tartaruga Comum, Tartaruga Careta, Tartaruga Cabeçuda, Tartaruga amarela, Careba Dura, Careba Amarela)
Adult Size Range: Length: 90-130 cm/ 35-50 in; **Weight:** 100-180 kg/ 220-400 lb
Range: All oceans; primarily subtropical and temperate waters; often associated with structures (i.e., reefs, wrecks, platforms); pelagic oceanic (surface dwelling in the open ocean) small juveniles; benthic neritic (bottom dwelling in coastal waters) large juveniles and adults

Kemp's Ridley, ***Lepidochelys kempii*** (**Spanish:** Tortuga Lora, Cotorra; **French:** Tortue de Kemp; **Portuguese:** Tartaruga de Kemp)
Adult Size Range: Length: to 70 cm/ 28 in; **Weight:** 35-50 kg/ 80-110 lb
Range: Gulf of Mexico, eastern USA, rarely in eastern North Atlantic; coastal, primarily subtropical and temperate waters; pelagic oceanic (surface dwelling in the open ocean) small juveniles; benthic neritic (bottom dwelling in coastal waters) large juveniles and adults

Olive Ridley, ***Lepidochelys olivacea*** (**Spanish:** Tortuga Golfina, Tortuga Olivacea, Parlama, Guaraguá, Maní; **French:** Tortue Olivâtre; **Portuguese:** Tartaruga Oliva, Tartaruga Olivácea, Tartaruga Pequena, Xibirro)
Adult Size Range: Length: 70-80 cm/ 28-32 in; **Weight:** 35-60 kg/ 80-130 lb
Range: Pacific, Indian and Atlantic Oceans, rarely in eastern North Atlantic; pelagic oceanic (surface dwelling in the open ocean); most often in tropical waters

Sources:

Seaturtle.org

Pritchard, P. C. H. and Mortimer, J. A. (1999) Taxonomy, External Morphology, and Species Identification. pp. 21-38. In: Eckert, K.L., K.A. Bjorndal, F.A. Abreu-Grobois, and M. Donnelly (Editors). 1999. Research and Management Techniques for the Conservation of Sea Turtles. IUCN/SSC Marine Turtle Specialist Group Publication No. 4. (for further details see http://www.iucn-mtsg.org/publications.htm)

Wyneken, J. The Anatomy of Sea Turtles. 2001. U.S. Department of Commerce NOAA Technical Memorandum NMFS-SEFSC-470, 172 pp.

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Illustrations by Tom McFarland and Dawn Witherington

Chapter 2: Handling

All Turtles

After capture, every turtle should be assessed to determine their general state of health and suitability for subsequent research procedures, including an examination of the oral cavity (see Chapter 4: Oral Cavity Anatomy). Remove any attached gear if applicable (see NMFS 2008), and attempt to resuscitate all comatose turtles (see Chapter 3: Resuscitation) when necessary. Successfully resuscitated turtles benefit from being held on deck as long as possible (up to 24 hours) when conditions permit to allow stress toxins to dissipate from the body. All captured turtles should be subject to standard processing protocols before release: identification, standard measurements, weight, photographs, flipper and PIT tags, and skin biopsies (in select cases). Some may be subject to the additional procedures described in this manual, depending on the results of their general assessment and the directed research needs.

Figure 2-1. Keep the turtle moist and in the shade (NMFS/SEFSC photo).

Turtles should be protected from temperature extremes of heat and cold, provided adequate air flow, and kept moist during sampling. Keep the skin and eyes moist while the turtle is on deck; cover the animal's body with a wet towel (Figure 2-1) and periodically spray it with water or apply petroleum or water-based lubricant jelly to the skin and carapace. If using towels, pay particular attention to the ambient temperature, as evaporative cooling may chill the animal under some conditions. If the turtle is to be held out of water for an extended period of time (e.g., transport, surgery) or in cool air temperatures, use petroleum or water-based lubricant jelly on the skin as necessary to avoid drying instead of using wet toweling. Keep the turtle in the shade, maintaining its body temperature between 60° and 90° F, similar to water temperatures at capture. If air temperatures are greater than 80° F, ensure that the turtle does not overheat; conversely, if air temperatures are less than 60° F, ensure that the turtle does not become hypothermic. Safely isolate the turtle and immobilize it on a cushioned surface such as a foam pad, an automobile tire or similar. The area surrounding the turtle should not contain any materials that could be accidentally ingested.

Transport turtles in individual containers to ensure that they are unable to injure themselves or each other. Do not transport turtles in open vehicles during excessive heat or cold. Do not pick up turtles by their flippers, but rather, lift them by grasping both sides of the carapace (a better support of their weight) or use a stretcher that provides adequate support. In order to minimize the

risk of either introducing a new pathogen into a population or amplifying the rate of transmission of an endemic pathogen from animal to animal, thoroughly clean containers in which turtles are being transported with soap and water and disinfect them with a mild bleach solution.

Conduct field and laboratory procedures using latex or similar disposable gloves whenever possible. Remove the gloves following the proper method: 1) Grip one glove on the outside of the glove near the cuff. Reflect and peel it down until it comes off inside out. Cup it with your other gloved hand. 2) Place 2 fingers of your bare hand inside the cuff of the glove that is still on your hand. 3) Peel that glove off so that it comes off inside out with the first glove inside it. During these steps, take care not to snap the glove during the removal so that material could spray or aerosolize. 4) Dispose of the gloves in an appropriate container and thoroughly wash your hands with soap and water.

All equipment (tagging equipment, tape measures, etc.) that comes into contact with sea turtle body fluids, cuts, or lesions must be disinfected between the processing of each turtle. Whenever feasible, equipment that does not contact fluids, cuts, or lesions should be disinfected between the processing of each turtle as well. To disinfect field equipment, use an appropriate disinfectant such as a freshly mixed 1:10 solution of household bleach (~5 – 6 % sodium hypochlorite). To prepare 1:10 bleach solution, add one volume of household bleach (e.g., 1 cup, liter) to 10 volumes of clean water (e.g., 10 cups, liters). Spray or soak equipment for at least 2 minutes for equipment disinfection and use fresh solution each time.

Figure 2-2. Green turtle displaying fibropapilloma tumors (Photo courtesy of the Turtle Hospital, Marathon, Florida).

NOAA Fisheries researchers (including fishery observers) must maintain a separate set of sampling equipment for handling animals displaying fibropapilloma (FP) tumors or lesions (Figure 2-2). Whenever an animal suspected of having FP tumors is encountered, care must be taken to ensure that the same equipment is not later used on other turtles. For most, this means that some equipment (e.g., calipers, scales) should not be used on affected turtles because one is not likely to have a duplicate set. If a spare set of calipers is not available, use a tape measure and record curved measurement only. Quarantine the tape measure and use the spare until the original tape measure can be disinfected. Use the same protocols for tagging pliers and PIT tag injectors – quarantine the equipment and then use spares thereafter. The PIT tag scanner may be used again after removing and disposing of the plastic bag around the PIT tag reader and replacing it with a new plastic bag. NOAA Fisheries observers in the field should place contaminated equipment (used on a turtle displaying FP tumors) into a plastic bag

thorough disinfection as soon as possible. Any equipment that comes into contact with animals displaying FP tumors must be disinfected using bleach solution (as described above).

During release, turtles should be lowered as close to the water's surface as possible, in water of similar temperature as capture, when fishing gear is not in use (if applicable) and engines are in neutral.

Leatherback Turtles

Exercise extra care when handling, sampling and releasing leatherback turtles during directed capture research activities (Figure 2-3), as field and laboratory observations indicate that they have more friable skin and softer bones than hardshell turtles. Leatherback turtles shall not be turned on their backs. Precautions shall be taken to ensure that animals are supported from underneath during handling and release.

The additional recommended monitoring protocols will be taken for animals captured during directed research activities. In order to improve monitoring of the animals during directed capture and to improve our basic understanding of the biology and medical status of leatherbacks, a designated observer should be on each capture outing team. Whenever possible, this observer should be a veterinarian; however, a dedicated observer with training in the techniques required for this position is also acceptable.

Figure 2-3. Handling a leatherback during directed research capture activities (NMFS/SEFSC photo).

Recommended Monitoring Protocols:

- Perform a gross examination upon capture, including assessment of body fat (subjective), activity, alertness, pre-existing injuries, weight and length.
- Record respiratory rate over a two-minute period, logged every 20 minutes.
- Record response to noxious stimuli (either tail pinch or blink response), logged every 20 minutes.
- Record heart rate determined by digital or Doppler detection on femoral artery, ultrasound, rectal pulse oximiter, or EKG, logged every 20 minutes.
- Record body temperature detected by anal probe inserted 15 cm, logged every 20 minutes.
- Assure cooling by running ambient seawater over the carapace and forelimbs during the time on deck.
- Collect two tubes of blood in a clot tube and urine or feces if possible.
- Relate changes in the animal's condition to the chief scientist so that an ongoing assessment of the animal's condition can be made.

The chief scientist for each outing will be trained by a veterinarian in the following information and procedures:

- Acceptable parameters for heart rate, respiration, temperature, and responsiveness, as defined by baseline data gathered in the field as well as in collaboration with veterinarians and colleagues from NMFS/SWFSC.

- Appropriate response to changes suggesting a need to abort further animal handling and initiate release.

- Safe water reintroduction and monitoring of a turtle in possible distress.

- Appropriate first aid measures for animals in distress. These measures may include intubation, artificial respiration, and administration of pharmaceuticals to stimulate respiration and/or cardiac contraction.

During laboratory procedures, monitor each turtle manually, noting its response to stimuli (surgical stimuli, eye reflex, withdrawal reflex) and respiratory intervals. Monitor the following parameters on each turtle using instruments such as electrocardiogram (ECG or EKG), blood gases, and cloacal temperature (to allow temperature corrections for blood gases).

Adverse reactions could be indicated by cardiac arrhythmia, cardiac arrest, respiratory arrest, seizures, or severe blood gas alterations. Veterinarians are still in the process of defining normal and altered blood gas parameters by establishing baselines, but alarming values would be recognized (Dr. Craig Harms, pers. comm.). The response to adverse reactions would depend on the type of reaction, but would likely involve basic supportive therapy including intubation and assisted respirations, IV fluids (for shock and to hasten elimination of drugs through renal excretion), anti-arrhythmic drugs (e.g., IV lidocaine for VPCs), cardioresuscitory drugs (e.g., IV epinephrine for cardiac arrest), or anti-seizure medication (e.g., IV diazepam).

Avoid any animal deemed to be in distress at any time during the pre-capture period. In addition to animal monitoring, include an emergency field kit for intervention on each directed capture research outing. This kit should be available to the field team veterinary observers or the chief scientist and should include:

- Oxygen canister and a demand breathing valve
- Endotracheal tubes
- Oral speculum and appropriate sized blade
- Water-based lubricant jelly
- Betadine® ointment or similar
- Gauze sponges
- Medical tape
- Isopropyl alcohol
- Needles and syringes (various sizes)

  Doxapram, epinephrine, lidocaine, furosemide, diazepam, dexamethasone sodium phosphate, sodium bicarbonate, and saline solution

Chapter 3: Resuscitation

If a turtle appears to be comatose or unresponsive, as determined by testing for bilateral responsiveness (Figure 3-1), attempt to revive the turtle (Figures 3-2a and b) before putting it back into the water. A fully conscious turtle has bilateral reflexes and has a central (e.g., brain) recognition of the stimulus. An unresponsive turtle will not have full bilateral responses nor central recognition of a stimulus. A comatose turtle will have lost all reflexes. To test eye reflexes, check for a blink response by gently touching the corner of the eye or eyelid. Pinch both front and rear flippers and the tail to check for response; a lack of bilateral response for any of these tests may indicate the need for resuscitation. Use the method of resuscitation described on the following Sea Turtle Resuscitation Guidelines (66 FR 67495, December 31, 2001). Regulations (66 FR 67495, December 31, 2001; 50 CFR 223.206) allow a fisherman to keep incidentally captured turtles on deck up to 24 hours for resuscitation purposes.

Figure 3-1. Testing eye reflex (NMFS/SEFSC photo).

Figures 3-2a and b. Resuscitation position with 15-30° elevation on (a) a cushioned surface and (b) on a standard automobile tire (NOTE: a slightly greater angle of head inclination than depicted in photo (b) would be preferable to better facilitate water drainage.) (NMFS/SEFSC photos).

Successfully resuscitated turtles benefit from being held on deck as long as possible, when conditions permit, to allow stress toxins to dissipate from the body. Keep the skin and the eyes moist while the turtle is on deck (Figure 3-2b) by covering the animal's body with a wet towel, periodically spraying it with

water, or by applying petroleum or water based lubricant jelly to its skin and carapace. Comatose or unresponsive turtles captured during directed research activities should be transported as quickly as possible to a rehabilitation facility whenever feasible.

A turtle that has shown no sign of life after 24 hours on deck (held in the shade, kept moist and its body temperature maintained above 60° F) may be considered dead. If the turtle cannot be revived before returning to port, it should be returned to the water, preferably in a non-fishing area. Mark the turtle (spray paint it or tag it) before returning it to the water.

In the past, an alternative method of resuscitation known as plastral pumping was recommended (see FR 43 32801, July 28, 1978; 57 FR 57354, December 4, 1992). This practice involved placing the turtle on its carapace and pumping the plastron with a hand or foot. However, we strongly discourage this technique, as further study determined that it may actually do more harm than good and should not be attempted during resuscitation (per 66 FR 67495, December 31, 2001). Plastral pumping may cause the airway to block and cause the viscera to compress the lungs which are located dorsally, thereby hindering lung ventilation.

Sea Turtle Resuscitation Guidelines

If a turtle appears to be unresponsive or comatose, attempt to revive it before release. Turtles can withstand lengthy periods without breathing; a comatose sea turtle will not move, breathe voluntarily, or show reflex responses or other signs of life. In other cases, an unresponsive turtle may show shallow breathing or reflexes such as eyelid or tail movement when touched. Use the following method of resuscitation in the field if veterinary attention is not immediately available:

- **Place the turtle on its plastron (lower shell) and elevate the hindquarters approximately 15 - 30 degrees to permit the lungs to drain off water for a period of 4 up to 24 hours. A board, tire or boat cushion, etc. can be used for elevation.**

- **Keep the turtle in the shade, at a temperature similar to water temperature at capture. Keep the skin (especially the eyes) moist while the turtle is on deck by covering the animal's body with a wet towel, periodically spraying it with water, or by applying petroleum jelly to its skin and carapace. Do not put the turtle into a container with water.**

- **Do not put the turtle on its carapace (top shell) and pump the plastron (breastplate) or try to compress the turtle to force water out, as this is dangerous to the turtle and may do more harm than good.**

- **Periodically, gently touch the corner of the eye or eyelid and pinch the tail near the vent (reflex tests) to monitor consciousness.**

- **Sea turtles may take some time to revive; do not give up too quickly. Turtles that are successfully resuscitated benefit from being held on deck as long as possible (up to 24 hours) to fully recover from the stress of accidental forced submergence.**

- **Release successfully resuscitated turtles over the stern of the boat, when fishing or scientific collection gear is not in use, the engine is in neutral, and in areas where they are unlikely to be recaptured or injured by vessels. A turtle that has shown no sign of life after 24 hours on deck may be considered dead and returned to the water in the same manner.**

NMFS/SEFSC Photos

References:

Federal Register, December 31, 2001. Government Printing Office, Washington DC 66 (250), pp. 67495- 67496.

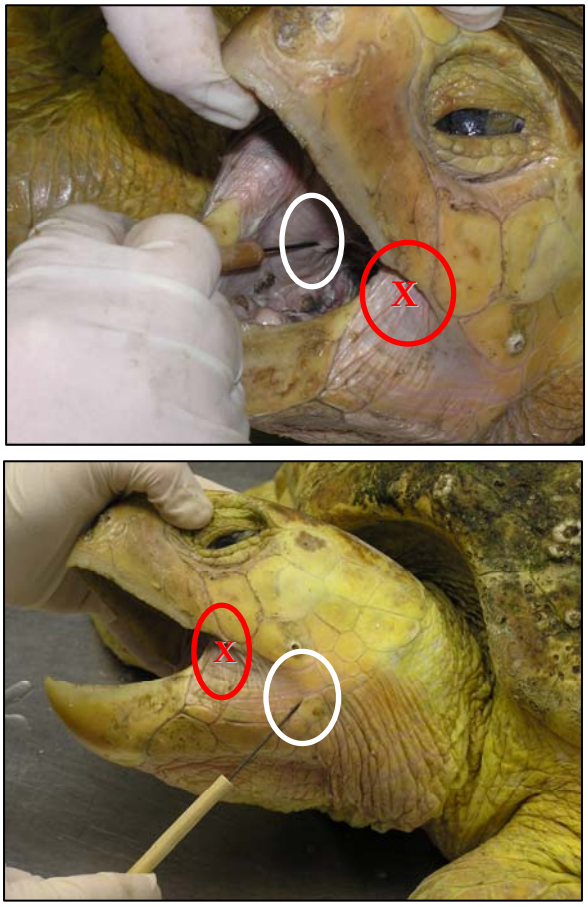
October 2008

Figure 3-3. Resuscitation guidelines (NMFS/SEFSC).

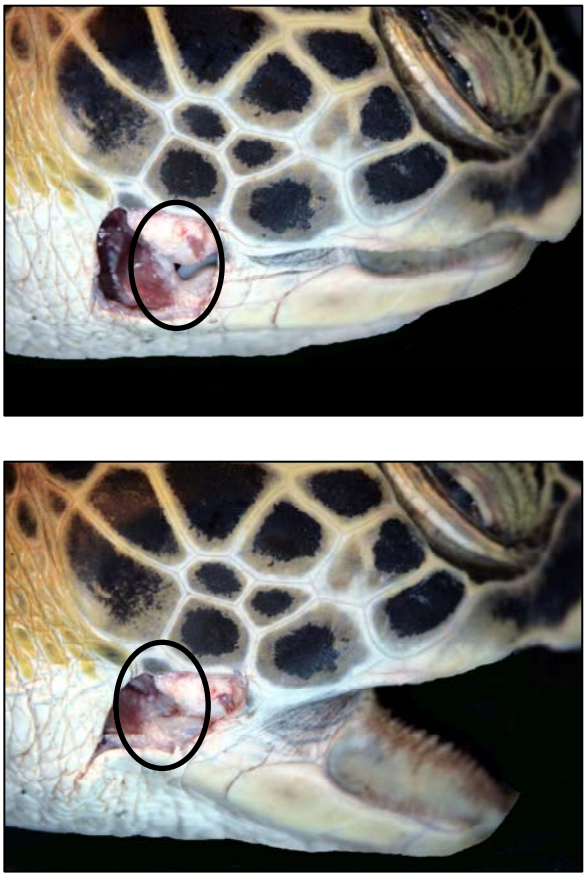
Chapter 4: Oral Cavity Anatomy

The oral cavity is described here to assist in performing general health assessments and to identify the location of hooks in incidentally captured turtles, especially to distinguish hooks that are swallowed from those lodged in the oral cavity (Figures 4-1 ─ 4-7). The anatomy details described here are intended primarily to provide the basic knowledge necessary to assess whether hook removal may cause further injury. Do not attempt to remove hooks when it appears that removal will cause further serious injury to the turtle. For example, the removal of hooks lodged in the jaw joint (Figures 4-1 and 4-2) the glottis, or in the esophagus where the insertion point is not visible may cause greater injury to the turtle than leaving the hook in place. For all hooked animals, follow the guidance in the NOAA Technical Memorandum NMFS-SEFSC-580, Careful Release Protocols for Sea Turtle Release with Minimal Injury (NMFS SEFSC 2008).

The upper and lower beak (Figure 4-3), or rhamphotheci, of hardshell sea turtles are keratinized and cover many of the bones of the upper jaw and dentary of the lower jaw. They differ among species and can be used for identification. The tongue (Figures 4-3 and 4-4) is a large, nonprotrusible, muscular organ fixed to the floor of the mouth. The glottis (Figures 4-4 and 4-5), the opening to the trachea and the valve to open and close the airway, is located at the back of the tongue. The esophagus (Figure 4-6) starts at the back of the mouth behind the tongue and links the oral cavity to the stomach. Most of the length of the esophagus is lined with sharp, keratinized papillae that angle toward the stomach. These are presumed to trap food, preventing food particles from being regurgitated when excess water is expelled. The roof of the mouth (Figure 4-7) is ventral to the braincase.



Figures 4-1a and b. Internal view (a) and external view (b) of jaw joint location, indicated by the pointer. The jaw joint should not be confused with "the corner of the mouth" indicated here in red. (NMFS/SEFSC photos).



Figures 4-2a and b. External view of jaw joint location with skin and muscle removed, shown with the jaws closed (a) and open (b). (Photos by J. Wyneken, Florida Atlantic University).

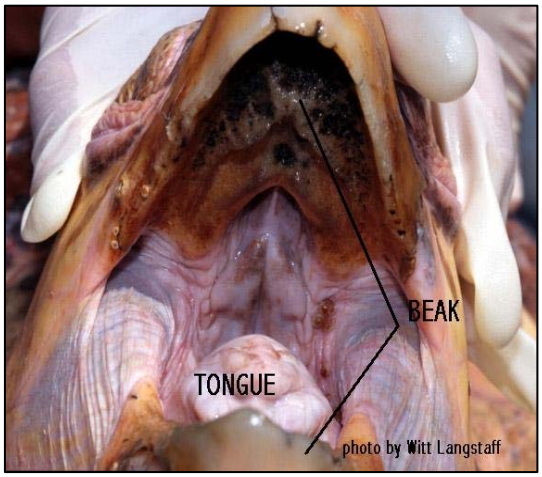


Figure 4-3. The upper and lower beak, or rhamphotheci, of a loggerhead (Photo by W. Langstaff).

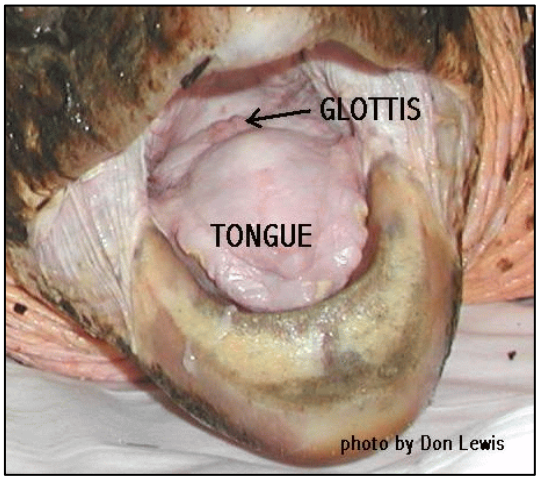
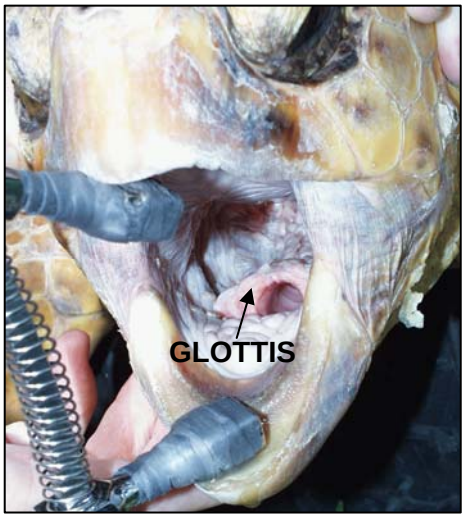
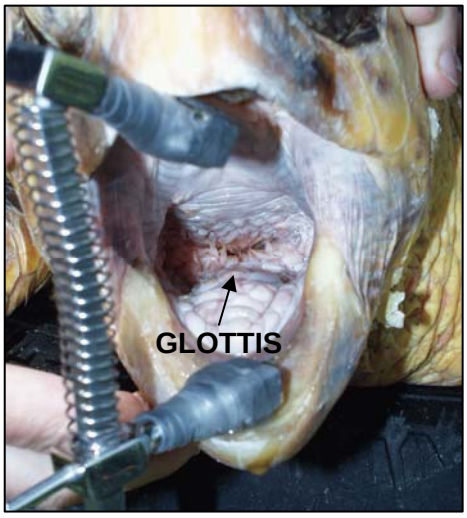


Figure 4-4. The tongue and glottis, which is closed in this photograph (Photo by D. Lewis).





Figures 4-5a and b. Glottis (a) open and (b) closed (Photos by C. Harms, N.C. State University).

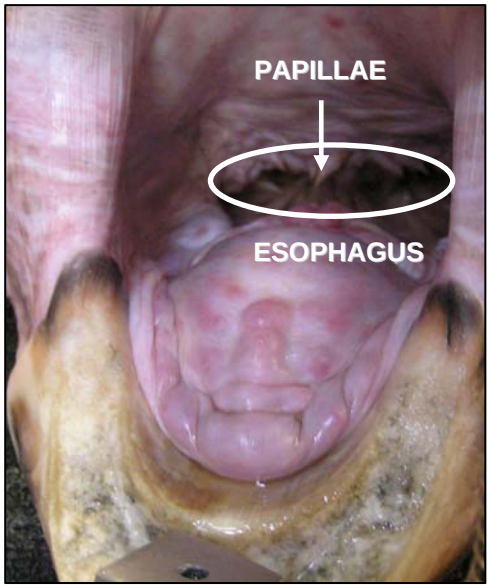


Figure 4-6. The entrance of the esophagus is marked by the presence of papillae (NMFS/SEFSC photo).

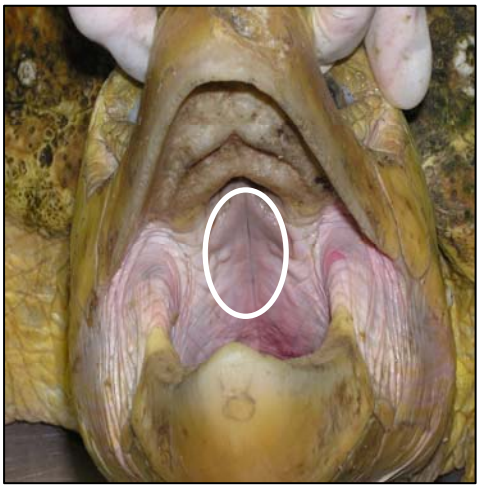


Figure 4-7. Roof of the mouth and upper jaw (NMFS/SEFSC photo).

Chapter 5: Morphometrics

Standard Measurements

If the turtle can be brought onboard or on land, take standard carapace measurements: CCL, SCL_{STD}, SCL_{MIN}, CCW, and SCW. Use a flexible fiberglass tape measure to take over-the-curve measurements and calipers for straight measurements; record in centimeters, rounded to the nearest 0.1 cm. For measurements over-the-curve (CCL and CCW), follow the curvature of the carapace. If barnacles affect these measurements, record this in the comments on the datasheet. For leatherbacks, generally only curved measurements are taken.

Figure 5-1. Curved carapace length taken with flexible fiberglass tape measure (NMFS/SEFSC photo).

Methodology to weigh turtles will differ slightly depending on the type of scale available, but in all cases, the turtle must be adequately restrained so there is no potential for injury from this procedure. The scale, sling or platform used should be disinfected between animals when practicable.

Figure 5-2. Straight carapace length (SCL) measurement, notch-to-tip (NMFS/SEFSC photo).

CCL – Curved Carapace Length, standard (notch-to-tip): Record the distance between the center of the nuchal scute and the posterior tip of the longest postcentral scute, following the curvature of the dorsal centerline (Figures 5-1 and 5-3). On leatherbacks, take the measurement alongside (not over the top) the central vertebral ridge (Figure 5-4).

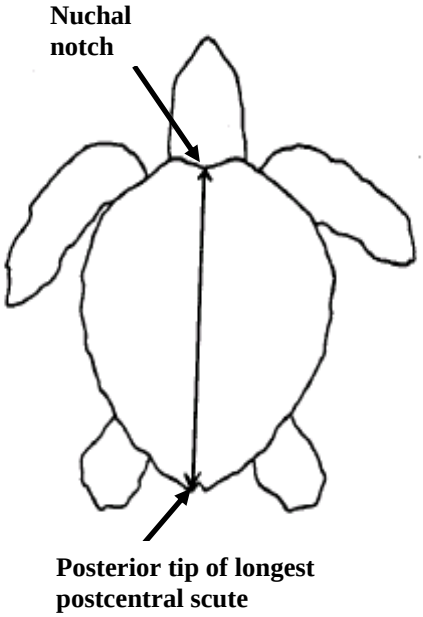


Figure 5-3. Carapace length (CCL and SCL) measurement, notch to tip [Figure modified from Bolten (1999)].

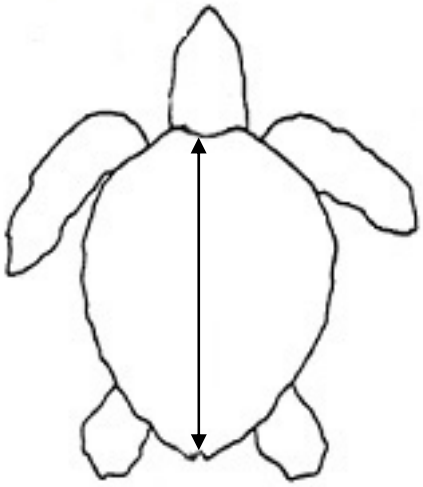


Figure 5-5. Carapace length (CCL and SCL) measurement, notch to notch [Figure modified from Bolten (1999)].

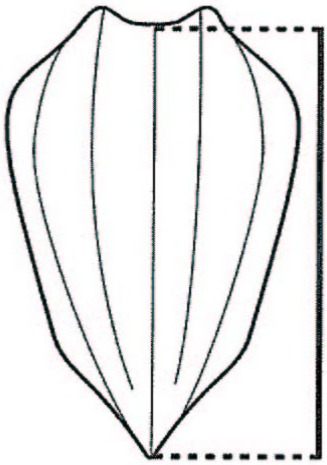


Figure 5-4. Curved carapace length (CCL) and straight carapace length (SCL) in leatherback turtles. In both cases, length is measured from the nuchal notch (anterior edge of the carapace at the midline) to the posterior tip of the caudal peduncle [Figure and caption text taken from Bolten (1999)].

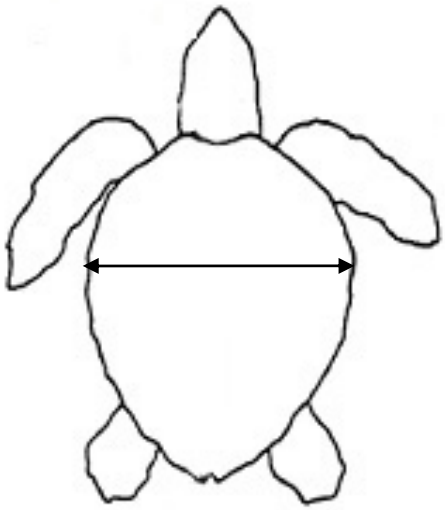


Figure 5-6. Carapace width (CCW and SCW) measurement [Figure modified from Bolten (1999)].

SCL_{STD} – Straight Carapace Length, standard (notch-to-tip): Record the distance between the center of the nuchal scute and the posterior tip of the longest postcentral scute (Figures 5-2 and 5-3).

SCL_{MIN} – Straight Carapace Length, minimal (notch-to-notch): Record the distance between the center of the nuchal scute and the notch between the two postcentral scutes (Figure 5-5).

CCW – Curved Carapace Width: On leatherbacks, measure the width from side ridge to side ridge (ridges depicted in Figure 5-4) at the widest point. On hardshell turtles, record the maximum distance between the lateral edges of the carapace, measured over the curvature of the shell, perpendicular to the longitudinal axis of the carapace at the widest point (Figures 5-6 and 5-7).

SCW – Straight Carapace Width: Record the maximum distance between the lateral edges of the carapace taken perpendicular to the longitudinal axis of the carapace at the widest point (Figures 5-6 and 5-8).

Figure 5-7. Curved carapace width (CCW) measurement (NMFS/SEFSC photo).

Figure 5-8. Straight carapace width (SCW) measurement (NMFS/SEFSC photo).

Additional Measurements

Additional measurements (maximum carapace length, maximum head width, maximum head length, body depth, plastron length, total tail length, plastron-to-vent length, vent-to-tip length, and circumference) may be taken as needed, following the protocols of Wyneken (2001).

Oral Cavity Measurements

Measures of the jaw and internal oral cavity anatomy may be taken to investigate oral cavity dimensions, particularly as they relate to a turtle's ability to swallow hooks of various sizes. All measures are taken using spring and/or dial calipers while the mouth is held open with a canine mouth gag (a type of oral speculum available from veterinary equipment suppliers). The canine mouth gag tips should be padded to reduce damage to the beak as the turtle bites down on the gag. All mouth measurement instruments should be cold sterilized using 2% chlorhexidine gluconate or similar between each use.

These oral cavity measures include:

Internal Gape Width: Measure is taken with spring calipers at the midpoint of the lateral oral commissures, the soft tissue connecting upper and lower jaws at the angles of the mouth, while the jaws are held open to their full extent with a canine mouth gag. Fixed spring caliper distance is then measured using dial calipers.

Esophagus Width: Measure is taken with spring calipers at the entrance of the esophagus (Figure 5-9), marked by the first presence of papillae. This distance is then measured with dial calipers. Note: this is a flexible opening, and the measurement represents a close approximation of the unstretched diameter of the esophagus width.

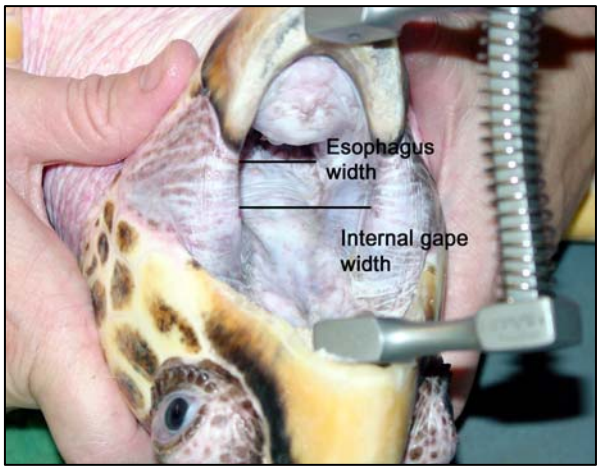


Figure 5-9. Internal oral cavity measurements: internal gape width, esophagus width (NMFS/SEFSC photo).

Figure 5-10. Gape Height (NMFS/SEFSC photo).

Gape Height: Measure is taken using dial calipers while jaws are held open to full extent with a canine mouth gag (Figure 5-10), representing the maximum internal distance between the distal points of the upper and lower jaw.

Upper Jaw Length: Measure is taken with dial calipers from the soft tissue at the insertion point of the rhamphotheca (keratinaceous beak) to the distal point of the upper jaw (Figure 5-11).

Lower Jaw Length: Measure is taken with dial calipers from the soft tissue at the insertion point of the rhamphotheca (keratinaceous beak) to the distal point of the lower jaw (Figure 5-12).

Figure 5-11. Upper jaw length (NMFS/SEFSC photo).

Figure 5-12. Lower jaw length (NMFS/SEFSC photo).

Chapter 6: Marking

Temporary Marking

Turtles may be temporarily marked using a non-toxic substance (e.g., paint, livestock paint sticks, non-toxic fingernail polish). No potentially harmful or toxic paints, such as xylene or toluene-based paints, or those containing tributyl tin and cyanide or copper cyanide, should be used. No reflective paints or paints with exothermic set-up reactions should be used. Paint should be applied without crossing the suture lines separating the scutes whenever possible.

Shell Etching

An etching tool such as a Dremel® with a pear-shaped bit can be used to place an etch or groove in the carapace of hardshell turtles. The bit and carapace should be disinfected before use, and the groove should not penetrate the scute. The groove could be marked with non-toxic paint if desired. Care should be exercised when choosing this technique, as discomfort may result from the procedure.

Flipper Tags

If a turtle is encountered without flipper tags, apply two new flipper tags to the trailing edge of the rear flippers just proximal to the first scale. If this site is unsuitable (lesions, scars, missing flippers, etc.), locate an alternate site along the trailing edge of a suitable flipper (i.e., the trailing edge of the front flipper(s) immediately proximal and adjacent to the first scale, or between the first and second large scales distal to the axilla). Turtles larger than 30 cm SCL should generally receive flipper tags. Experienced taggers may be comfortable tagging smaller animals in some cases. Extra care should be taken when positioning the tag in smaller animals to allow room for growth, although the tag should be positioned to allow for growth on all turtles. Check carefully for previous tagging scars on both front and rear flippers and note if present.

There may be circumstances where a previously applied tag will need to be removed prior to applying a new one. If a tag is damaged, covered in fouling organisms (e.g., barnacles) that cannot be removed, or if the tag appears to be in danger of coming off, the tag should be removed and replaced with a new tag. There may also be situations where a tag may be improperly placed (i.e., overgrown with tissue or tearing out), or injurious to the animal. In these situations, the tag should be carefully removed and replaced at the discretion of the tagger if they feel that removal will not cause further injury. Generally, the tag can be removed using two pairs of pliers to uncrimp the tip, but wire or bolt cutters may be necessary. If a previously applied tag is removed, the identification number should be recorded, and the tag should be reported to the original tagging project and the Cooperative Marine Turtle Tagging Project (CMTTP). Return the voided tag to the CMTTP or program coordinator.

To apply self-piercing, self-locking Inconel® alloy flipper tags:

> **(1)** Remove a tag from the strip (Figure 6-1) and record its identification number on the tagging form. Be careful not to bend the tag from its original shape. Only peel back enough tape on the

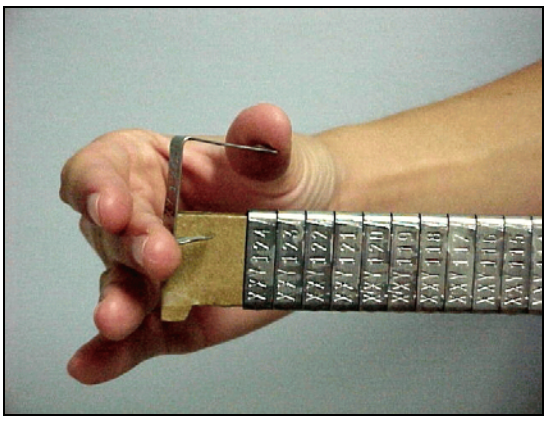


Figure 6-1. Remove cleaned tag from strip (NMFS/SEFSC photo).

Figure 6-2. Inserting the tag into the applicator (NMFS/SEFSC photo).

strip to remove one or two tags at a time to prevent loss of remaining tags. Scrub all tags with hot, soapy water to remove the oily residue present when shipped from manufacturer and disinfect with isopropyl alcohol or 10% povidone-iodine solution prior to use. Tags provided to NMFS/SEFSC observers will be cleaned before distribution.

(2) Hold the applicator in one hand. With the pointed (piercing) side of the tag facing the depression in the jaw of the pliers and with the hole placed adjacent to the depression, place the end of your index finger of the other hand inside the tag against the bend. Pull the tag straight back into the open jaws of the applicator, aligning the point opposite the small depression (Figure 6-2). A firm pull will be needed to snap the tag completely into the correct position. Take care not to squeeze the applicator together before you are ready to tag the turtle or the tag will fall out. Swab the tag, applicator tips, and tagging site with 10% povidone-iodine solution.

(3) *Rear Flipper Tagging (preferred site):* Locate the correct site (Figure 6-3) to apply the tag (the trailing edge of the rear flipper just proximal to the first scale). Juvenile and subadult hardshell turtles can be placed on their carapace to facilitate access to the tagging site. If someone is available to help, they should hold the turtle and restrain the flipper while the tag is applied. Be sure to position the tag so there will be adequate overhang (approximately 1/3 the length of the

Figure 6-3: Applying an Inconel® tag to the rear flipper of a loggerhead turtle (NMFS/SEFSC photo).

tag) after it is attached to the flipper (Figure 6-4).

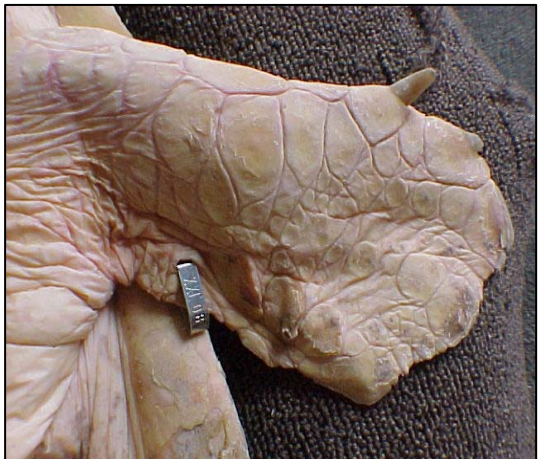


Figure 6-4. Inconel® tag applied to the rear flipper of a sea turtle (NMFS/SEFSC photo).

Front Flipper Tagging: Although the rear flipper is the preferred location, there may be circumstances where the front flipper is tagged instead. Place the turtle on its plastron and locate the correct site to apply the tag (the trailing edge of the front flipper(s) immediately proximal and adjacent to the first scale (Figure 6-5), or between the first and second large scales distal to the axilla). If someone is available to help, they should hold the turtle and restrain the flipper while the tag is applied. Be sure to position the tag so there will be adequate overhang (approximately 1/3 the length of the tag) after it is attached to the flipper.

(4) Apply the tag by squeezing the applicator together in a firm, steady manner. The tag point will pierce the flipper and lock into place with the tip bending securely over the opposite side like a staple point. Squeeze the applicator together with some force in order to fully lock the tag; it may be helpful to use both hands. If the tag does not lock, grasp it once again with the pliers and apply more pressure. You can use the tips of the pliers to pinch down on the end of the tag's tip to ensure that the tip is securely locked. If you cannot get the tag to lock, remove it and apply another tag to the same flipper. A tag that is not applied properly will be shed quickly.

Figure 6-5. Applying an Inconel® tag to the front flipper of a loggerhead turtle (NMFS/SEFSC photo).

(5) Repeat the entire procedure and apply a second tag at the same site on the other flipper (Figure 6-6). All turtles should be double tagged in this manner using consecutive tag numbers

Figure 6-6. Two rear flipper tags (NMFS/SEFSC photo).

whenever possible. If a tag is damaged for any reason, please record this information on the tagging form and return the damaged tag. If the recommended tagging site has been injured or is unsuitable for tag application, use an alternate site along the trailing edge of the flipper.

PIT Tags

Currently, NMFS/SEFSC is using sterile-packed single use 125 kHz Destron PIT tags. These inert tags are 12 mm x 2.1 mm glass encapsulated RFID tags. They are positioned inside the turtle where loss or damage due to abrasion, breakage, corrosion over time is virtually non-existent (Balazs 1999).

Scanning Protocol

All turtles encountered should be checked for PIT tags. Rarely, a turtle may have more than one PIT tag. PIT tag scanners in use by the SEFSC generally are capable of reading frequencies of 125 kHz, 128 kHz, 134.2 kHz, and/or 400 kHz. Researchers should avoid using AVID encrypted tags; these encrypted tags cannot be read by all scanners, and few scanners capable of reading encrypted tags are widely in use by researchers in the field.

Figure 6-7. Scanning for internal PIT tags (NMFS/SEFSC photo).

(1) Keep the PIT tag scanner inside a plastic sealed bag at all times during use to prevent it from getting wet. Scan a sample tag to verify that the PIT tag reader is working properly. The button on the scanner needs to be continuously depressed throughout the scanning process, and the screen may display "WORKING" or similar (depending on the type of scanner) when functioning properly.

(2) Place the PIT tag scanner directly on the turtle's skin; on leatherbacks you may have to press hard into the skin with the reader, as the tag may be deep. For hardshell turtles, slowly scan the dorsal surface of both front flippers (Figure 6-7), the shoulder and neck areas, and rear flippers. Attempt to scan the ventral surfaces, especially all four flippers and the neck, as some projects tag in the rear flippers or other locations; small turtles can be turned over for access to ventral surfaces. For leatherbacks, scan the dorsal musculature of both forelimbs, the shoulder region and the top of the neck. It is important to slowly move the scanner multiple times, allowing it to cycle through different tag frequencies to avoid missing a tag.

(3) If a PIT tag is detected, record the identification code exactly as it appears on the scanner display, including any hyphens that may appear as part of the code. ID codes usually are hexadecimal (digits 0-9 and letters A-F) and are 10 bytes (125, 128, or 400 kHz tags) or 15 bytes (134.2 kHz tags) long. Double check to make sure you have recorded the ID code exactly as it appears on the reader display. Please be especially careful with letters and numbers

that easily are confused, such as the letter O and the number Ø. Record all tag IDs (there could be more than one PIT tag). If the scanner display reads "AVID" or the ID reads inconsistently, you may have detected an encrypted AVID tag. Encrypted tags may display a 16 byte alphanumeric code (0-9 and A-Z) on non-AVID reader displays. Record what you see on the viewer and insert a new PIT tag in the opposite shoulder/flipper.

(4) Wipe off the plastic bag. If a tag ID code remains on the display, press the scanner button again until it reads "no tag found" to extend the battery life, although the PIT tag scanner automatically turns itself off eventually. When not in the field, store the unit with the plastic bag open so that humidity does not accumulate and damage the unit. Replace or recharge batteries as needed, and do not store the unit for long periods with the batteries installed.

Application Protocol

Turtles larger than 30 cm SCL should receive PIT tags if scanning reveals no PIT tags present. In some cases, experienced taggers may feel comfortable tagging smaller turtles in the triceps superficialis muscle. The tag should occupy less than 20% of the muscle's total volume and 1/5 of its length, and it should not be located near the ends of the muscle (J. Wyneken pers. comm.). To determine if a small turtle should be tagged in the triceps superficialis, pinch the muscle forward and assess the tag size relative to the muscle size.

(1) Scan the PIT tag before opening the package to ensure that it is a functional tag. Double check that the number on the display matches the label.

(2) Record the PIT tag number on the datasheet and peel off the self-adhesive label on the PIT tag package, if available, and attach it to the datasheet.

(3) Remove the loaded needle from the sterile wrapper and insert it into the injector, or remove the preloaded syringe and needle if using these, taking care not to depress the plunger.

(4) Swab the PIT tag injection location and the end of the injector with 10% povidone-iodine solution

(5) Place the tag into musculature, where it will become encapsulated. Leatherbacks should be tagged in the center of the dorsal musculature (triceps complex) of the forelimb (Figures 6-8 and 6-9); insert the entire needle perpendicular to the skin (Note: The preferable site for leatherbacks is the musculature above the right forelimb, as some nesting research projects only scan the right side). Hardshell turtles should be tagged in the triceps superficialis muscle (Figures 6-10 and 6-11); pierce the skin of the flipper with the needle and insert the entire needle parallel to the surface just under the skin and into the muscle. Slide the plunger forward. (Note: The preferable site for Kemp's ridleys is the left triceps superficialis muscle to maximize the chances of tag detection, as the nesting project in Rancho Nuevo scans the left front flipper).

(6) Put your thumb over the injection site and apply pressure while carefully removing the needle. Dispose of the needle in a sharps container. If the

injection site bleeds, swab it with 10% povidone-iodine solution and apply pressure until the bleeding stops.

(7) Scan the flipper for the PIT tag to ensure that it is functioning in the turtle.

Figure 6-8. Leatherback turtle PIT tagged in the dorsal musculature (Photo courtesy of S. Eckert, Duke University).

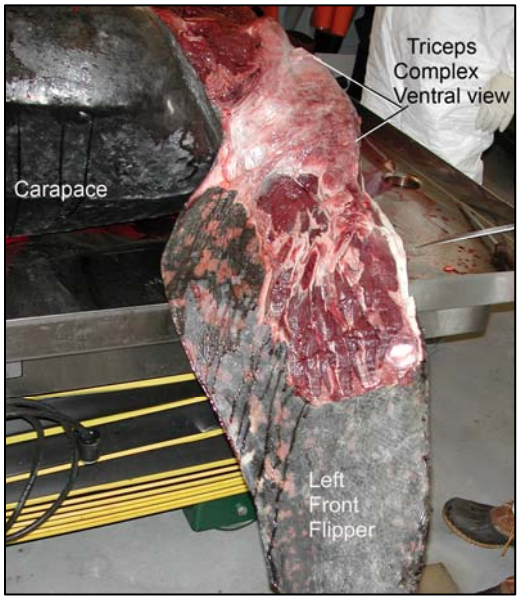
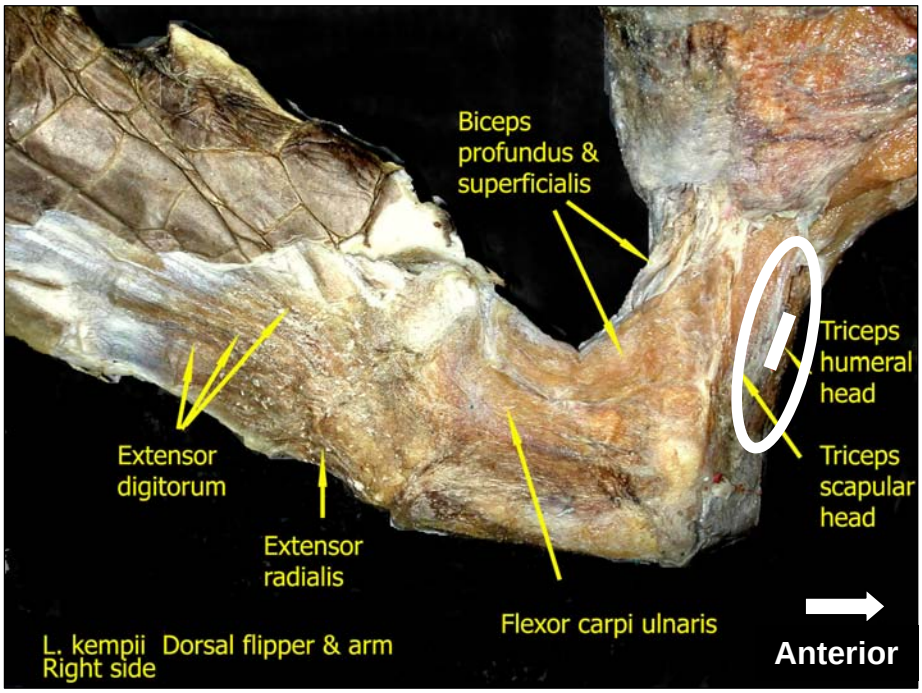
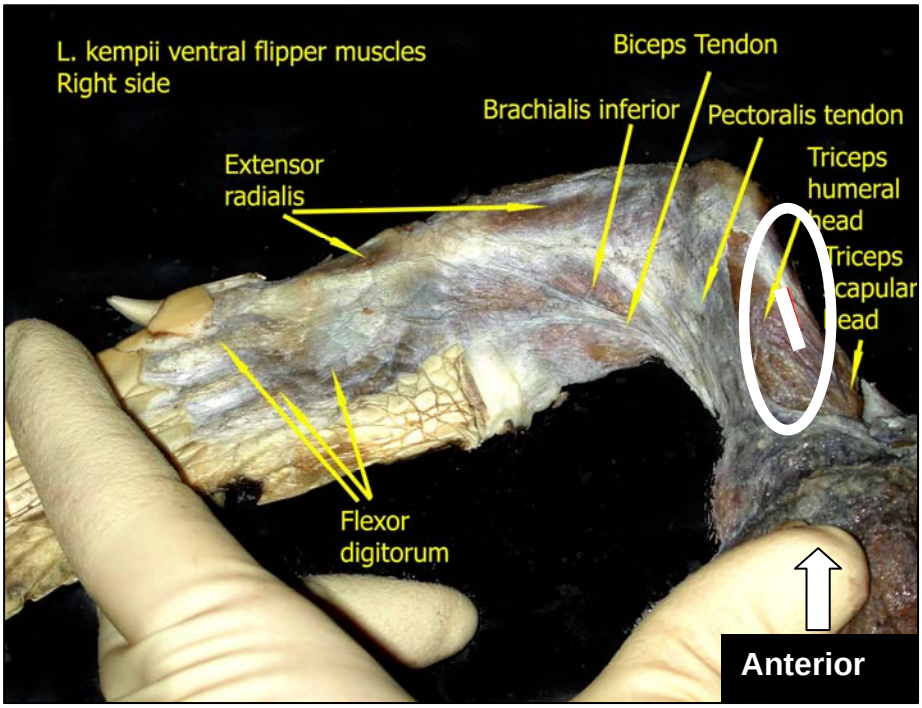


Figure 6-9. Annotated leatherback musculature depicting the triceps complex. (Photo courtesy of J. Wyneken, Florida Atlantic University).

Figure 6-10. Inserting PIT tag into the triceps superficialis muscle of a Kemp's ridley (NMFS/SEFSC photo).





Figures 6-11a and b. PIT tag placement (white line) in hardshell turtles, shown in dorsal view (a) and ventral view (b) of a dissected Kemp's ridley flipper (Photos and annotations by J. Wyneken, Florida Atlantic University).

Carapace Tagging

Tagging leatherbacks externally allows researchers, observers and fisherman to report tag sightings even when they are unable to bring the turtle onboard. Color-coded tagging, such as Floy® dart tags, would allow for the easy identification of an animal that had interacted with a fishery when encountered again on the high seas or on a nesting beach. These tags have a stainless steel applicator tip and a nylon dart head. Further detailed descriptions of Floy® dart tags can be found at: http://www.floytag.com/images/floycatalog.pdf. The tagging protocol is relatively simple and has been used for a number of years in marine and freshwater fish species.

1) Clean harpoon applicator tip and dart anchor thoroughly with 10% povidone-iodine wipes.

2) Load tag into applicator.

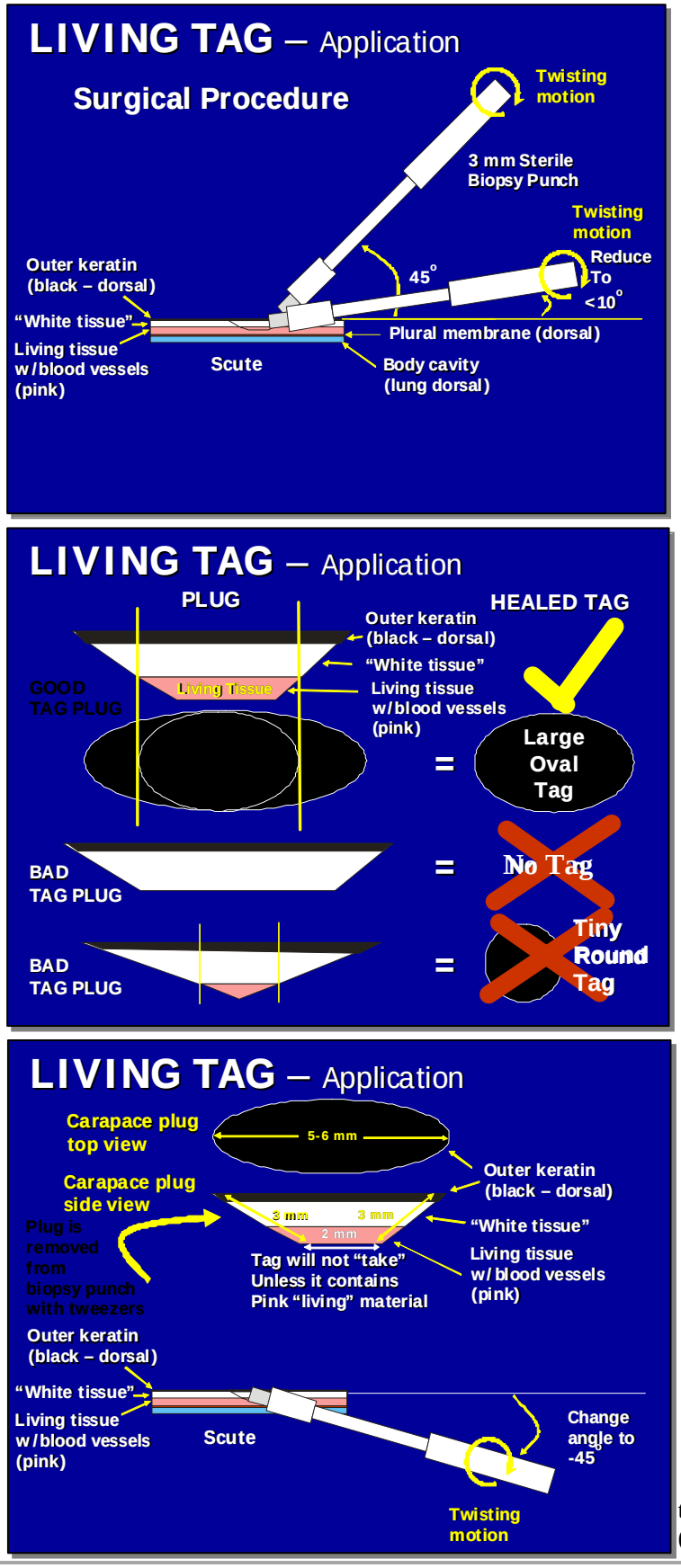
3) Tag animal with a quick, forceful jab high on carapace adjacent to the central ridge to optimize visibility.

Living Tags

Living tags provide a permanent marking method for sea turtles, and they are particularly useful with post hatchlings and small juveniles that cannot be marked using traditional tagging methods. A living tissue plug is removed from the plastron and transplanted into the carapace, leaving a permanent, identifiable light spot that grows with the animal on the contrasting dark carapace.

At least 24 hours prior to tagging, thoroughly scrub the carapace and plastron with clean water, antibacterial soap, and a scrub brush (e.g., toothbrush). Flakes of keratin, if present, can be scraped off with a scalpel blade held perpendicular to the carapace. Clean the area with fresh water and dry with a paper towel just prior to tagging (Figures 6-12a and b). Wear latex or similar disposable gloves and keep the area and equipment clean. Select a standard scute location on the carapace to receive the living tag plug. The ideal location is usually fairly central in the scute, and the topography of the carapace should match that of the plastron plug (i.e., do not take a plastron plug from a flat area and then take a peak from the carapace; a flat carapace location should receive a flat

Figures 6-12a and b. Cleaning the (a) carapace and (b) plastron (NMFS/SEFSC photos).



Figures 6-13a-c. Living tag procedure (NMFS/SEFSC diagrams).

plastron plug). For loggerheads, NMFS/SEFSC has found that the best tags were taken from the relatively thick tissue of the humeral or pectoral scutes on the plastron, so that bone was not sampled.

The order that the living tissue is obtained does not matter; the carapace or the plastron can be sampled first. The goal is to minimize the time required for the procedure. First, the method of taking carapace tissue is described. Place the blade of a sterile 3 ─ 6 mm biopsy punch at the carapace surface forming an approximate 45º angle. Using moderate force and a twisting action (twisting reduces the amount of force required to cut through the carapace material), let the biopsy punch cut into the carapace material to a depth of approximately 1 ─ 2.5 mm (Figure 6-13a).

Depth control is critical to obtaining a good plug. You must reach the area containing "pink" living tissue (only one mm thick on a seven-month-old Kemp's ridley, two mm in a 120 g loggerhead) that is vascularized. If you go too deep, you may puncture the lung (carapace) or enter body cavity (plastron). Once at the correct depth, reduce the angle of the biopsy punch to approximately five degrees, push forward two to three mm, and then angle back up to the surface, creating a plug that is 5 to 6 mm in length, three mm wide and 2.5 mm in depth (Figure 6-13b).

The side profile of the plug should be layered (Figures 6-13b and c) with a layer of black or brown/white keratin, layer of "white" bone/cartilage, and a thin layer of "pink" vascularized (blood vessel) material. Take care to ensure that no pigmented keratin material contaminates the white and pink layers of the plug, and if the plug is temporarily placed aside, it should be placed with the keratin (shell) side down to avoid contaminating the "living" areas of the plug. Only tag plugs with suitable living tissue will form good living tags; tag plugs with no living tissue will not take, and those with little living tissue will form small, potentially undetectable healed living tags. Depending on the skill level of the tagger, oval shaped tags may remain oval or heal in a circular shape. The shape, size and area of the living tissue on the tag plug and tag hole dictate the final shape and size of the living tag. Unless reciprocal transplants are to be done, discard the carapace plug (Figure 6-14); attempting to transplant carapace plug into plastron hole is rarely successful on Kemp's ridleys, but has been found to work well on loggerheads.

Figure 6-14. Removing the carapace tissue plug (NMFS/SEFSC photo).

Next, select an all white or cream-colored scute from the plastron matching the profile of carapace surface where the tag will be placed as the source of the living tag plug. Use a 3 ─ 6 mm biopsy punch to remove a plastron plug, and use forceps disinfected with 70% isopropyl alcohol to handle the plug (Figure 6-15). Clean off forceps in alcohol between handling the carapace plug and the plastron plug. The presence of moisture will cause tissue adhesive to foam or clump; excess moisture (including blood) should be blotted from the area to receive glue. Use veterinary

Figure 6-15. Removing the plastron tissue plug. (NMFS/SEFSC photo).

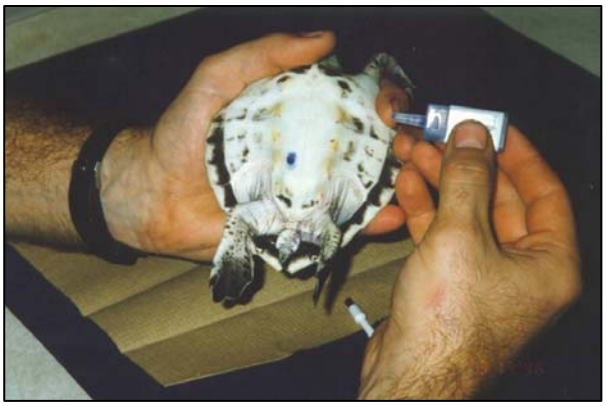


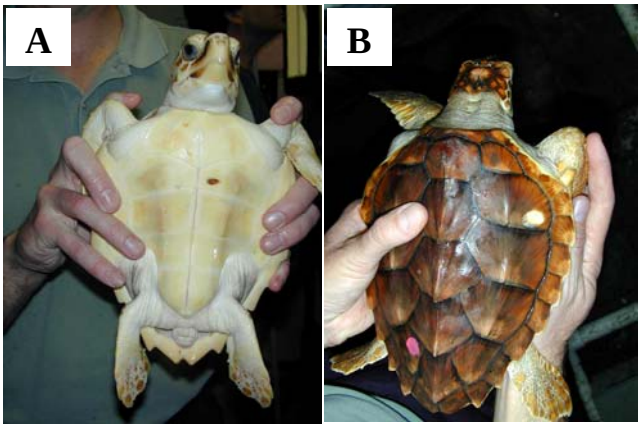
Figure 6-16. Sealing plastron with tissue glue (NMFS/SEFSC photo).

Figure 6-17. Inserting the plastron tissue plug onto the carapace (NMFS/SEFSC photo).

quality tissue adhesive (such as Nexaband® or Vetbond®) to fill and seal the empty plastron donor hole where the plug was taken if there is no reciprocal transplant, ensuring that all edges are sealed with one continuous film (Figure 6-16).

Insert the plastron plug into the carapace hole and press it into place. Rotate the plastron plug in the carapace hole to get the best fit possible, and then press down the plug to expel any liquid that might have pooled in the hole (Figure 6-17). If the plastron plug is a good match for the carapace hole, pressing down will create a slight vacuum that will hold the tag in place until it can be glued. Blotting with a paper towel helps remove any excess liquid that will interfere with the tissue glue. Apply veterinary quality tissue adhesive (such as Nexaband® or Vetbond®) around the perimeter of the tag, taking care not to allow the glue to flow over the complete surface of the tag or get under the plastron plug. If glue gets under the plastron plug, or if too much glue is used and the surface of the tag is completely covered, the tag will not "take." Use only enough glue to seal the perimeter of the plastron plug to the edges of the carapace hole, and do not try to wipe off excess glue, as the tag may stick to the wiping object and pull out.

Turtles should be left out of the water for 15 – 30 minutes after tagging to allow the glue to dry. It takes about six weeks for the living tag to heal before it can be determined whether or not the tissue graft was successful (Figures 6-18a and b).



Figures 6-18a and b. A 14-month-old loggerhead showing living tags on (a) the plastron and (b) the carapace. (Photos by J. Wyneken, Florida Atlantic University).

Wire Tags

Successfully tagging large numbers of hatchlings presents a significant challenge. Coded Wire Tags (CWTs) provide a reliable method for marking hatchlings, and they have been used extensively in captive reared and wild Kemp's ridley hatchlings (Higgins et al. 1997). CWTs have also been used in larger turtles, such as yearling Kemp's ridleys from the NOAA Kemp's ridley headstart project (1978-1992). A small section of coded wire is injected using a specialized tag injector into the dorsal surface of the front flipper near the claw (Figure 6-19).

The tags may be either non-magnetized or magnetized at the time of insertion, but the wire tag must be magnetized for detection with a handheld magnetometer. A non-magnetized tag can be magnetized immediately before detection by passing a magnet over the front flippers where the tags would be implanted, or before tagging by using a pre-magnetized roll of wire or using a magnetized head on the tag injectors (Higgins et al. 1997, Fontaine et al. 1993).

CWTs may be inscribed with binary or decimal coding. They each may be coded with a unique label or more often, each tag on a spool of coded wire is identical, allowing for the identification of groups and not individuals. To read the code on a CWT, dissection and examination under a microscope is necessary. Therefore, the code on CWTs is only obtained when a turtle is recovered dead, allowing for dissection and removal of the tag.

CWTs are detected using a wand type tag detector (magnetometer) or by x-ray radiography. To detect a tag using a wand tag detector, make sure that there are no metal objects (e.g., jewelry, watches) in the area, as they can interfere with magnetic tag detection. Test the unit by passing it over a metal with a known magnetic content and confirm an audible beep. If possible, position the turtle at least one meter away from the ground, sand, metal equipment, vehicles, electronic circuits, walls with pipes or reinforcement steel, etc. Immobilize the turtle and extend the flipper away from the turtle's body and hold the detector perpendicular to the leading edge of the flipper next to the body. Pass the wand over the surface of the flipper keeping it perpendicular to the leading edge of the flipper, keeping it as close to the flipper surface as possible without touching it (Figure 6-20) . Scan each surface of both flippers at least three times up and down the length of the flipper. If no tags are detected after three passes along the entire flipper, try several short passes back and forth in the area of the claw. If a suspected tag is detected (the wand beeps), carefully pass the wand over the suspected tag site to confirm consistent multiple readings. Check around the flipper to make sure there are no metal sources that could cause a false positive reading.

If no tag is detected after examining both surfaces of the front flippers, pass a magnet over the flippers in an attempt to magnetize a non-magnetized wire tag (Figure 6-21). The magnet should be passed in only one direction in parallel sweeps from the leading edge of the flipper towards the trailing edge in overlapping sweeps. Take care not to sweep the magnet perpendicular from the leading edge to the trailing edge, as this could result in a failure of the tag to take a magnetic charge or the un-magnetization of a previously magnetized tag. After passing the magnet over each flipper surface, follow the previously described procedure for tag detection.

A detailed description of the protocol for wire tagging and detection (Higgins et al. 1997) can be found at: http://www.sefsc.noaa.gov/seaturtletechmemos.jsp and http://galveston.ssp.nmfs.gov/publications/pdf/279.pdf.

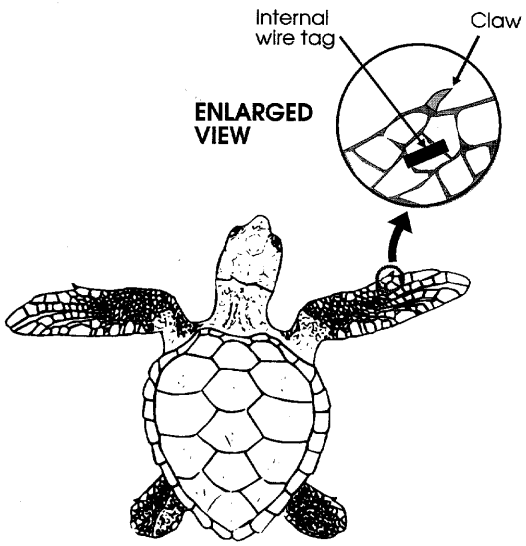


Figure 6-19. Implantation location of internal wire tag in hatchling flipper (Diagram from Higgins 1997).

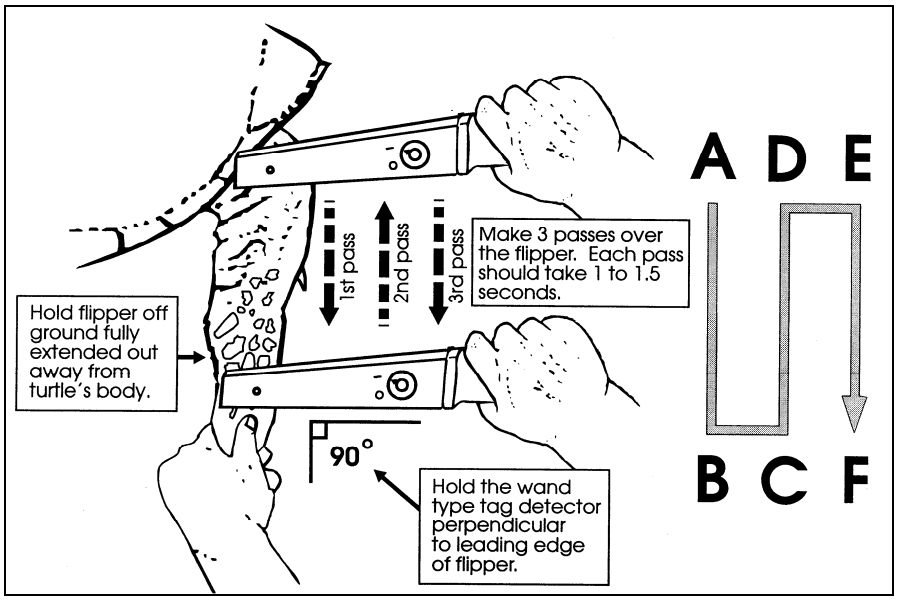


Figure 6-20. Proper positioning of wand over the turtle's flipper for magnetized wire tag detection (Diagram from Higgins 1997).

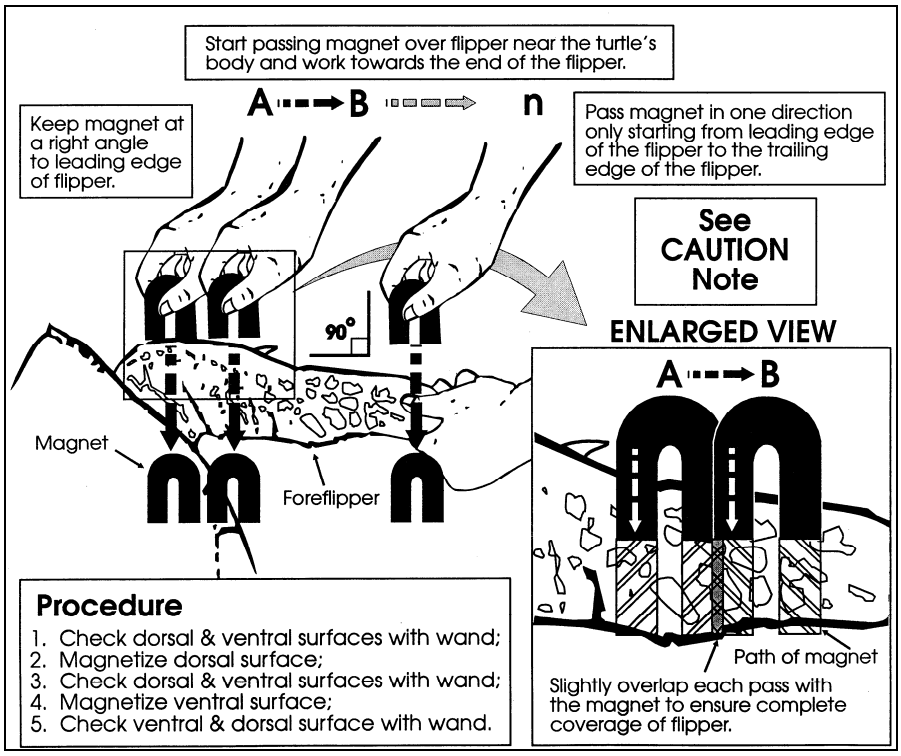


Figure 6-21. Proper technique for magnetizing a previously non-magnetized wire tag in a flipper (Diagram from Higgins 1997).

Oxytetracycline Marking

In certain circumstances, sea turtles may be injected with the antibiotic oxytetracycline (Figure 6-22). Oxytetracycline marks the bones of the sea turtle at the time of injection so they can be used in future aging studies if the turtle strands dead. One dose administered prior to hook removal, skin biopsy, and tagging could offer the same beneficial prophylactic effects as presurgical antibiotics may offer in preventing post-surgical infections as well.

The quantity of tetracycline to be administered depends on the weight of the animal, which can be estimated from its straight carapace length (SCL_{N-T}) if the actual weight is unknown. Estimated dosage quantities are provided on the Dosage Cards for Kemp's ridleys (Table 6-1), loggerheads (Table 6-2), and green sea turtles (Table 6-3). These values are based on length-weight regression models derived from morphometric data collected from wild-caught turtles in the coastal waters of North Carolina. As such, we do not recommend using the estimated dosage provided on turtles outside the Atlantic Ocean and Gulf of Mexico to account for potential differences in length-weight relationships among populations. Measure the straight carapace length of the turtle in cm and use the Dosage Cards to find the corresponding dosage (25 mg/kg assuming an oxytetracycline concentration of 200 mg/ml). If the actual weight of the turtle is known, or if you are using a different concentration of the drug, calculate the dosage using the formula:

Dosage (ml) = Weight (kg) x 25 (mg/kg) / concentration (mg/ml).

Verify that product has not expired, as this product can become toxic after expiration, and confirm the product's concentration. Wear disposable gloves and draw the necessary dosage from the bottle with a disposable syringe. Use a 3-cc syringe for antibiotic quantities 0.6 – 2.9 ml and a 5-cc syringe for larger quantities.

Figure 6-22. Oxyetracycline injection (NMFS/SEFSC photo).

On a restrained turtle, clean the injection site with 10% povidone-iodine solution. Insert the needle in the right front dorsal shoulder musculature (latissimus dorsi, terres major, and deltoides) in a single injection site using a sterile, disposable syringe and a 20-gauge 1" needle. Animals with a SCL > 70 cm should have their dosage split into two equal volumes to administer in each shoulder. Before injecting the tetracycline, pull back on the syringe plunger to make sure the needle is not in a blood vessel. If there is no blood coming into the syringe, apply continuous force to the plunger to administer the antibiotic. If blood does enter the syringe, readjust the needle placement by partially retracting the needle

(do not remove entirely) and changing the angle of insertion. Check again to verify the needle is not in a blood vessel before administering the antibiotic. After removing the needle, apply pressure with a 10% povidone-iodine swab in the area to stop any bleeding and prevent infection. Dispose of the needle and syringe in a sharps container.

OXYTETRACYCLINE DOSAGE FOR KEMP'S RIDLEY SEA TURTLES

SCL N-T (cm)	Drug Dosage (ml)		SCL N-T (cm)	Drug Dosage (ml)
20	0.2		43	1.4
21	0.2		44	1.5
22	0.2		45	1.6
23	0.3		46	1.7
24	0.3		47	1.7
25	0.3		48	1.8
26	0.4		49	1.9
27	0.4		50	2.1
28	0.5		51	2.2
29	0.5		52	2.3
30	0.5		53	2.4
31	0.6		54	2.5
32	0.6		55	2.6
33	0.7		56	2.8
34	0.8		57	2.9
35	0.8		58	3.0
36	0.9		59	3.2
37	0.9		60	3.3
38	1.0		61	3.5
39	1.1		62	3.6
40	1.1		63	3.8
41	1.2		64	3.9
42	1.3		65	4.1

Table 6-1. Oxytetracycline dosage card for Kemp's ridley sea turtles assuming a dose of 25 mg/kg and an oxytetracycline concentration of 200 mg/ml. Dosage (ml) = Weight (kg) x 25 (mg/kg) / concentration (mg/ml). Dosage calculations are based on estimated weight from Kemp's ridley morphometric data regressions where: Weight (kg) = $0.0006(SCL)^{2.6121}$ with $R^2 = 0.894$.

OXYTETRACYCLINE DOSAGE FOR LOGGERHEAD SEA TURTLES

SCL N-T (cm)	Drug Dosage (ml)		SCL N-T (cm)	Drug Dosage (ml)
40	1.3		73	6.7
41	1.4		74	7.0
42	1.5		75	7.3
43	1.6		76	7.5
44	1.7		77	7.8
45	1.8		78	8.1
46	1.9		79	8.4
47	2.0		80	8.7
48	2.1		81	9.0
49	2.2		82	9.3
50	2.4		83	9.6
51	2.5		84	9.9
52	2.6		85	10.2
53	2.8		86	10.5
54	2.9		87	10.8
55	3.1		88	11.2
56	3.2		89	11.5
57	3.4		90	11.9
58	3.5		91	12.2
59	3.7		92	12.6
60	3.9		93	12.9
61	4.1		94	13.3
62	4.3		95	13.7
63	4.5		96	14.0
64	4.7		97	14.4
65	4.9		98	14.8
66	5.1		99	15.2
67	5.3		100	15.6
68	5.5		101	16.0
69	5.8		102	16.4
70	6.0		103	16.8
71	6.2		104	17.3
72	6.5		105	17.7

Table 6-2. Oxytetracycline dosage card for loggerhead sea turtles assuming a dose of 25 mg/kg and an oxytetracycline concentration of 200 mg/ml. Dosage (ml) = Weight (kg) x 25 (mg/kg) / concentration (mg/ml). Dosage calculations are based on estimated weight from loggerhead morphometric data regressions where: Weight (kg) = $0.022\ (SCL)^2 - 1.1789(SCL) + 22.751$ with $R^2 = 0.897$.

OXYTETRACYCLINE DOSAGE FOR GREEN SEA TURTLES

SCL N-T (cm)	Drug Dosage (ml)		SCL N-T (cm)	Drug Dosage (ml)
20	0.1		57	2.6
21	0.2		58	2.8
22	0.2		59	2.9
23	0.2		60	3.1
24	0.2		61	3.2
25	0.2		62	3.4
26	0.3		63	3.5
27	0.3		64	3.7
28	0.3		65	3.8
29	0.4		66	4.0
30	0.4		67	4.2
31	0.5		68	4.4
32	0.5		69	4.6
33	0.6		70	4.8
34	0.6		71	4.9
35	0.7		72	5.1
36	0.7		73	5.4
37	0.8		74	5.6
38	0.8		75	5.8
39	0.9		76	6.0
40	1.0		77	6.2
41	1.0		78	6.5
42	1.1		79	6.7
43	1.2		80	7.0
44	1.3		81	7.2
45	1.3		82	7.5
46	1.4		83	7.7
47	1.5		84	8.0
48	1.6		85	8.3
49	1.7		86	8.6
50	1.8		87	8.8
51	1.9		88	9.1
52	2.0		89	9.4
53	2.1		90	9.8
54	2.3		91	10.1
55	2.4		92	10.4
56	2.5			

Table 6-3. Oxytetracycline dosages for green sea turtles assuming a dose of 25 mg/kg and an oxytetracycline concentration of 200 mg/ml. Dosage (ml) = Weight (kg) x 25 (mg/kg) / concentration (mg/ml). Dosage calculations are based on estimated weight from green sea turtle morphometric data regressions where:
Weight (kg) = $0.0002(\text{SCL})^{2.861}$ with $R^2 = 0.777$.

Chapter 7: Electronic Tags

Electronic tags allow researchers to remotely monitor information such as position, movement patterns, dive behavior, survival, and environmental parameters. Satellite tags are used to collect data on location, depth, and/or temperature. Deployment length is dependent on battery size and will vary depending on research question and animal size. Sonic tags emit an acoustic signal that can be received underwater with a hydrophone. Triangulation of the acoustic signal allows researchers to determine an animal's location. Radio tags emit a radio signal on a specific frequency that can be detected by an antenna when a turtle surfaces. Radio tags provide location information via triangulation of the signal above the water.

Electronic tags, including sonic, radio, satellite transmitting, and archival tags are attached to sea turtles via two methods: direct and tethered. All tags and attachment materials should weigh less than five percent of a turtle's weight, and tags should be streamlined to minimize any effects of drag. Researchers must make attachments as hydrodynamic as possible. Tag dimensions vary by manufacturer and tag type, but should be proportional to turtle size and consistent with weight restrictions. Each attachment must be made so that there is no risk of entanglement. The lanyard (if used) length must be less than 1/2 of the carapace length of the turtle to avoid entanglement in the turtle's front flippers and prevent the turtle from biting the tag. It must include a corrodible, breakaway link that will corrode and release the tag-transmitter after the tag-transmitter life is finished. Adequate ventilation around the head of the turtle must be provided during the attachment of tags if attachment materials produce fumes. To prevent skin or eye contact with harmful chemicals used as attachment materials, turtles must not be held in water during the application process. Ideally, turtles will be and held no longer than two hours; however, there may be weather or logistical events that may require bringing turtles to shore for tagging and temporary holding. In that event, turtles should be released as weather permits, no more than one day after capture.

Electronic Tag Specifications

Below is a list of tag types currently in use or being considered for use by the NMFS/SEFSC. Specific manufacturers and models are not listed here because of the dynamic nature of this field of technology. Attachment methods are constantly refined and improved by researchers; the methods defined here have been tested and approved by NMFS/SEFSC, but do not constitute an exhaustive list of potential acceptable methods. Argos transmitting satellite and archival tags operate within approved frequencies, 401.618 MHz to 401.680 MHz. Sonic tags operate in the 25 – 80 kHz range, and radio tags use a range of 164 – 166 MHz.

Tag Attachment Protocols

Tether Attachment Protocol (see Epperly et al. 2007)

Hardshell turtles

Tethers for satellite, radio, or sonic tags are attached to the trailing edge at the rear of the carapace (Figure 7-1) to reduce drag while

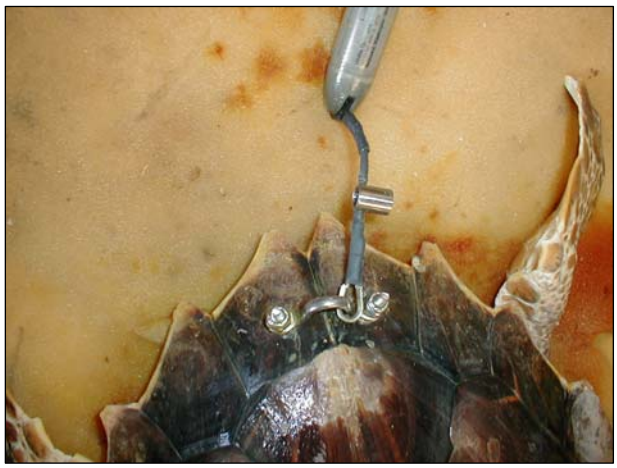


Figure 7-1. Attachment of an archival satellite tag on a hardshell turtle (NMFS/SEFSC photo).

being towed by the turtle. Tags are streamlined and as light as possible (< five percent of body weight) to keep drag to a minimum. When handling the turtle and equipment, use disposable gloves and change them often to maintain the most sterile environment possible.

After removing epibionts and thoroughly scrubbing the area with water and povidone-iodine disinfectant, drill a 0.5 cm hole through one of the turtle's pygal bones, as well as the overlying scutes, with a drill bit soaked in povidone-iodine disinfectant for 15 minutes prior to use. Pass a plastic electrician's tie through the hole and secure. Transmitters should be housed in bullet-shaped buoys (approximately 10 cm diameter and 10 cm in height) secured to one end of a tether that consists of one mm diameter stainless steel fishing leader. Connect the tether to the plastic electrician's tie in the turtle's shell with a ball-bearing swivel and two short lengths of either 30 lb braided fishing line (e.g., Spiderwire®) or 30 lb test monofilament fishing line. This configuration will allow the turtles to break free if either the buoy or tether becomes entangled in submerged or floating debris or bottom structure.

1. Immobilize the turtle and clean dorsal and ventral surfaces of postcentral scutes using a scouring pad and scrub brush, and if needed for barnacle removal, a chisel. Activate tag.

2. Pour ~ two oz 10% povidone-iodine to thoroughly soak the hardware into a bag containing the hardware, exclusive of the nylon parts, and a new drill bit, and soak for at least 15 minutes, agitating frequently. Use alcohol swabs to clean the nylon parts, as iodine breaks down nylon over time, while alcohol is inert.

3. Saturate sterile gauze sponges with 10% povidone-iodine and cleanse the dorsal and ventral surfaces of the postcentral scutes several times over a 15 minute period.

4. Install a 3/16" titanium drill bit into the portable drill and align the eyestrap (pad eye) on the postcentral scutes. Be sure to position the eyestrap as far forward (toward head) as possible on the postcentral scutes to capture the underlying bone. However, be cognizant that you will be drilling at an angle; do not drill so far up as to intercept the integument on the ventral surface (Figure 7-1). Using the holes of the eyestrap as a guide, drill once quickly through the scute. Use a blood clotting gel such as Clotisol® or ferric subsulfate to stop bleeding, if necessary, after first cleaning the dropper tip with an alcohol swab.

5. Flood the area thoroughly with 10% povidone-iodine.

6. Select an appropriate length 1/8” bolt and insert it through the eyestrap. If the bolts are too long, insert them from the bottom so that they can be trimmed later. Use nylon washers against the carapace and the plastron and a stainless washer between the eyestrap and the nut or head of the bolt.

7. Thread thimble of tag tether over eyestrap before inserting the second bolt.

8. Repeat steps six and seven for the second hole.

9. Turn the turtle onto its carapace, being careful to protect the tag (try to keep it in the PVC sleeve), and secure the bolts with washers and lock nuts using a wrench.

10. Use bolt cutters to cut off any excess length of the bolts if necessary.

Leatherback Turtles- Pygal Tether Attachment (Figure 7-2)

1. Immobilize the turtle and activate the tag. When handling the turtle and equipment use disposable gloves, changing them often to maintain the most sterile environment possible.

2. Pour enough 10% povidone-iodine into a hardware bag containing two 1/4” X 1 5/8” acetal polyoxymethylene resin (e.g., Delrin®) disks, a new 5/16” drill bit, and 1/4” outer diameter surgical tubing to coat items. Agitate the bag frequently to disinfect the hardware.

3. Saturate sterile gauze sponges with 10% povidone-iodine or use 10% povidone-iodine scrubs and cleanse the dorsal and ventral areas of turtle in the pygal region (Figure 7-2). Do this several times.

4. Install the drill bit into a portable drill and drill a single hole through the center of the pygal region. Use a blood clotting agent such as Clotisol® or ferric subsulfate to stop bleeding, if necessary, after first cleaning the dropper tip with an alcohol swab.

5. Flood hole thoroughly with 10% povidone-iodine.

6. Swab outside of surgical tubing with a triple antibiotic ointment such as Neosporin® and pass surgical tubing through the hole until it is flush at the top.

7. Cut excess surgical tubing flush at the bottom using scissors or line cutters.

8. Thread monofilament tether through an acetal polyoxymethylene resin (e.g., Delrin®) disk that has been swabbed with triple antibiotic ointment on the bottom.

9. Pass monofilament through surgical tubing. Lubricate monofilament with triple antibiotic ointment if needed.

10. Secure monofilament at the bottom with the second acetal polyoxymethylene resin disk (swabbed with topical antibiotic ointment) and a crimp below the disk so that the tether is tight and secure. Cut off any excess monofilament.

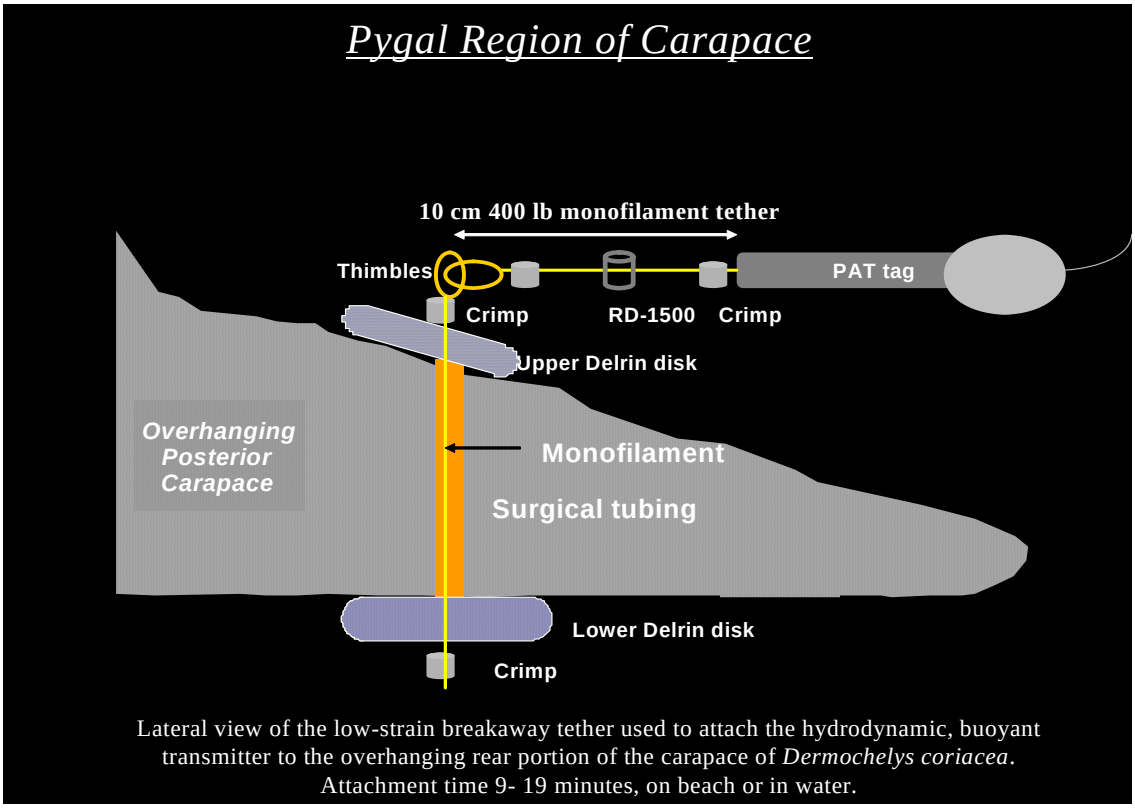


Figure 7-2. Attachment of archival tag using a tether through the pygal region of a leatherback (NMFS/SEFSC diagram).

Harness Attachment Method (Figure 7-3, Method developed by Scott Eckert and adapted from Eckert and Eckert 1986)

Note: Recent concerns about the effects of drag have been raised regarding this harness attachment method. Drag effects are currently being researched, and new materials and attachment methods are currently under investigation. NMFS **does not** currently use or endorse this method of attachment pending futher research.

1. Activate satellite tag.

2. Place plastron strap under the posterior end of plastron (approximately 10 – 20 cm from the edge.

3. Feed each end of the plastron strap through the loop of their respective vinyl tube covered shoulder strap.

4. Center the elastic tubes with the four D-rings on the carapace with two D-rings forward for the shoulder straps and one D-ring to each side for the plastron strap.

5. Secure the plastron strap at each end to the D-rings, making sure that tension of elastic tubes is not too great and allows for growth of the turtle without allowing movement of the harness. The attached loops for the shoulder straps should be just below the D-ring.

6. Feed the vinyl tube with each shoulder strap under the front flippers and curve the tubes over the turtle's carapace.

7. Secure the shoulder straps to the remaining D-rings until an appropriate amount of tension is present in the elastic tubes which will allow for growth of the turtle yet ensure the harness will remain in place. Some trimming of the vinyl tubes may be needed to properly secure the shoulder straps.

8. Attach the transmitter plate to the vinyl tubes ahead of shoulder D-rings and loosely attach with four large cable ties. Do not secure yet, as some adjustments may still be needed for the harness.

9. Check overall tension on shoulder straps, the plastron strap, and the elastic tubes. Make any needed adjustments at this time. Do not over tighten the harness. The harness should be secure on the turtle but still allow for growth.

10. Once all the straps are properly adjusted, secure the shoulder strap loops to the plastron strap on each side of the turtle several centimeters below the D-rings for the plastron strap using small cable ties. A hole will need to be punched with an awl (or similar tool) through the hole in the loop and the plastron strap for the cable ties.

11. Next, secure the plastron strap below each of the D-rings with a cable tie by punching a hole with the awl through the tensioned plastron strap and its loose end below the D-ring.

12. Secure the shoulder straps with cable ties below their D-rings.

13. Tighten cable ties for the transmitter plate to the vinyl tubes.

14. Trim all excess strap material and cable ties.

Figure 7-3. Satellite tag attached using the harness attachment method (NMFS/SEFSC photo).

Direct Attachment Protocol

Epoxy Attachment for Satellite Tags on Hardshell Turtles (see Godley et al. 2002)

Holding – Use a tub to safely hold the turtle in a natural prone position while attaching the transmitter. The tub size will vary based on the size of the animal (e.g., a plastic fish box for small animals or a plastic pool or tank for large animals). Place a cushioned pad on the bottom of the tub to cushion the turtle. The tub will serve to comfortably restrict movement of the turtle during the attachment procedure and can be used aboard a boat or on land. A wet cloth draped over the turtle's eyes to completely block vision often reduces the turtle's desire to move. Shelter the turtle from

direct sunlight, wind, and rain with a tarp during the attachment procedure.

Preparing the carapace – Remove epibionts (barnacles, algae, etc.) from the carapace at the mounting and bonding site of transmitter. In general, the ideal location to place the transmitter is the point where the first and second vertebral scutes meet (Figure 7-4). This section of the carapace rises to a maximum point above the sea surface each time the turtle breathes, and the base antenna on the transmitter will break the plane of the water's surface. Alternatively, transmitters may be attached directly to the second vertebral scute on the carapace (Papi et al. 1997, Polovina et al. 2000, Griffin 2002). Attachment media may also encompass sections of the first and third vertebral scutes, as well as the first and second costal scutes. Thoroughly scrub these areas with a scrub brush and 10% povidone-iodine, rinse with fresh water, dry with a towel, and then lightly sand with sandpaper. When smooth, lightly wipe the entire area with an alcohol pad or a small amount of acetone.

Mounting the transmitter on the carapace – Activate the transmitter in the lab prior to entering the field. Coat all surfaces of the transmitter except the bottom with anti-fouling paint if desired, and cover saltwater switches with electrical or masking tape. The size and weight of the satellite transmitter used will depend on the size of the turtle. Large tags will be attached to the carapace using a two-part epoxy, or a combination of two-part epoxy and fiberglass resin and cloth (< 200 g). The tag and attachment materials should not exceed five percent of the turtle's body weight.

Use a two-part cool setting epoxy (e.g., Power-Fast®) to secure the transmitter on to the carapace. The epoxy components are discharged from the cartridge in equal amounts via a caulk gun, and are incorporated in a specialized mixing nozzle so no modification of amounts is required. There is no danger of setting too quickly. Use a small amount of epoxy (< 50 g) to create an even base for the transmitter to rest and to secure it to the carapace. Drying time will vary between 20 – 60 minutes depending on ambient temperature and humidity. Secure small tags with the epoxy alone; apply additional epoxy or two coats of fiberglass material on larger transmitters to ensure a long attachment life (i.e., one year). When the base has hardened, fiberglass cloth and resin (e.g., Power-Fast® or Bondo®) may be used to further secure the transmitter to the carapace from the edges and/or top to the surrounding scutes. If using fiberglass cloth and resin, use 20 drops of catalyst to two oz of fiberglass resin and mix liberally for about 15 seconds.

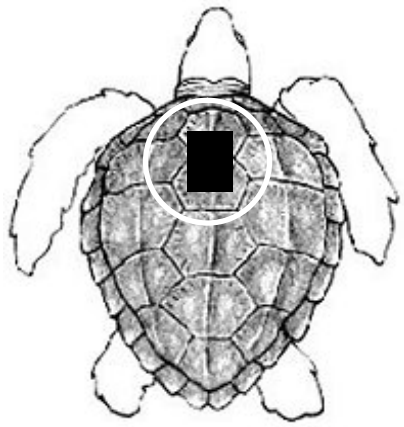
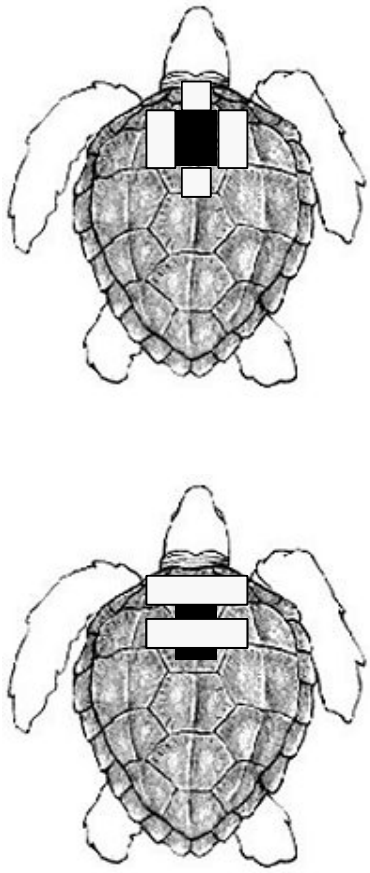


Figure 7-4. Position of satellite transmitter attachment on turtle's carapace (Diagram by C. McClellan, Duke University).

The amount of catalyst may change based on ambient temperatures and humidity, and will be tested in advance to determine hardening time. Use a liberal coat of mixed resin on the transmitter and carapace where pre-cut strips



Figures 7-5a and b. Placement of (a) the first layer and (b) the second layer of fiberglass (Diagrams by C. McClellan, Duke University).

of fiberglass cloth will be applied in two layers over the transmitter, allowing each layer to dry completely (approximately 15 – 20 minutes). Use two five cm wide by 11 cm wide by 5 cm long squares of fiberglass cloth in the first layer, one piece on each edge of the tag (Figure 7-5a). The second layer consists of two 5 cm wide by 13 cm long strips of fiberglass cloth, one over the tag and one across the front of the tag (Figure 7-5b).

Take care to prevent fiberglass resin from running off the shell or coming in contact with the turtle's skin or eyes by wiping up drips immediately. Maintain adequate ventilation while using fiberglass media (e.g., Bondo®). A coat of fiberglass anti-fouling paint may be applied over attachment media to prevent fouling on these materials. When the attachment materials are dry, remove the tape from the saltwater switches and polish with sandpaper to remove any residual grime. Sand the fiberglass as well to remove any sharp edges. Release the turtle at or near the point of capture. Ideally, turtles will be tagged on the boat and held no longer than 1.5 hours, barring unforeseen weather or logistical events (Figure 7-6).

An alternative attachment method is to use a roll of 1.0 cm diameter adhesive (e.g., Sonic Weld®, Ed Greene and Company) around the bottom edge of the transmitter to form a well, followed by application of epoxy resin (e.g., Foil Fast®, Rawlplug Company) epoxy to the entire bottom surface of the transmitter within the well using a glue gun. Heat generated by curing epoxy has not been noticed by researchers during the application process. Preparation and setting time is approximately one hour, after which turtles are released in close proximity to where they were collected.

Figure 7-6. A satellite tagged loggerhead ready for release (Photo by C. McClellan, Duke University).

Direct Satellite Tag Attachment for Leatherbacks

1. Immobilize the turtle and activate the tag. When handling the turtle and equipment, use disposable gloves and change them often to maintain the most sterile environment possible. Attachment methods may vary depending on tag design; one suggested attachment method is described here.

2. Pour enough 10% povidone-iodine to thoroughly soak the hardware (e.g., four 1/4" X 1 5/8" acetal polyoxymethylene resin (e.g., Delrin®) disks and a new three mm drill bit) in a bag. Agitate the bag frequently to disinfect the hardware.

3. Saturate sterile gauze sponges with 10% povidone-iodine or use 10% povidone-iodine scrubs and cleanse the central ridge area of turtle (Figure 7-7). Do this several times.

4. Install drill bit into a portable drill and drill two small holes through the ridge. If necessary, use a blood clotting agent such as Clotisol® or ferric subsulfate to stop bleeding by applying drops into the holes after first cleaning the dropper tip with an alcohol swab. The hole will only penetrate a few millimeters horizontally through the carapace ridge and will not enter the body cavity.

5. Flood holes thoroughly with 10% povidone-iodine.

6. Thread one monofilament or coated wire tether through an acetal polyoxymethylene resin disk that has been swabbed with triple antibiotic ointment (e.g., Neosporin®) on the bottom.

7. Swab outside of the tether monofilament with triple antibiotic ointment and pass through the hole.

8. Once passed through the hole, secure the monofilament with a second acetal polyoxymethylene resin disk (swabbed with triple antibiotic ointment) and a crimp so that the tether is tight and secure. Cut off any excess monofilament.

9. Repeat steps six through eight for the second monofilament tether.

Figure 7-7. Direct carapace attachment on leatherback (Photo courtesy of Sandra Ferraroli, Centre National de la Recherche Scientifique).

Sonic and Radio Transmitter Attachment

General information – Transmitters will be programmed by the manufacturer and tested in the lab prior to entering the field. Activation of the transmitter simply involves removing a magnet. Coat the transmitter with anti-fouling paint before attaching to the turtle.

Holding the turtle in a prone position – Use a container to safely hold the turtle in a natural prone position while attaching the transmitter. The container size will vary depending upon the size of the animal and could range from a

plastic fish box for small animals to a plastic pool or tank. The container will serve to comfortably restrict movement of the turtle to a minimum during the attachment procedure and can be used aboard a boat or on land. Place a cushioned pad on the bottom of the container and shelter turtles from direct sunlight, wind, or rain with a tarp during the attachment procedure. A wet cloth draped over the turtle's eyes to completely block vision often reduces the turtle's desire to move.

Mounting the sonic transmitter on the carapace – In general, locating the transmitter on the posterior section of the carapace will reduce drag and will keep the transmitter submerged even when the turtle surfaces to breathe (Figure 7-8). Sonic transmitters are available in various sizes enabling us to tag both small and large sea turtles (loggerhead, green, and Kemp's ridley). Given that the transmitter and attachment materials cannot exceed five percent of the turtle's body weight, transmitters will be placed only on turtles > 20 cm SCL.

Attachment media will encompass sections of the last vertebral scute as well as the last costal scute. Remove epibionts (barnacles, algae, etc.) from the carapace at the site of transmitter attachment using a hoof pick or other blunt instrument. Thoroughly scrub these areas, rinse with fresh water, dry, and then lightly sand with sandpaper. When smooth, lightly wipe the entire area with an alcohol pad or a small amount of acetone. Use a two-part cool setting epoxy (e.g., Power-Fast®) to secure the transmitter on to the carapace. The epoxy components are discharged from the cartridge in equal amounts via a caulk gun and are incorporated in a specialized mixing nozzle, so no modification of amounts is required, and there is no danger of setting too quickly. Use a small amount of epoxy (< 20 g) to create an even base for the transmitter to rest and to secure it to the carapace. Taper the attachment media to prevent it from catching on rocks or fishing nets. Drying time will vary between 20 – 60 minutes, depending on ambient temperatures and humidity. When the attachment materials are dry, release the turtle at or near the point of capture.

Mounting the radio transmitter on the carapace – Radio transmitters are available in various sizes, enabling tagging of both small and large sea turtles. The transmitter and attachment materials should not exceed five percent of the turtle's body weight. Therefore, transmitters should be placed only on turtles > 20 cm SCL. Small (e.g., ~30 g cylindrical) transmitters can be attached directly to the carapace of smaller turtles (25 – 40 cm SCL) or tethered to the posterior end of the carapace of larger turtles (> 40 cm SCL). Larger (e.g., ~60 g rectangular) transmitters can be attached directly to the carapace of larger turtles (> 40 cm SCL).

Use a two-part cool setting epoxy (e.g., Power-Fast®) to secure the transmitter to the

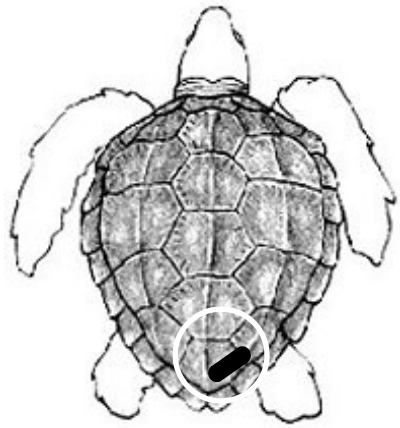


Figure 7-8. Position of sonic transmitter attachment (Diagram by C. McClellan, Duke University).

carapace. The epoxy components are discharged from the cartridge in equal amounts via a caulk gun and are incorporated in a specialized mixing nozzle, so no modification of amounts is required, and there is no danger of setting too quickly. Use a small amount of epoxy (< 20 g) to serve the dual function of creating an even base for the transmitter to rest and securing it to the carapace. Taper the attachment media to prevent it from catching on rocks or fishing nets. Drying time will vary between 20 – 60 minutes, depending on ambient temperatures and humidity. When the attachment materials are dry, release the turtle at or near the point of capture.

Stomach Temperature Pill

Satellite-linked data recorders (e.g., Mk10-AL, 93 x 51 x 22 mm, 125 g; Wildlife Computers Redmond, WA) and stomach temperature pills (e.g., STP3, 21.5 mm diameter, 63 mm length; Wildlife Computers, Redmond, WA) may be deployed in turtles > 105 cm in length. Currently these devices are used to record temperature as described here, but future advancements may allow multiple parameters to be sampled.

Adult loggerhead turtles maintain internal body temperatures several degrees higher than ambient water temperature (T_W) (Sato et al. 1994). Ingestion of prey at ambient T_W has the effect of rapidly lowering stomach temperature (T_S), such that fluctuations in T_S may be used to identify a feeding event. The magnitude of the decrease in T_S and time necessary for T_S to recover to previous levels following prey ingestion reflects both prey size and ambient T_W. The STP3 possesses four thermistors to detect T_S, and a transmitter to relay T_S data to a satellite-linked data recorder, such as the MK10-AL, mounted on the turtle's carapace. The T_S data are intercepted and archived by the MK10-AL instrument. Data recognition software then analyzes the T_S data to identify large fluctuations indicative of ingestion. When one of these events is recognized, software then pick 6 points that characterizes the fluctuation, records the time the fluctuation occurred, the depth at which the fluctuation was recorded, and the ambient Tw. This information is transmitted, along with location data and dive behavior data, the next time the Mk10-AL uplinks to an Argos satellite when the turtle surfaces to breathe.

The satellite-linked data recorder will be attached to the turtle's carapace between the first and second vertebral scutes using Power-Fast® marine epoxy or similar. Stomach temperature pills will be inserted in animals (must be > 105 cm CCL) to a depth of 20 – 25 cm into the esophagus using a lubricated flexible rubber tube. Generally it is not necessary to restrain the turtle with a net while inserting the STP3. Rather, use nylon webbing straps to hold the mouth open. One person holds strap on upper jaw and another person holds strap on lower jaw while a third person uses the lubricated rubber tube to push the pill into the turtle's esophagus.

The insertion of the pill is a quick procedure, and the turtle's mouth is usually held open for less than one minute. A damp cloth is placed over the turtle's eyes to keep it calm during the procedure. Ensure that the pill is properly sized for the animal to prevent potential intestinal blockage. Previous studies have shown that an STP3 inserted in this manner is eventually pushed into the stomach by peristaltic action and food ingestion (Southwood et al. 2005), causing no residual effects.

Chapter 8: Biopsy Sampling

Biopsies, the sampling of single or multiple tissues, are routinely collected to:

- Provide information relative to the life history of the population being studied – Skin biopsies have been collected for genetic studies, while bone biopsies have been collected for aging studies;

- Better understand the nature of a lesion and determine the most appropriate therapy – Single or multiple samples are collected, determined by the type of lesion biopsied;

- Determine sex – Small pieces of gonadal tissue can be evaluated histologically to determine the sex of the animal;

- Evaluate the animal for contaminants – Both fat and liver biopsies provide a way to monitor organochlorine contaminants in wildlife populations. Biopsies also may be obtained from other visceral structures, usually through a laparoscopic incision;

- Conduct stable isotope analysis – Analysis of stable isotope levels of carbon and nitrogen provides insight into diet, foraging behavior and potentially distributional patterns; and

- Conduct biochemical analyses – Muscle biopsies can be evaluated to determine aerobic and anaerobic metabolic capacity, thermal tolerance, or stable isotope analysis.

Skin Biopsy

Protocol for Turtles Boated or on Land

Small hardshell turtles should be turned onto their carapaces briefly to facilitate skin biopsy sampling; this may not be possible for large turtles. The sample site should be along the posterior edge of a rear flipper in soft tissue, not a scale. If a rear flipper is not accessible, samples can be taken from the front flippers as well. Thoroughly soak and scrub the area with 10% povidone-iodine solution followed by an isopropyl alcohol wipe, then thoroughly swab again with 10% povidone-iodine solution prior to sampling. A new, sterile biopsy tool should be used for each turtle to prevent cross-contamination.

The researcher should wear gloves to protect the hand that is holding the flipper and the sampling surface. A vial cap, plastic dive slate, or other plastic surface cleaned with 70% isopropyl alcohol should be placed beneath the sampling site as a hard surface against which to press. Press a new biopsy punch firmly into the flesh just along the posterior edge and rotate one complete turn, cutting all the way through the flipper to the plastic surface (Figure 8-1). Repeat the tissue punch process with the same punch to obtain two plugs from each animal. An alternative method is to remove a plug of skin from the shoulder region using a sterile 6 mm biopsy punch to cut a skin plug and forceps and surgical scissors to extract and trim the sample.

Figure 8-1. Skin biopsy taken from trailing edge of rear flipper (NMFS/SEFSC photo).

Place the tissue plugs into the vial containing a suitable storage solution, such as saturated NaCl solution with or without 20% DMSO. If the sample does not come out of the corer easily, place it into the vial by inserting a new, clean wooden applicator stick through the hollow handle of the biopsy punch, shaking the punch in the vial, or snapping the tip off of the biopsy punch and placing the entire tip in the vial. Wipe the punched area with 10% povidone-iodine solution. If necessary, a blood clotting agent, such as ferric subsulfate or Clotisol®, or a cyanoacrylate tissue glue such as Nexaban® (Veterinary Products Lab, Phoenix, AZ, USA) or an over-the-counter equivalent such as Super-Glue® or Krazy-Glue® can be used for hemostasis. Using a pencil, label a piece of waterproof paper with the date, species, id, master tag, and trip number if applicable, and place in the vial. Label the outside of the vial using a permanent marker with date, species, id, and master tag and seal the label with clear tape. To prevent spillage, wrap laboratory sealing film, such as Parafilm®, around the cap of the vial. Place vial within a labeled sample bag (e.g., Whirl-pak®) and close.

Wear gloves each time you collect a sample and handle the buffer vials. The NMFS/SEFSC observer programs currently use a saturated sodium chloride solution for tissue sample storage, but some programs may use 20% dimethyl sulfoxide (DMSO) buffer saturated with sodium chloride instead. If you are using DMSO buffer, it is nontoxic and nonflammable, but handling the buffer without gloves may result in exposure, producing a garlic/oyster taste in the mouth along with breath odor. This substance soaks into skin very rapidly along with any dissolved contaminants. Do not store the buffer where it will experience extreme heat, and do not freeze the sample. The buffer must be stored at room temperature or cooler, such as in a refrigerator.

Protocol for Turtles Not Boated

When a turtle that cannot be boated is alongside the vessel, a corer attached to a biopsy pole is used to obtain a biopsy sample. The sampling gear consists of a 12’ anodized aluminum breakdown biopsy pole, such as the NOAA/Epperly Biopsy Pole, or similar biopsy harpoon and a disinfected stainless steel biopsy corer.

Assemble the pole sections together if necessary to attain the desired pole length. The corers should be stored in ethanol-cleaned vials. Clean the end of the threaded stud on the biopsy pole section with an alcohol swab. Carefully remove the corer from its vial and screw it tightly on the end of the stud of the biopsy pole.

No more than two biopsies should be conducted per animal, and if you are unsuccessful obtaining a sample after two

attempts, no further attempt should be made (as required by permit conditions). Suitable sampling sites for hardshell turtles include the flippers, shoulders, and pelvic regions. A forceful jab perpendicular or oblique to the body is needed to penetrate the skin of most turtles (Figure 8-2). There are nerve bundles high on the shoulders near the carapace that should be avoided, as should the heavily vascularized armpit area. The best method to obtain biopsy samples from leatherbacks is to scrape a ribbon of tissue from the carapace with the corer, leaving a gray superficial scar that will heal well over time. Do not target the carapace, head and neck, or limbs with a jabbing motion when sampling leatherbacks.

Due care should be taken not to strike anyone when handling the pole onboard. Unscrew the corer from the pole, and place the entire corer with tissue sample into the sample vial. Do not attempt to remove the tissue from the corer. Clean the adapter stud with an alcohol swab and label the vial as previously described.

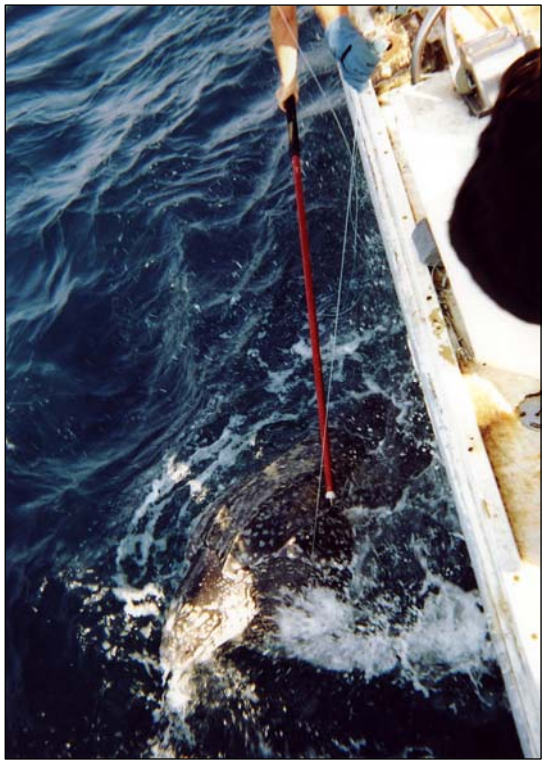


Figure 8-2. Taking a biopsy from a leatherback not boated (NMFS/SEFSC photo).

Lesion Biopsy

Samples may be taken to better understand the nature of a lesion and determine the most appropriate therapy. Single or multiple samples are collected, determined by the type of lesion biopsied. The methods used to collect and preserve the sample vary, depending upon the nature of the lesion and which diagnostic tests will be performed. For histologic evaluation, samples are fixed in 10% neutral buffered formalin (NBF). Samples to be examined for microbial isolation attempts are first cleansed with sterile saline before being placed in an appropriate transport media or sterile container for shipment to a diagnostic laboratory. Never freeze tissues undergoing histologic examination to preserve them, as this will result in tissue damage due to crystallization.

Fat Biopsy

Subcutaneous fat is collected from the inguinal region (Figure 8-3). Only a veterinarian or other highly trained individual using sterile surgical instruments should conduct this procedure. This procedure should not be performed on any compromised animals (e.g., those that are emaciated, with heavy parasite loads or bacterial infections) unless medically advised or necessary based on the experimental design of a health related study. After manually restraining the turtle, scrub the inguinal area with 10% povidone-iodine solution.

Infuse lidocaine hydrochloride (e.g., Phoenix Pharmaceuticals, Inc., St. Joseph, MO, USA), up to 2 mg/kg, intradermally and subcutaneously around the proposed incision sites in the inguinal areas ten minutes prior to the procedure to block any pain and discomfort to the turtle. Pull the rear flipper on the side of the incision back and toward the opposite side, causing the skin to remain taut. Make a two cm incision in the inguinal fo ssa using a disposable scalpel blade; blunt dissection of the connective tissue will be accomplished using surgical scissors. After grasping the connective tissue layer with forceps, use the surgical scissors to cut sharply down into the subcutaneous fat. Use the connective tissue layer to assist with gripping the fat with the forceps (as the consistency of the fat makes it difficult to seize it), and excise an approximately 0.4 – 4.0 g (~0.44 –4.4 cc) of the fat, which will then be placed in hexane-rinsed aluminum foil and immediately frozen at -80°C.

To close the incision, use a buried, simple continuous (or continuous horizontal mattress) subcuticular pattern using a monofilament nominally absorbable suture, such as one of the three following (Govett et al. 2004): polyglyconate (e.g., Maxon™, US Surgical, Norwalk, CT, USA), or poliglecaprone 25 (e.g., Monocryl™, Ethicon, Somerville, NJ, USA), or polydioxanone (e.g., PDS II™, Ethicon), followed by cyanoacrylate tissue glue on the surface. Depending on the size of the biopsy, it may be necessary to close the fat layer to eliminate dead space and reduce the chances of seroma or hematoma formation.

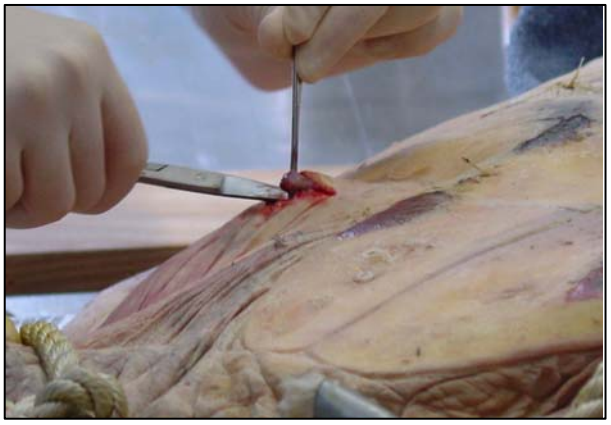


Figure 8-3. Taking a fat biospy sample (NMFS/OPR photo).

To reduce post-surgical complications (i.e., infections), a single dose of antibiotic (Table 8-1) may be administered prior to surgery. A non-steroidal, anti-inflammatory drug (e.g., ketoprofen at 2 mg/kg IM, MacLean et al. 2008) may be administered to reduce post-operative pain. If administered to green turtles, be especially watchful, as an older related anti-inflammatory compound, flunixin meglumine (e.g., Banamine®), can be lethal to green turtles (D. Mader, pers. comm.).

Drug	Dosage	Source
ceftazidime	20 mg/kg IM	Stamper et al. 1999
oxytetracycline	25 mg/kg IM	Harms et al. 2004
enrofloxacin	20 mg kg oral	Jacobson et al. 2005
ticarcillin	50 or 100 mg/kg IM	Manire et al. 2005
amikacin	5 mg/kg IM	Carpenter 2005

Table 8-1. Several antibiotic choices to reduce post-surgical complications.

Muscle biopsy

Surgical muscle biopsy (Southwood et al. 2003, Southwood et al. 2006)

Muscle tissue may be collected for biochemical analyses to determine aerobic and anaerobic metabolic capacity, thermal tolerance, or stable isotope analysis. Muscle tissue may be obtained from either the iliotibialis muscle of the rear flipper (Figure 8-4a) or the deltoidus muscle (Figure 8-4b), which protracts and abducts the front flippers during swimming. Only a veterinarian or other highly trained individual using sterile surgical instruments should conduct this procedure. This procedure should not be performed on any compromised animals (e.g., those that are emaciated, with heavy parasite loads or bacterial infection) unless medically advised or necessary based on the experimental design of a health related study.

Figures 8-4a and b. Muscle tissue may be excised from (a) the iliotibialis muscle or from (b) the deltoidus muscle (Photos from A.L. Southwood).

Thoroughly clean the incision area with 95% ethyl alcohol and 10% povidone-iodine solution. Inject up to 2 mg/kg 2% lidocaine (e.g., Vetoquinol Inc., Lavaltrie, QC) intramuscularly, intradermally, and subcutaneously into the incision area 10 minutes before the sample is to be taken. Make a 1.5 cm incision in the skin using a disposable scalpel blade and use surgical scissors for blunt dissection to expose muscle. Grasp muscle tissue with tissue forceps and use surgical scissors to excise approximately 200-300 mg of muscle tissue. Wrap the excised tissue in aluminum foil or place in a suitable storage vial and freeze in liquid nitrogen immediately. Use monofilament absorbable suture (e.g., polygloconate, MaxonTM, US Surgical, Norwalk, CT, USA; polydioxanone, Ethicon PDS IITM, Piscataway, NJ, USA; or poliglecaprone, Ethicon MonocrylTM) to close the incision area. A simple interrupted pattern with 3-0 suture may be used to pull muscle tissue together and horizontal mattress using 2-0 suture may be used to close the skin incision. Treat incision area with topical antibiotic cream (e.g., povidone-iodine ointment or triple antibiotic ointment) and give the turtle a single dose of antibiotic (Figure 7-4) at a site other than the incision site to reduce the risk of infection. Samples should be stored in an ultrafreezer at -80ºC.

Non-Surgical Muscle Biopsy

When a small sample is sufficient, an alternative non-surgical method, which is

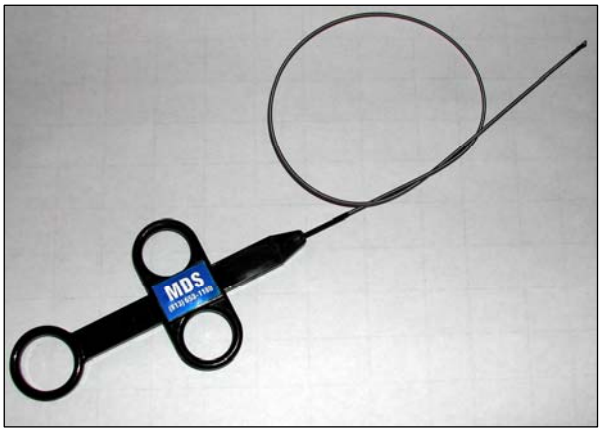
possible to conduct in the field, is to take a muscle biopsy sample in the shoulder region after thoroughly cleaning the area with 10% povidone-iodine solution and alcohol. Collect one sample on each side of the neck using a sterile 6 mm biopsy punch to the depth of the corer. Hold the sample with forceps and trim using surgical scissors. The samples should be placed in a suitable storage vial and stored in an ultrafreezer at -80ºC. If bleeding occurs, a blood clotting agent, such as ferric subsulfate or Clotisol® can be used, or the region may be cauterized or sutured if necessary.

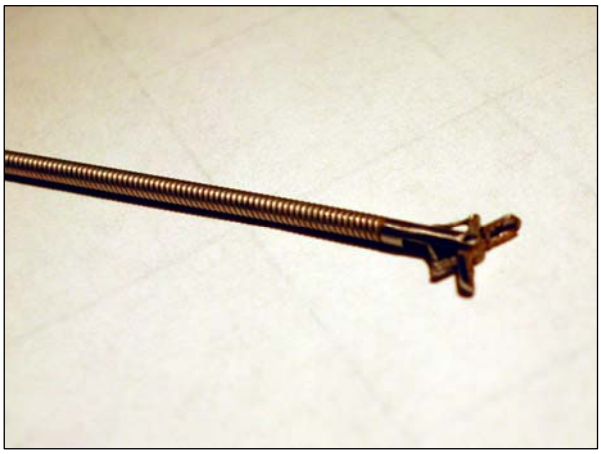
Biopsies Taken During Laparoscopy

Laparoscopies (Chapter 15: Laparoscopy) are performed to identify the sex of the animal, as well as to collect tissues for health assessments and for histology to confirm sex identification. It is possible to sample tissues such as the gonads, liver, kidney, spleen, and mesenteric fat, as well as any lesions. Below we describe in detail the methods for two of these; the methods for the other tissues will be done with similar care and attention to the well being of the turtle.

Gonad Biopsy

This procedure can be performed in the course of laparoscopy for sex determination, but should only be conducted by a veterinarian or other highly trained individual. This procedure should not be performed on any compromised animals (e.g., those that are emaciated or having heavy parasite loads, bacterial infections, etc.) unless medically advised or necessary based on the experimental design of a health related study. Propofol may be administered (5 mg/kg IV, MacLean et al. 2008) as a short-acting (depending on ambient temperature considerations) general anesthetic prior to the procedure. A nonsteroidal anti-inflammatory drug (e.g., ketoprofen, 2 mg/kg IM, MacLean et al. 2008) may be administered to reduce post-operative pain with no sedation, but special care should be taken with green turtles, as an older related anti-inflammatory compound, flunixin meglumine (e.g., Banamine®), can be lethal in that species (D. Mader, pers. comm.). A single pre-surgical dose of antibiotic (Table 8-1) may be administered to reduce the chances of post-surgical infections.





Figures 8-5a and b. Endoscopic cup biopsy forceps used for gonad biopsies (Photo by J. Vaughan, Florida Atlantic University).

Follow the procedure for laparoscopy in Chapter 15. Once the gonad is identified, extend the incision about three to four mm, attach the biopsy guide over the scope or open a biopsy port if the trocar is so equipped, and feed the biopsy tool into its port. Using an endoscopic cup biopsy forcep (Figures 8-5a and b), sample a one to two mm piece of the side of the cranial 1/3 of the gonad (about 1/3 the way down), avoiding vascular areas (the gonad sits on top of some of the renal blood vessels). Also, make sure the paramesonephric duct (i.e., the oviduct in females) is not lying on the sampling site. Sampling 1/3 of the way down from the cranial pole of the gonad will avoid accessory ducts (epididymus, vas deferens, Wolfian ducts, etc.), thus allowing access to the greater concentrations of follicles in the caudal ends of the ovaries. In addition, if one were to sample all the way cranially, this may disrupt the epididymus/vas deferens of males. Using a clean hypodermic needle, retrieve samples from the forcep cup, place into microcentrifuge tubes (e.g., Eppendorf®) filled with 10% buffered formalin, and store at room temperature. If any bleeding occurs (it is exceedingly rare for it to bleed beyond the surface sampling site), administer 10 ml/kg of intracoelomic fluids (e.g., Lactated Ringer's solution, 0.9% saline solution). After completing the examination, remove all air prior to suturing the wound. Close the incision as described in Chapter 15. Label the biopsy sample tubes with a permanent marker on the top and the side and properly package them prior to shipping.

Liver Biopsy

Liver biopsy samples for toxicology analysis may be collected in the course of laparoscopy for sex determination. This procedure should not be performed on any compromised animals (e.g., those that are emaciated or having heavy parasite loads, bacterial infections) unless medically advised. Propofol may be administered (5 mg/kg IV, MacLean et al. 2008) as a short-acting (depending on ambient temperature considerations) general anesthetic prior to the procedure. A nonsteroidal anti-inflammatory drug (e.g., ketoprofen, 2 mg/kg IM, MacLean et al. 2008) may be administered to reduce post-operative pain with no sedation, but special care should be taken with green turtles, as an older related anti-inflammatory compound, flunixin meglumine (e.g., Banamine®), can be lethal in that species (D. Mader, pers. comm.). A single pre-surgical dose of antibiotic (Table 8-1) may be administered to reduce the chances of post-surgical infections.

Follow the procedure for laparoscopy in Chapter 15. After laparoscopic examination of the gonads (if applicable), leave the laparoscope and sleeve in place and make a second one cm skin incision in the same inguinal space as the laparoscope. Advance a second trocar into the body cavity at a location that can be verified by the laparoscope as safe from any internal organ contact. Once the trocar is in the body cavity, advance a 4-mm cup biopsy instrument into the field of view and guide it to the liver. Take the biopsy at a location at the margin of the liver with minimal observable vascularity, avoiding the vascular areas (the gonad sits on top of some of the renal blood vessels). Make sure the paramesonephric duct (that will be the oviduct in females) is not lying on the sampling site. Using an endoscopic cup biopsy forcep, sample a one to two mm piece of the liver by firmly clamping the desired tissue with the cutting cup biopsy tip and retracting until the

tissue comes away. Obtain two biopsies of approximately 0.1 g (one to two mm) each from each turtle. Use a hypodermic needle to get the samples out of the forcep cup and into microcentrifuge tubes (e.g., Eppendorf®) filled with 10% buffered formalin. Observe the biopsy site directly for hemorrhage; if clotting fails to occur rapidly, insert a small piece of absorbable gelatin sponge hemostatic device (e.g., Gelfoam® Pharmacia & Upjohn, Kalamazoo, MI, USA) via the instrument port, and apply to the biopsy site to promote clotting. Close the incision as described in Chapter 15.

Release

Hold turtles receiving propofol out of water for at least one hour following the conclusion of the procedure, and do not return to the water until fully responsive. Hold all animals temporarily in tanks to ensure normal swimming and diving activity have returned prior to release.

Chapter 9: Blood Sampling

Blood samples should be taken by experienced personnel, and care should be taken to ensure no injury results from sampling. Turtles that are severely injured or compromised should not be sampled unless specifically authorized or during treatment by a veterinarian. Make a maximum of two attempts on each side of the neck, limiting needle insertion attempts to a total of four. Extract a maximum of 3 ml blood/kg of body weight; this cumulative blood volume should not be exceeded within a 45-day period of time. If the turtle cannot be adequately immobilized for blood sampling, efforts to collect blood must be discontinued.

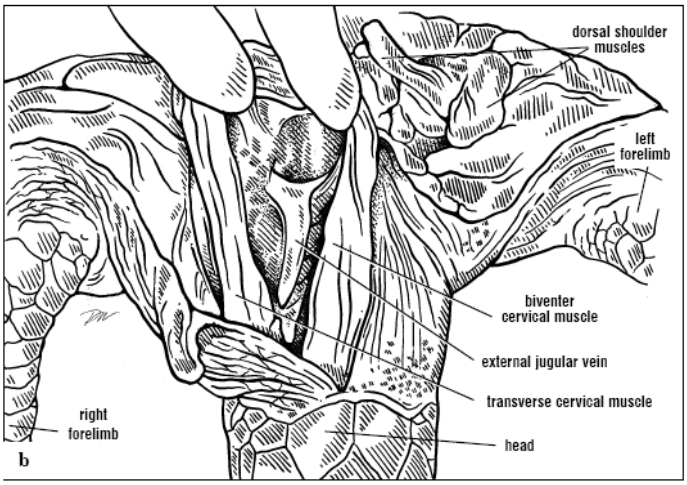


Figure 9-1. External jugular vein, biventer cervical and transverse cervical muscles in green turtle (Diagram from Wyneken 2001).

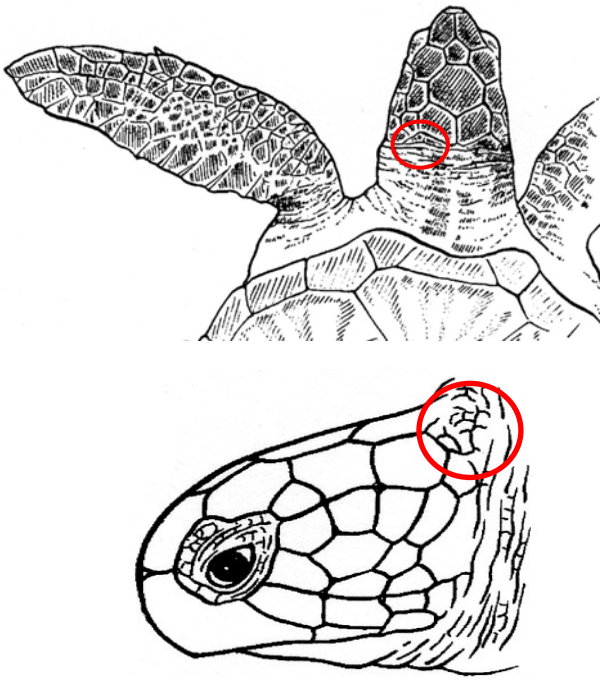
Hardshell Turtles

The external jugular vein (often termed the dorsal cervical sinus) is a commonly used blood collection site in sea turtles (Wyneken 2001). This vein is located relatively dorsal and superficial in the neck, and the biventer cervical and transverse cervical muscles are good landmarks for its location (Wyneken 2001). Rinse and clean the neck region with water and 10% povidone-iodine solution, isopropyl alcohol or other antiseptic prior to sampling. Restrain the turtle and pull the head gently forward and downward until it is fully outstretched to facilitate the filling of the bilateral cervical sinus. Use a new, disposable syringe and needle or a vacuum tube, needle, and holder system to collect the sample. A general guideline for needle selection to obtain blood samples is: for turtles less than

0.5 kg, use a 23-gauge 0.5” needle; for turtles 0.5 – 5 kg, use a 21-gauge 1” needle; for turtles larger than 5.0 kg, use a 21-gauge 1.5” needle.

Insert the needle on either side of the midline of the neck (depending on the size of the turtle, from 0.5 – 3.0 cm lateral to the midline) about 1/3 to 1/2 of the distance between the back of the head from the anterior edge of the carapace (Figure 9-2).

Figure 9-2. Blood sampling from the bilateral cervical sinus using a vacuum tube and needle (Photo courtesy of J. Wyneken, Florida Atlantic University).



Figures 9-3a and b. Blood sampling site for green sea turtles (Diagram from Wyneken 2001, annotations by A. Southwood)

In green and hawksbill sea turtles, the external jugular is smaller in diameter and branches little when compared with the anatomy in other cheloniids (Wyneken 2001). Some researchers have found that particularly with green turtles, the ideal sampling site is anterior to this position just behind the head (Figures 9-3a and b). To draw blood from this site, insert the needle at a very shallow angle (≤ 30°) lateral to the supraoccipital crest at the base of the skull where the scales form a noticeable "V" shape, directing the needle at shallow angle straight back towards the carapace along the medial line (A. Southwood, pers. comm.). The correct location (Figure 9-4) is within the "valley" between the biventer and transverse cervical muscles (Figure 9-1).

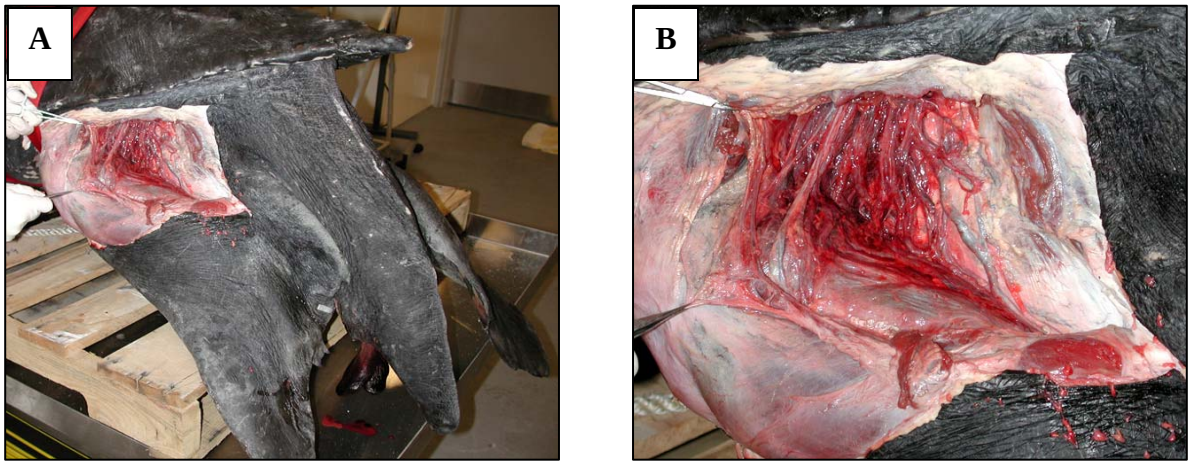
Insert the needle approximately 45 – 90° to the plane of the neck and do not move the needle laterally to locate the vessel, as this will cause tissue damage. Once the needle is inserted, apply suction and move the needle slowly up and down until the sinus is located. Do not remove the needle from the neck while still applying suction, as this can contaminate the sample. Once the blood has been obtained, withdraw the needle and insert it into the rubber tip of an appropriate vacuum tube to transfer the sample. Dispose of the needle and syringe in a sharps container.

Place the samples on ice until they can be processed. Place the vials adjacent to, but not directly on the ice to prevent freezing and lysis of red blood cells, which adversely affects plasma biochemistry analysis and some serological assays. In the laboratory, spin down blood samples for 10 min in a centrifuge and then pipette the separated plasma into a cryogenic vial. Wash the remaining red blood cells with an equal amount of 2.5% saline solution and then pipette into a second cryogenic vial. The samples should be stored at -80°C.

Figure 9-4. Blood sampling from superficial veins lateral to the supraoccipital crest in a green sea turtle (Photo courtesy of A. Southwood).

Leatherbacks

Leatherbacks are generally blood sampled in the rear flipper (Figures 9-5a and b) in a nexus (vein bundle) located approximately five cm from the edge of the carapace and one cm interior medial of the tibia (Dutton 1995, Wyneken pers. comm.). Alternatively, blood can be sampled from the interdigitary vessels, with optimal needle insertion points approximately one inch distal to the junctions of each pair of phalanges (Wallace and George 2007). Sampling from the dorsal cervical sinus is not recommended by some in leatherbacks, as it is relatively deep compared with other species, but it can be performed using an 8.9 cm (3.5") spinal needle (Harms et al. 2007) if the researcher is experienced with this procedure.



Figures 9-5a and b. Location of the rear flipper nexus (a) for blood sampling and (b) a closeup view (Photos courtesy of J. Wyneken, Florida Atlantic University).

Chapter 10: Cloacal and Microbiologic Lesion Sampling

In order to conduct bacterial culture and/or antimicrobial susceptibility testing, cloacal and lesion samples may be examined. Temporarily overturn each turtle to be sampled onto its carapace and restrain. First, scrub the external opening of the cloaca with 10% povidone-iodine to disinfect the area. After securely gripping the tip of the tail, insert a sterile culturette tip (e.g., BBL CultureSwab™, Becton Dickinson and Company, Sparks, MD) approximately one inch into the cloaca. For culture of external lesions, gently insert the culturette tip into the deepest area of the wound. In both cases, rotate the culturette tip approximately 360° and remove it from the cloaca or lesion (Figure 10-1). Immediately place the culture into a sterile transport medium (e.g., BBL Port-A- Cul™ Tubes, Becton Dickinson and Company, Sparks, MD) for overnight shipment to a laboratory for bacterial culture and antimicrobial susceptibility testing. Some media tubes also may be stored between at -80° C in liquid nitrogen prior to testing.

Figure 10-1. Cloacal culture (NMFS/SEFSC photo).

Chapter 11: Fecal Sampling

Fecal samples may be collected for diet analysis or to evaluate the presence, diversity, and species composition of any internal parasites using sodium nitrate flotation and sedimentation. When sampling for diet content analysis, turtles will be held in a suitable holding pool (in a shaded area with water temperatures similar to those of the water temperatures at capture) for a period of up to 48 hours to obtain a defecated sample.

Sampling for parasite analysis will occur either after turtles have defecated during biological sampling or by digital extraction of feces from the cloaca. Those turtles that do not defecate during the sampling period may be temporarily overturned onto the carapace and restrained.

After cloacal cultures have been obtained, fecal samples can be taken if desired (see Chapter 10: Cloacal and Microbiologic Lesion Sampling). Insert one finger while wearing lubricated latex gloves into the cloaca of the turtle to feel for the presence of a fecal mass (Figure 11-1). If one is detected, remove it, place it into either a polyethylene bag or a conical centrifuge tube and place it on ice. Label bags and tubes (e.g., turtle identification number, date, and species), and then ship the sample on ice to the laboratory.

Figure 11-1. Collection of fecal sample for parasite analysis (NMFS/SEFSC photo).

Chapter 12: Epibiota Sampling

Using a hoofpick/scraper, rounded putty knife or other blunt instrument, carefully pry off barnacles or other epibiota from the turtles' carapace, taking care not to remove the underlying scute (Figure 12-1). Barnacles may also be removed by placing the narrow tip of a wooden skewer at the base of the barnacle and gently rotating so that the skewer tip lodges between the barnacle and the carapace of the turtle. Apply gentle leverage in order to pry the barnacle from the carapace. If necessary, several entry/leverage points may be used to ensure that the barnacle is removed from the carapace without causing damage; however, in some instances, the keratin to which the barnacles are attached may already exist in a 'sloughing' state and become dislodged. For epibiota present in areas other than the carapace, use the corner of a plastic putty knife or forceps tips to gently pry up the edge of the specimen in question, and then pull the entire organism away from the epithelium. If bleeding occurs, apply pressure to the affected area using a 10% povidone-iodine swab. Seal the sample in a freezer-style plastic storage bag, and using a permanent marker, label the bag (e.g., turtle identification number, date, and species). The epibiota also can be placed in sample jars filled with ethanol.

Figure 12-1. Epibiota sampling from loggerhead carapace (NMFS/SEFSC photo).

Chapter 13: Keratin Sampling

Keratin may be collected from the outermost edge of the eight most posterior marginal scutes of the carapace for mercury analysis (Day 2003). Scutes free of fouling organisms/epibiota, and those that appeared to have keratin of sufficient thickness and texture to provide a sufficient sample mass while minimizing the risk of penetrating through the keratin layer, should be targeted for sampling (Figure 13-1). A relatively thin edge of keratin, where the keratin and underlying bone can be discriminated, is usually present where the dorsal and ventral surfaces of a scute meet. Thus, it is possible to avoid scraping too deeply, causing injury to the turtle and contaminating the sample with untargeted tissues.

To obtain a keratin sample, place each turtle on its plastron briefly on a slightly elevated platform with approximately 15 – 20 cm of the posterior edge of the carapace overhanging the edge of the platform. While one researcher is restraining the turtle's rear flippers, two other researchers will prepare to collect the sample. Before taking the keratin sample, scrub two cm or more of carapace dorsal and ventral to the edge of the scutes vigorously with a plastic scrubbing pad to remove sloughing keratin. If there are no areas free of epibiota, use a plastic scraper to clear the target area as thoroughly as possible prior to scrubbing. Afterward, rinse the scrubbed area with high purity distilled water and isopropanol, and then remove any remaining foreign matter and debris using cellulose based cleanroom wipes or cotton gauze, distilled water, and isopropanol or high purity 95% ethanol. Finally, remove the lateral edge of the prepared marginal scutes by shaving off the edges of the scutes parallel to the edge being sampled using a disposable, sterile scalpel blade. Keratin splinters may also be collected by carefully sliding a sterile biopsy punch along the outer edge of the scute, parallel to the body axis. Allow the shavings to fall directly into a polyethylene sample bag held by a second researcher wearing Kevlar gloves to prevent injury.

Figure 13-1. Collecting keratin sample. Turtle in this photo is positioned on its carapace to better highlight the sampling region (Photo by M. Godfrey, North Carolina Wildlife Resources Commission).

Typically, the posterior lateral corner of each scute will yield the thickest sample without penetrating the keratin and contaminating the sample with untargeted tissue. This should yield small shavings or splinters of keratin ~ one mm in thickness totaling ten cm total of one mm thick shavings. Label the outside of the polyethylene bag (e.g., turtle identification number, date, and species), and then freeze the sample at -20 °C.

Chapter 14: Gastric Lavage

(Adapted from Forbes 1999)

Although there are several procedures to analyze the feeding habits of wild sea turtles, the preferred method is gastric lavage or stomach flushing (Forbes 1999). Gastric lavage allows for the retrieval of undigested food from the esophagus and anterior portions of the stomach for content analysis. This simple technique to sample the gut contents of wild turtles has been conducted on hardshell turtles ranging in size from 25 to 115 cm curved carapace length (Forbes 1999).

Restrain turtles by placing them briefly in an inverted position on a cushioned surface, such as an automobile tire or padded slant board. If using a tire, it should be placed on a platform raised several feet above the ground to allow easier access to the turtle during the lavage process. Prior to lavage, adjust the turtle's position so that the cranial anterior part of the body is lower than the caudal posterior to allow gravity to assist with collection of esophageal contents. Depending on the size and activity level of the turtle being lavaged, one or two individuals will restrain the flippers and hold the head so that the neck and esophagus remain in line with the longitudinal axis of the body.

Prompt the turtle to open its mouth by gently tugging on the skin of the throat, offering a bite block, or working two lengths of soft, large-diameter rope in between the jaws to hold the jaws apart from one another (see NMFS 2008 for mouth opening tools and techniques). Once the mouth is open, insert an appropriately sized standard veterinary canine oral speculum just posterior to the anterior tip of the rhamphotheca to keep the jaws from closing. The powerful jaws of larger loggerheads may necessitate use of a short length of 5 cm diameter PVC or similar bite block to keep the mouth open. Both the bars of the oral speculum and any pipe used for this purpose should be wrapped with soft, rubber tape or tubing to prevent damage to the rhamphotheca.

Once the mouth is securely open and the turtle's position has been stabilized, prepare to insert two lengths of clear, flexible, vinyl tubing lubricated with vegetable oil or water-based lubricating gel into the esophagus, passing to either side of the oral speculum. The first tube, used to retrieve food items from the esophagus, should be one m in length with a wall thickness of two mm and inner diameter of three to five cm, depending upon the size of the turtle. The second tube, used to introduce water into the esophagus to flush out food particles, should be three m in length with a wall thickness of two mm and inner diameter of five mm. Round the ends of both lengths of tubing by melting them with a flame and allowing them to cool prior to use to ensure that the tubing will not damage the walls of the esophagus during insertion. Align the tubes exterior to the turtle to pre-measure the distance to the caudal margin of the pectoral scute of the plastron, roughly corresponding to the level of the stomach, and mark the distance on the tube for that particular turtle with either tape or erasable marker. The tubes should be passed no farther than this mark, or no farther than they will pass without resistance. Although the lengths of tubing may partially obstruct the glottis during the lavage process, take care not to

Figure 14-1. Gastric lavage (NMFS/SEFSC photo).

accidentally introduce the ends of the tubing into the glottis opening. An alternative method will be to lubricate a soft plastic veterinarian's stomach tube with vegetable oil and cautiously insert it into the mouth and throat area.

To initiate lavage, pump freshwater into the esophagus using a double-action, veterinary stomach pump while the introduction tube is gently moved up and down the length of the esophagus (Figure 14-1). If water does not begin to flow from the retrieval tube within a few seconds after introducing water into the esophagus, or if return water flow is low, adjust the position of the retrieval tube to remove any obstruction. If the situation does not resolve, water flow into the esophagus should be stopped. Barring any such difficulties, gently move the retrieval tube up and down the esophagus for 30 – 45 seconds while water flowing through the tube is collected in a bucket. The actual lavaging of an individual turtle should not exceed three minutes. Strain the bucket contents through a fine-mesh sieve, and preserve any food particles in 10% buffered formalin for future analysis.

After completion of lavage, stop the water flow and elevate the posterior of the turtle slightly to allow the introduction and retrieval tubes to drain. Once the tubes have drained, remove the introduction tube first, followed by the retrieval tube and the mouth gag or PVC pipe. At this point, elevate the anterior part of the turtle's body slightly relative to the posterior to allow any remaining water to drain into the esophagus, away from the glottis, so that the turtle can take a breath.

Equipment (e.g., lavage tubes) must be disinfected between animals. Lavage tubes must be thoroughly cleaned prior to disinfection. Disinfectants should be used according to directions, ensuring that contact time with disinfectant according to label directions is sufficient. Disinfection can be compromised if items are contaminated with debris and/or have rough or porous surfaces. Researchers should clean items prior to disinfection and increase the exposure time for rough or porous items. Additionally, a separate set of equipment must be used for healthy animals and those with health problems (e.g., fibropapilloma tumors).

Chapter 15: Laparoscopy

Laparoscopies are performed to identify the sex of the animal and for health assessments (Figures 15-1a – c). Only individuals thoroughly trained in the laparoscopy of marine turtles, or directly supervised by individuals so trained should conduct this procedure. Aseptic techniques should be used at all times to prevent infection. This procedure should not be performed on any compromised animals (e.g., those that are acutely emaciated or obese, overheated, or have heavy parasite load or severe bacterial infection).

Large Juvenile and Adult Turtles

Maintain the turtle at temperatures similar to capture temperature and restrain the turtle briefly on its carapace in an inverted or lateral position for surgery (Figure 15-1). Following a surgical scrub (either three alternating applications of 70% ethanol and surgical iodine scrub, 70% isopropanol, povidone-iodine scrub, and chlorohexidine wipe), inject a local anesthetic (lidocaine, maximum of 2 mg/kg) into the muscle and dermis of the peritoneal wall of the prefemoral fossa. At operating temperatures above 78º F, allow a minimum of 10 minutes and a maximum of 45 minutes after the lidocaine injection prior to surgery. For lower temperatures, allow greater drug effect onset times (e.g., allow a minimum of 15 – 20 minutes when operating between 72 – 78° F).

Make a one to two cm incision just through the skin; use the trocar and sleeve to push through the muscles and peritoneal wall into the body cavity. Be careful to avoid an entry that is too far posterior (where the trocar might strike the kidney) or an entry that goes too deep (where the trocar might strike the lung or intestine). After achieving entry into the peritoneal cavity, verify the location of the trocar with the laparoscope prior to inflating the body cavity with filtered air. Inflation (known as insufflation) is sometimes necessary to visualize the internal organs.

After completing the examination, remove all air prior to suturing the wound. Intracoelomic fluids (sterile 0.9% saline or other IV fluid solution at up to 3% body weight or 30 ml/kg) may be administered as supportive peri-operative care to maintain fluid and electrolyte balance and to help displace air in the body cavity during evacuation.

Use a single deep suture and two superficial sutures to seal the wound using a monofilament nominally absorbable suture (Govett et al. 2004), such as: polyglyconate (Maxon™, US Surgical, Norwalk, CT, USA), or poliglecaprone 25 (Monocryl™, Ethicon,
Somerville, NJ, USA), or polydioxanone (PDS II™, Ethicon). The suture size depends on the size of the turtle, but will be 2-0, 3-0 or 4-0. The deep suture is a horizontal mattress pattern to eliminate dead space, and the superficial sutures may be either a buried, subcuticular horizontal mattress or external simple interrupted, horizontal mattress, or cross mattress, depending on surgeon preference.

Figure 15-1a, b and c. Laparoscopy for sex identification (Photos by M. Godfrey, NCWRC).

Drug	Dosage	Source
ceftazidime	20 mg/kg IM	Stamper et al. 1999
oxytetracycline	25 mg/kg IM	Harms et al. 2004
enrofloxacin	20 mg kg oral	Jacobson et al. 2005
ticarcillin	50 or 100 mg/kg IM	Manire et al. 2005
amikacin	5 mg/kg IM	Carpenter 2005

Table 15-1. Several antibiotic choices to reduce post-surgical complications.

A single pre-surgical dose of antibiotic (Table 15-1) may be administered to reduce the chances of post-surgical infections. Propofol may be administered (5 mg/kg IV, MacLean et al. 2008) as a short-acting general anesthetic prior to the procedure. Turtles that receive propofol should be held out of water for at least one hour following the conclusion of the procedure and should not be returned to water until it is established that they are fully responsive. A nonsteroidal anti-inflammatory drug (e.g., ketoprofen, 2 mg/kg IM, MacLean et al. 2008) may be administered to reduce post-operative pain with no sedation; be especially attentive if used on green turtles, as an older related anti-inflammatory compound, flunixin meglumine (e.g., Banamine®), can be lethal in that species (D. Mader, pers. comm.). All wild turtles should be held in tanks temporarily to ensure that normal swimming and diving activity have returned prior to their release.

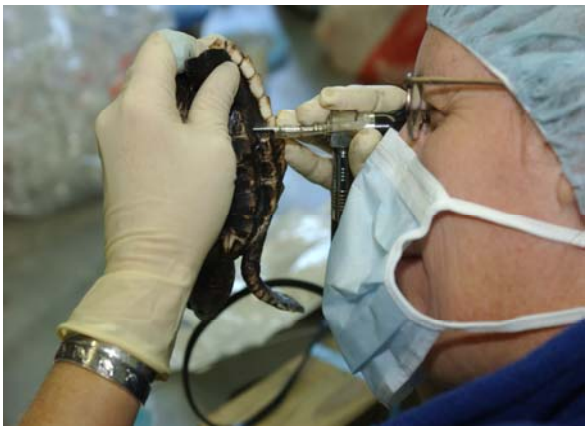
Post-hatchling and Small Juvenile Turtles (Wyneken et al. 2003, 2007)

The laparoscopic method for sex determination has been modified for use in sea turtles as small as 120 g (Figures 15-2a and b). When post-hatchling turtles reach a minimum size of 120 g, withhold food for 24 hours and give one to two drops of infant simethecone orally 12 – 24 h prior to the procedure. Prior to laparoscopy, clean the turtle posterior to

the head with disinfectant soap and water, then give them a surgical scrub (either three alternating applications of 70% ethanol and surgical iodine soap, or soap and water, 70% isopropanol, povidone-iodine scrub, and chlorhexidine wipe). A minimum of 10 – 15 minutes prior to surgery, and no longer than 40 min prior to surgery, inject 0.25 ml 10% lidocaine (1mg/ml) for 120 g turtles (1.3 – 2.1 mg/kg) around the incision site in the anterior inguinal fossa to block any pain or discomfort the turtle might experience during the procedure.

Right handed observers usually use the right prefemoral fossa while left handed people may prefer to enter through the left inguinal fossa. Depending on state permit conditions, the analgesic butorphanol may be administered (0.1mg/kg SQ) approximately 10 minutes prior to surgery. When deemed appropriate, an antibiotic such as ceftazidime (20 mg/kg IM) may be administered in the shoulder musculature to reduce the risk of post surgical infection.

It is simplest to hold small turtles by hand and position each so the head is facing down; the viscera are displaced by gravity away from the incision site and are not



Figures 15-2a and b. Laparoscopy for sex identification in loggerhead post-hatchling (Photo 15-2a by J. Foote, Mote Marine Lab and 15-2b by S. Taylor, Duke Marine Laboratory).

covering the gonads. For post-hatchlings, omit the use of a trocar and sleeve, as they require a larger incision. Use a simple longitudinal incision to open the skin with a simple 0.5 cm cut and follow with a stab incision made with closed 4.5” straight sharp-point surgical scissors. This does the least damage to the inguinal muscle.

Examine the internal organs, especially the gonads and gonadal ducts of the turtles using a 2.7 mm 30º rigid orthopedic endoscope (e.g., Medical Diagnostic Systems, Brandon, Florida). Landmarks for

gonads and ducts are established by the lung tip and kidney, posteriorly. Internal anatomical characteristics, such as relative size, color, shape, attachment of the gonad, and accessory duct (Müllerian duct) form are then recorded. Remove the scope and close the incision with one to three simple interrupted sutures.

Use absorbable suture material [e.g., Maxon™ (polyglyconate), Monocryl™ (polyglyconate) and/or Vicryl™ (polyglactin) 000 or 0000 size with an attached cutting needle works well] often with cyanoacrylate tissue glue, closing both the muscle and skin at the same time. The cut edges are everted slightly. Finally, apply triple antibiotic ointment to the site to prevent any post-surgical infection, and coat each turtle with a water-based lubricating gel to prevent the turtle from drying out. Return the turtles to the water the next day and feed. Typically, the post-hatchling turtles eat enthusiastically following this procedure. Release into oceanic waters one to three weeks following surgery. If a turtle does not feed and/or floats with its flippers out to the sides, seek veterinary assistance quickly, as the turtle may have developed an infection.

Chapter 16: Imaging

Turtles may be examined using a variety of imaging techniques [e.g., ultrasonography (U/S), radiography (x-rays), computed tomography (CT), and magnetic resonance imaging (MRI)] to assess their health and to gather baseline images. These imaging devices are similar to those used in human medicine, and the procedures are non-invasive. Transport, handling, restraint and positioning for imaging shall be consistent with safe handling procedures detailed in Chapter 2: Handling. Wet towels may interfere with image clarity, but may be used on non-affected areas of the turtles, depending on ambient air temperatures. Dry towels used over the head and eyes or for restraint may calm the turtle, will not interfere with the image, and do not pose the risk of evaporative cooling. It may be advised to fast the animal for 48 hours, depending on the animal's condition and veterinary guidance.

Figure 16-2. Turtle restrained for MRI procedure (NMFS/SEFSC photo).

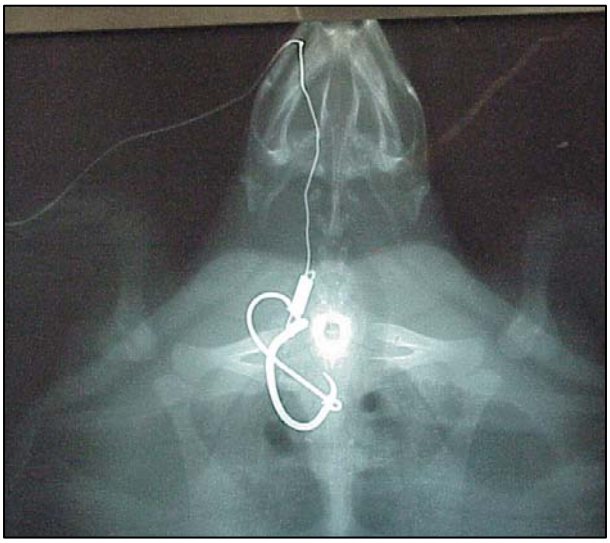


Figure 16-1. X-ray image of ingested fishing hook (Photo courtesy of J. Flanagan, Houston Zoo).

Ultrasonic examination is non-invasive, quick, and generally does not require an anesthetic. Ultrasonic imaging has been used widely on sea turtles (Owens 1999), primarily to evaluate a turtle's gonadal condition and determine reproductive status. Radiography (Figure 16-1) has wide ranging application for health assessment, including diagnosis after injury or ingestion of foreign materials (e.g., fishing hooks, plastic). CT scans and MRIs (Figure 16-2) offer high resolution images. Methodology for these imaging procedures will differ depending on the technology used and operator preference; therefore, no specific guidelines for these procedures are detailed here. Standard radiation safety protocols for personnel shall be followed for radiographic and CT imaging.

In some cases, the turtle may need to be physically or chemically restrained or anesthetized [e.g., 3 - 5 mg/kg propofol IV; or mededtomidine (0.15 - 0.18 mg/kg IV) / ketamine (5 - 6 mg/kg IV) reversed with atipamezole (0.7 - 0.9 mg/kg IM) (after Chittick et al. 2002)], at the discretion of the attending veterinarian. Ultimately, determining the need for sedation, drug selection and dosage is solely the decision of the attending veterinarian. While sedated, the animal's heart rate should be monitored (Figure 16-3) using an ultrasonic doppler flow detector (e.g., Model 811-BTS, Parks Medical Electronics, Inc., Aloha, Oregon, USA) or other appropriate methods whenever possible.

Figure 16-3. Monitoring heart rate (NMFS/SEFSC Photo)

References

Balazs, G.H. 1999. Factors to consider in the tagging of sea turtles, pp 101-109. *In:* Eckert, K.L., Bjorndal, K.A., Abreu-Grobois, F.A., and Donnelly, M. (Eds.) Research and Management Techniques for the Conservation of Sea Turtles, IUCN/SSC Marine Turtle Specialist Group Publication No. 4.

Bolten, A.B. 1999. Techniques for measuring sea turtles, pp 110-114. *In:* Eckert, K. L., Bjorndal, K.A., Abreu-Grobois, F.A. and Donnelly, M. (Eds.) Research and Management Techniques for the Conservation of Sea Turtles, IUCN/SSC Marine Turtle Specialist Group Publication No. 4.

Carpenter, J.W. 2005. Exotic Animal Formulary, 3rd Edition. St. Louis, MO: Elsevier Saunders, p. 55.

Chittick, E.J., Stamper, M.A., Beasley, J.F., Lewbart, G.A., and Horne, W.A. 2002. Medetomidine, ketamine, and sevoflurane for anesthesia of injured loggerhead sea turtles: 13 cases (1996–2000) Journal of the American Veterinary Medical Association 221(7):1019-1025.

Code of Federal Regulations, 50 CFR 223.206, October 1, 2003. Government Printing Office, Washington DC, pp. 164-170.

Day, R.D. 2003. Mercury in loggerhead sea turtles, *Caretta caretta*: Developing monitoring strategies, investigating factors affecting contamination, and assessing health impacts. Master's thesis, College of Charleston, South Carolina, 145 p.

Dutton, P.H. 1995. Molecular evolution of sea turtles with special reference to the leatherback, *Dermochelys coriacea*. Ph.D. Thesis, Texas A&M University, College Station. 137 pp.

Eckert, S. A. and Eckert, K.L. 1986. Harnessing Leatherbacks. Marine Turtle Newsletter No. 37: p. 1-3.

Epperly, S., Stokes, L., and Dick, S. 2004. Careful release protocols for sea turtle release with minimal injury. NOAA Technical Memorandum NMFS-SEFSC-524, 42p.

Epperly, S.P., Wyneken, J., Flanagan, J.P., Harms, C.A., and Higgins, B. 2007. Attachment of popup archival transmitting tags to loggerhead sea turtles (*Caretta caretta*). Herpetological Review 38(4): 419-425.

Federal Register, December 31, 2001. Government Printing Office, Washington DC 66 (250), pp. 67495-67496.

Federal Register, December 4, 1992. Government Printing Office, Washington DC 57 (234), pp. 57354-57358.

Federal Register, July 28, 1978. Government Printing Office, Washington DC 43 (146), pp. 32800-32811.

Fontaine, C. T., Revera, D. B., Williams, T.D., and Caillouet, C.W. Jr. 1993. Detection, verification and decoding of tags and marks in head started Kemp's ridley sea turtles, *Lepidochelys kempii*. NOAA Tech. Memo. NMFS-SEFC-334. U. S. Dept. Commerce, 40 pp.

Forbes, G.A. 1999. Diet Sampling and Diet Component Analysis, pp 144-148. *In:* Eckert, K. L., Bjorndal, K.A., Abreu-Grobois, F.A. and Donnelly, M. (Eds.) Research and Management Techniques for the Conservation of Sea Turtles, IUCN/SSC Marine Turtle Specialist Group Publication No. 4.

Godley B.J., Richardson, S., Broderick, A.C., Coyne, M.S., Glen, F., and Hays, G.C. 2002. Long-term satellite telemetry of the movements and habitat utilisation by green turtles in the Mediterranean. Ecography 25, 352-362.

Govett, P.D., Harms, C.A., Linder, K.E., Marsh, J.C., and Wyneken, J. 2004. Effect of four different suture materials on the surgical wound healing of loggerhead sea turtles (*Caretta caretta*). Journal of Herpetological Medicine and Surgery 35(4): 6-11.

Griffin, D.B. 2002. GIS analysis of inter-nesting habitat, migratory corridors, and resident foraging areas of the loggerhead sea turtle (*Caretta caretta*) along the southeast coast. Internship Report, College of Charleston, Charleston, SC, pp. 64.

Harms, C.A., Papich, M.G., Stamper, M.A., Ross, P.M., Rodriguez, M.X., and Hohn, A.A. 2004. Pharmacokinetics of oxytetracycline in loggerhead sea turtles (*Caretta caretta*) after single intravenous and intramuscular injections. Journal of Zoo and Wildlife Medicine 35(4): 477-488.

Harms, C.A., Eckert, S.A., Kubis, S.A., Campbell, M., Levenson, D.H., Crognale, M.A. 2007. Field anaesthesia of leatherback sea turtles (*Dermochelys coriacea*). The Veterinary Record 161: 15-21.

Higgins, B.M., Robertson, B.A. and Williams, T.D. 1997. Manual for mass wire tagging of hatchling sea turtles and the detection of internal wire tags. NOAA Technical Memorandum NMFS-SEFSC-402, ix plus 66p.

Jacobson, E., Gronwal, R., Maxwell, L, Merrit, K, Harman, G. 2005. Plasma concentratiosn of enrofloxacin after single-dose oral administration in loggerhead sea turtles (*Caretta caretta*). Journal of Zoo and Wildlife Medicine 36: 628-634.

MacLean, R.A., Harms, C.A., Braun-McNeill, J. 2008. Propofol anesthesia in loggerhead (*Caretta caretta*) sea turtles. Journal of Wildlife Diseases 44:143-150.

Manire, C.A., Hunter, R.P., Koch, D.E., Byrd L., Rhinehart H.L. 2005. Pharmacokinetics of ticarcillin in the loggerhead sea turtle (*Caretta caretta)* after single intravenous and intramuscular injections. Journal of Zoo and Wildlife Medicine 36: 44-53.

National Marine Fisheries Service Southeast Fisheries Science Center. 2008. Careful release protocols for sea turtle release with minimal injury. NOAA Technical Memorandum NMFS-SEFSC-580, 130 pp.

Owens, D.W. 1999. Reproductive cycles and endocrinology. *In* Research and Management Techniques for the Conservation of Sea Turtles. Eckert,K.L., K.A. Bjorndal, F.A. Abreu-Grobois, and M. Donnelly (Editors). IUCN/SSC Maine Turtle Specialist Group Publication No. 4: 119-123.

Papi, F., Luschi, P., Crosio, E., and Hughes, G.R. 1997. Satellite tracking experiments on the navigational ability and migratory behaviour of the loggerhead turtle *Caretta caretta*. Marine Biology 129: 215-220.

Polovina, J.J., Kobayashi, D.R., Parker, D.M., Seki, M.P., and Balazs, G.H. 2000. Turtles on the edge: movement of loggerhead turtles (*Caretta caretta*) along oceanic fronts, spanning longline fishing grounds in the central North Pacific, 1997-1998. Fisheries Oceanography 9: 71-82.

Pritchard, P.C.H. and Mortimer, J.A. 1999. Taxonomy, external morphology, and species identification, pp. 21-38. *In:* Eckert, K. L., Bjorndal, K.A., Abreu-Grobois, F.A. and Donnelly, M. (Eds.) Research and Management Techniques for the Conservation of Sea Turtles, IUCN/SSC Marine Turtle Specialist Group Publication No. 4.

Sato, K., Sakamoto, W., Matsuzawa, Y. Tanaka, H., and Naito, Y. 1994. Correlation between stomach temperatures and ambient water temperatures in free-ranging loggerhead turtles, *Caretta caretta*. Marine Biology 118: 343-351.

Southwood, A.L., Darveau, C.A., Jones, D.R. 2003. Metabolic and cardiovascular adjustments of juvenile green turtles to seasonal changes in temperature and photoperiod. *Journal of Experimental Biology* 206: 4521-4531.

Southwood, A.L., Andrews, R.D., Paladino, F.V., and Jones, D.R. 2005. Effects of diving and swimming behavior on body temperatures of pacific leatherback turtles in tropical seas. Physiological and Biochemical Zoology 78(2): 285-297.

Southwood, A.L., Reina, R.D., Jones, V.S., Speakman, J.R., Jones, D.R. 2006. Seasonal metabolism of juvenile green turtles (*Chelonia mydas*) at Heron Island, Australia. *Canadian Journal of Zoology* 84(3): 125-135.

Stamper, M.A., Papich, M.G., Lewbart, G.A., May, S.B., Plummer, D.D., Stoskopf, M.K. 1999. Pharmacokinetics of ceftazidime in loggerhead sea turtles (*Caretta caretta*) after single intravenous and intramuscular injections. Journal of Zoo and Wildlife Medicine 31: 32-35.

Wallace, B.P. and George, R.H. 2007. Alternative techniques for obtaining blood samples from leatherback turtles. Chelonian Conservation and Biology 6(1): 147-149.

Wyneken, J. 2001. The Anatomy of Sea Turtles. NOAA Technical Memorandum NMFS-SEFSC-470, 172p.

Wyneken, J., Garner, M.M., and Harms, C. A. 2003. Tracking Natural Sex Ratios and Posthatchling Gonadal Development in Posthatchling Loggerhead Sea Turtles (*Caretta caretta*) Using Laparoscopy, Gross Morphology, and Histology. Proceedings of the Association of Reptilian and Amphibian Veterinarians, pp. 112-115.

Wyneken, J., Epperly, S.P., Crowder, L.B., Vaughan, J., and Esper, K.B. 2007. Determining sex in posthatchling loggerhead sea turtles using multiple gonadal and accessory duct characteristics. Herpetologica 63(1): 19-30.

14 Appendix B: *Protocol for Use of Shortnose, Atlantic, Gulf, and Green Sturgeons*
http://www.nmfs.noaa.gov/pr/pdfs/species/kahn_mohead_2010.pdf

A Protocol for Use of Shortnose, Atlantic, Gulf, and Green Sturgeons

Jason Kahn and Malcolm Mohead

U.S. Department of Commerce
National Oceanic and Atmospheric Administration
National Marine Fisheries Service

NOAA Technical Memorandum NMFS-OPR-45
March 2010

Atlantic sturgeon (Robert Michelson, Photography by Michelson, Inc.)

Gulf sturgeon (Oscar Sosa, *New York Times*)

Green sturgeon (Thomas Dunklin)

Cover: shortnose sturgeon (credit: Robert Michelson)

A Protocol for Use of Shortnose, Atlantic, Gulf, and Green Sturgeons

Jason Kahn and Malcolm Mohead

NOAA Technical Memorandum NMFS-OPR-45
March 2010

U.S. Department of Commerce
Gary Locke, Secretary of Commerce

National Oceanic and Atmospheric Administration
Jane Lubchenco, Ph.D., Administrator of NOAA

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Introduction

The goal of the National Marine Fisheries Service (NMFS) protocols for the use of sturgeon is standardization of research practices to benefit the recovery of Gulf of Mexico (Gulf), green, Atlantic, and shortnose sturgeon while also minimizing potentially negative impacts of research. As with *A Protocol for the Use of Shortnose and Atlantic Sturgeon* (Moser *et al.* 2000a), these protocols provide guidelines for consistent and safe sampling methods when conducting research on sturgeon. They were developed from a comprehensive review of the best available scientific information at the time of publication, including peer reviewed journals, technical memorandums, species status reviews, interviews with researchers, and empirical evidence provided by researchers. Currently, some state agencies have been delegated authority for issuing research permits for Gulf and green sturgeon. However, due to previous lack of protocols established for these species, they were incorporated into this document.

The majority of research conducted on sturgeon falls into several categories: capturing, handling, holding, standard research, anesthetization, tagging, gastric lavage, sex identification and stage of maturation, and age estimation. First, sturgeon must be captured, which may also require consideration of the waterway sampled to mitigate impacts on other federally listed threatened or endangered species. NMFS has determined that measuring, passive integrated transponder (PIT) tagging, and genetic sampling are essential procedures to provide NMFS with the most basic information on each fish and therefore those procedures are strongly recommended. After those procedures are completed, other discretionary research might include telemetry tagging, gastric lavage, sex identification, and age estimation. These discretionary procedures should use either chemical or physical anesthesia, potentially increasing risks to sturgeon.

These protocols were developed to allow for safe, non-lethal research on sturgeon, balancing the necessary negative impacts of research while still allowing researchers to gather information vital to the recovery of listed species under the Endangered Species Act (ESA). These protocols are based on a thorough and comprehensive review of the best available scientific information on current research methods and the subsequent risk to these species. When researchers or managers have reason to exceed recommendations in this document using less known or riskier techniques, NMFS recommends first using surrogate Acipenserids or hatchery-reared sturgeon. When researchers or managers feel non-recommended methods must be conducted on wild listed or candidate species, the researchers should consult with the appropriate permitting agency in order to justify why their methodology is necessary to provide information for the recovery of these species.

Non-Targeted Species Concerns in the Research Area

When sampling shortnose, Atlantic, Gulf, and green sturgeon, the potential exists for researchers to encounter other ESA or Marine Mammal Protection Act (MMPA) listed species, in addition to other locally or state protected species. These circumstances will vary with location and NMFS encourages consultation with the appropriate management authority in all cases.

When other ESA protected species are potentially present in an action area, the researcher must contact NMFS or the US Fish and Wildlife Service (USFWS) for clarification on the likelihood to adversely impact any listed species, or destroy or adversely modify any critical habitat for that species. The presence of listed species may require researchers to alter sampling plans to avoid taking listed fish, such as Pacific or Atlantic salmonids, or mammals, such as Stellar sea lions or manatees.

In many other locations, marine mammals, protected under the MMPA but not the ESA, may be present. The MMPA places a moratorium, with certain exceptions, on the taking and importing of marine mammals and marine mammal products. In 1981, Congress amended the MMPA to allow the incidental, but not intentional, taking of small numbers of marine mammals by U.S. citizens who engage in a specified activity (other than commercial fishing) within a specified geographical region. If marine mammals, including non-ESA listed pinnipeds or cetaceans, have the potential to be taken incidental to scientific research activities on sturgeon (e.g., there is a chance of entanglement), the researcher should consult with NMFS under section 101(a)(5) of the MMPA to determine if an incidental take authorization is warranted. Contact: Office of Protected Resources, Silver Spring, Maryland (301-713-2289).

In other instances, predators may frequent sampling areas posing threats to listed sturgeon species. In such cases, nets must be monitored at all times and pulled if predators are evidenced. Pinnipeds have been seen feeding on listed sturgeon by researchers (Fernandez 2008, Marty Gingras, California Department of Fish and Game, pers. comm.), potentially other predatory species such as odontocetes and sharks could take sturgeon while trapped in gillnets or trammel nets. If there are reasons to believe sturgeon could be harmed by predators while captured in gillnets or trammel nets, those nets should be continuously monitored.

Capture

Researchers most often capture Gulf, Atlantic, green, and shortnose sturgeon using a variety of gears including gillnets (drift and anchored), trammel nets, seine nets, trawls, trot lines, pound nets, and electrofishing. Nets of varying length and mesh size are chosen to target different life stages of sturgeon (Mason and Clugston 1993, DeVries 2006).

Generally, sturgeon are hardy, allowing some research methods lethal to other fish. These methods can still be stressful to sturgeon, occasionally resulting in lethal and, more often, sub-lethal effects. For example, during pre-spawning activities, capture and handling is thought to have resulted in immediate downstream migration or aborted spawning runs (Moser and Ross 1995, Kynard *et al.* 2007, Gail Wippelhauser, Maine Department of Marine Resources, pers. comm.). Also, during periods of warm water or low dissolved oxygen (DO), fish have been lethally stressed (Hastings *et al.* 1987, Secor and Gunderson 1998). NMFS recommends capturing adult sturgeon while they are still in their winter staging areas, but does not recommend targeting sturgeon during their upstream spawning migration due to the risks of aborted spawning runs. However, when the purpose of the research is to document the size of the spawning run, managers must determine whether the information to be gained is worth the risk posed by the research.

Dissolved Oxygen, Temperature, and Salinity

For all sturgeon species, research has revealed that survival is affected by a relationship between temperature, DO, and salinity and this vulnerability may be increased by the research-related stress of capture, holding, and handling. The following environmental information is considered relevant for establishing recommendations for directed sampling on early life stages to adult life stages of sturgeon.

Jenkins *et al.* (1993), Secor and Gunderson (1998), Niklitschek (2001), Secor and Niklitschek (2001 and 2002), and Niklitshek and Secor (2009a and 2009b) demonstrated shortnose and Atlantic sturgeon survival in a laboratory setting was affected by reduced DO, increased temperature, or increased salinity. Other researchers have demonstrated similar relationships between temperature, DO, and salinity in green sturgeon (Van Eenennaam *et al.* 2005, Allen *et al.* 2006, Allen and Cech 2007). Likewise, Altinok *et al.* (1998), Sulak and Clugston (1998), Sulak and Clugston (1999), and Waldman *et al.* (2002) reported high temperatures, low DO, and high salinities result in lower survival of Gulf sturgeon.

Though there may be differences between populations in different geographical regions, optimal growth for both Atlantic and shortnose sturgeon has been shown to occur at 70% oxygen saturation with a temperature of approximately 20°C (Niklitschek 2001). Shortnose sturgeon have also been shown to experience significant reductions in food consumption when temperatures exceed 25.8°C (Niklitschek 2001). Green sturgeon require cooler temperatures, growing optimally between 15° and 19°C, and experiencing reduced growth rates between 20° and 24°C (Mayfield and Cech 2004). However, larval green sturgeon grow more optimally at 24°C compared to 19°C (Allen *et al.* 2006). Gulf

sturgeon also appear dependent on temperature for optimal growth, fasting during hot summer months and feasting during winter when water temperatures and DO in the Gulf of Mexico and tributaries are more optimal (Sulak and Randall 2002).

Considerable work has been conducted on temperature tolerances of sturgeon (Wang *et al.* 1985, Wehrly 1995, Kynard 1997, Campbell and Goodman 2004, Cech and Doroshov 2004, Van Eenennaam *et al.* 2005, Ziegeweid *et al.* 2007, Sardella *et al.* 2008). In recent work on critical thermal maximum, Ziegeweid *et al.* (2007) demonstrated hatchery-raised young of year shortnose sturgeon can tolerate between 28° and 30°C, while the maximum safe temperature limits for adults ranges between 28° and 31°C. Kynard (1997) also notes empirical temperatures of 28° to 30°C in summer months creates unsuitable shortnose sturgeon habitat. Atlantic sturgeon experience lower survival when water temperatures exceed 28°C (Niklitshek and Secor 2005). Mayfield and Cech (2004) estimated the lethal water temperature for green sturgeon in the wild at 27°C. Sardella *et al.* (2008) found green sturgeon lethal limits in a laboratory is approximately 33°C, in freshwater and sea water, although the maximum respiratory response evidenced is 26° to 28°C. Although Gulf sturgeon reside in freshwater during summer months where water temperatures range from 28° to 32°C, there have been no studies estimating lethal temperature limits for Gulf sturgeon. It is worth noting, however, the healthiest population of Gulf sturgeon occurs in the Suwannee River, where temperatures are generally maintained at 28°C by springs in parts of the river.

There is no clear evidence to suggest minimum water temperatures negatively affect sturgeon when captured beyond the early life stages. Therefore, this document identifies only upper water temperature restrictions to establish safe sampling limits for threatened or endangered sturgeon. However, when air temperatures are below freezing, handling procedures should be limited to less than two minutes to prevent exposure of a sturgeon's skin to freezing temperatures.

Because warm water can hold less DO, percent oxygen saturation is a measurement that accounts for water temperatures and DO concentrations, providing a general index of how much DO is available to sturgeon under various environmental conditions. All three measures are used in this document to highlight risks to sturgeon survival (Table 1). The 24 hour LC50 (concentration lethal to 50% of the test fish) of DO for shortnose sturgeon is documented between 2.2 and 3.1 mg/L at temperatures ranging from 22°C to 29°C (Campbell and Goodman 2004). Secor and Niklitschek (2002) reported the critical DO concentration for Eurasian sturgeons to be 4.5 mg/L at 24°C, but also found 3.6 mg/L DO critical at 20°C. Following a similar pattern, critical concentrations of DO between 4.3 and 4.7 mg/L were found for shortnose and Atlantic sturgeon at temperatures ranging from 22° and 27°C respectively. Further, acute lethal effects to shortnose and Atlantic sturgeon were observed when DO was 3.3 mg/L at temperatures between 22° and 27°C (Secor and Niklitschek 2002). Survival of Atlantic sturgeon was observed to be 100% in water temperatures of 26°C with 7 mg/L DO; however, 12% survival was observed in waters with 3 mg/L DO at the same temperature (Secor and Gunderson 1998). Even when water temperatures were only 19°C and DO was 3 mg/L, 25% of the Atlantic sturgeon died. Similar to reduced growth rates experienced by shortnose sturgeon when

temperatures are above 25°C, both shortnose and Atlantic sturgeon growth is impaired when DO is less than 4.7 mg/L (Secor and Niklitschek 2002). Jenkins *et al.* (1993) confirmed 12% mortality for 339 mm juvenile sturgeon when held at 2.5 mg/L DO and 22.5°C, while no sturgeon died when DO was above 4 mg/L at any temperature. Likewise, Secor and Gunderson (1998) found the DO level required avoiding mortality was 5 mg/L. Specific DO tolerance levels have not been established for green or Gulf sturgeon, although hypoxia for many *Acipenser* species has been documented to begin at 4 mg/L (Cech *et al.* 1984, Jenkins *et al.* 1993, Secor and Gunderson 1998). Similarly, Cech and Crocker (2002) identified hypoxia for sturgeon as 58% oxygen saturation.

Table 1. Water temperature, dissolved oxygen, percent oxygen saturation of the water, and survival rates of sturgeon tested.

Authors	Species	Temp (°C)	DO (mg/L)	% Saturation	Effects
Jenkins *et al.* 1993	Shortnose	22.5	2.5	29%	88% survival
Campbell and Goodman 2004	Shortnose	22 – 29	2.2 – 3.1	25 - 41%	50% survival
Secor and Niklitschek 2002	Atlantic and shortnose	22 – 27	3.3	38 – 42%	Acute lethal effects
Secor and Gunderson 1998	Atlantic	26	3	37%	12% survival
Secor and Gunderson 1998	Atlantic	19	3	33%	75% survival
Secor and Niklitshek 2002	Eurasian	24	4.5	54%	Critical DO concentration, onset of sub-lethal effects
		20	3.6	40%	
Secor and Niklitshek 2002	Atlantic and shortnose	22 – 27	4.3 – 4.7	50 – 60%	Critical DO concentration, onset of sub-lethal effects

NMFS recognizes the synergistic effects of water temperature and DO present difficulties when establishing finite levels for safe sturgeon sampling (Table 1). It is clear from reported empirical catch data and scientific literature, higher temperatures and lower DOs stress sturgeon even if the percent oxygen saturation remains constant or increases. Water temperature and DO can be responsible for mortality events. Each individual sturgeon will react differently to changes in environmental conditions such as water quality, salinity, and stress associated with capture and handling, which compounds the difficulty of conducting a risk assessment.

Using data reported from capture of shortnose and Atlantic sturgeon from the 1970s to present and the critical thresholds and LC50s reported in the scientific literature as reference points, NMFS established safe environmental limits for capturing and handling sturgeon species. NMFS recommends not capturing or handling Gulf, Atlantic and shortnose sturgeon when DO concentrations are below 4.5 mg/L. Green sturgeon should not be captured or handled when DO concentrations are below 5 mg/L. Additionally, NMFS recommends not sampling for Gulf, shortnose, or Atlantic sturgeon when temperatures exceed 28°C and green sturgeon should not be captured when water temperatures exceed 25°C. When establishing these recommendations, NMFS also considered the percent oxygen saturation of water and recommends not sampling for Gulf, Atlantic, or shortnose sturgeon when oxygen saturation is below 55% or green sturgeon when oxygen saturation is below 58%. Sampling at higher temperatures or lower DO levels may be possible if the percent oxygen saturation in water is maintained at these levels.

Gillnets and Trammel Nets

Researchers typically use gillnets and trammel nets to capture sturgeon. These netting techniques, while potentially lethal for many species of fish, are somewhat safer for sturgeon. However, given the implications of water temperature, DO, and percent oxygen saturation, both soak times and mesh size are important factors considered for safely capturing and handling sturgeon. Mesh size that is too small for the targeted life stage is more likely to constrict gills resulting in mortality via suffocation. The mesh size chosen for gill netting sturgeon, therefore, should be carefully considered and appropriate for the species and life stage targeted. Experimental nets with multiple mesh sizes may be appropriate for researchers to discover the safest and most effective mesh size. For example, due to disproportionately high reports of mortality using ten inch stretch mesh with Atlantic sturgeon (Balazik *et al.* 2009), this size mesh should not be used to sample adult Atlantic or Gulf sturgeon.

Safe net soak times are influenced by water temperature, DO, and, to a lesser extent, salinity. While there are no publications documenting the effects of soak times on mortality rates of sturgeon, there is consensus amongst sturgeon researchers that shorter soak times are safer than longer soak times (Mark Collins, South Carolina Department of Natural Resources; Matt Fisher, Delaware Division of Fish and Wildlife; Dewayne Fox, Delaware State University; Chris Hager, Virginia Institute of Marine Science; Doug Peterson, University of Georgia; William Post, South Carolina Department of Natural Resources; Mike Randall, United States Geological Survey (USGS); and Ken Sulak, USGS, pers. comm.). By monitoring signs of stress such as excessive redness, mucous production, or lethargy, experienced researchers will often shorten net deployment regardless of measured environmental conditions (Kathryn Hattala, New York State Department of Environmental Conservation; Tom Savoy, Connecticut Department of Environmental Protection; and Doug Peterson, University of Georgia, pers. comm.).

When using anchored gillnets while targeting Atlantic and shortnose sturgeon, soak times of 14 hours are safe when water temperatures at the sampling depth are under 15°C. However, soak times should not exceed four hours in waters up to 20°C, two hours in

waters up to 25°C, and one hour in waters up to 28°C at the sampling depth (Table 2). Similar effects were alluded to in Moser *et al.* (2000a), but were not clearly defined. Gulf sturgeon net set durations should not exceed four hours under any conditions. Mortalities have been documented in the empirical records of researchers while fishing above 20°C at net set durations ranging from 45 minutes to 24 hours. However, mortalities have been extremely rare when fishing nets less than two hours and at temperatures between 20° and 25°C. The one hour soak time at water temperatures between 25° and 28°C (Table 2) accommodates standard research practices of netting at slack tides (i.e., the occurrence of relatively still water at the turn of the low tide). There have been only two recorded sturgeon mortalities documented when fishing in this manner.

Table 2. Appropriate fishing protocols for Gulf, Atlantic, and shortnose sturgeon.

Net set duration (hours)	Temperature at sampling depth	Minimum DO at sampling depth	% oxygen saturation at sampling depth
14†	Up to 15°C	4.5 mg/L	55%
4	15° to 20°C	4.5 mg/L	55%
2	20° to 25°C	4.5 mg/L	55%
1	25° to 28°C	4.5 mg/L	55%
No sampling	Over 28°C	4.5 mg/L	55%

† Net set duration for Gulf sturgeon should not exceed four hours for all temperatures up to 20°C.

When fishing for green sturgeon, NMFS recommends that gill net fishing not be conducted in the Sacramento River, California all year to prevent interactions with listed salmonids and to also protect green sturgeon during their upstream migrations. NMFS also recommends that no gillnetting or trammel netting occur in the Feather River between October 31st and March 1st of each year to protect spawning salmonids. When fishing for green sturgeon in other locations, the risk of interactions between gillnets or trammel nets and listed salmonids or pinnipeds requires the nets to be manned at all times. Additionally, pinnipeds are protected by the MMPA and the presence of gillnets in the water could pose an entanglement risk and require an Incidental Take Authorization (Section 101(a)(5) of the MMPA). NMFS recommends net soak times should not exceed four hours in water temperature up to 19°C, two hours between 19° and 23°C, and one hour for water temperature between 23° and 25°C (Table 3).

Table 3. Appropriate fishing protocols for green sturgeon.

Net soak times (hours)	Temperature at sampling depth	Minimum DO at sampling depth	% oxygen saturation at sampling depth
4	Up to 19°C	5 mg/l	58%
2	19° to 23°C	5 mg/l	58%
1	23° to 25°C	5 mg/l	58%
No netting	Over 25°C	5 mg/l	58%

When following the protocols in Table 2 between 2005 and 2009, East Coast sturgeon researchers recorded over 3,800 captures of shortnose sturgeon resulting in no mortality.

However, while fishing outside of these recommended criteria, the same researchers experienced a 0.6% mortality rate of captured shortnose sturgeon. This is the same mortality rate documented for shortnose sturgeon captured between 2000 and 2004 when researchers followed the Moser *et al.* (2000a) protocols.

When drift gillnetting, nets are allowed to drift on the rising tide or in slack tide until just after high tide for approximately thirty minutes to several hours, depending on the location and swiftness of the tide. Water quality conditions and net soak times for drift gill nets are the same as for anchored gillnets. However, drift nets must be tended because of the risk of gear entanglement or loss of gear resulting in ghost nets. For drift gillnet fishing, gear should be pulled immediately if it is obvious a sturgeon has been captured.

Electrofishing

Electrofishing gear poses documented risks and potentially lethal effects to all sturgeon species (Moser *et al.* 2000b, Holliman and Reynolds 2002). Sturgeon have exceptional electro-sensory abilities and actively avoid electrofishing gear (Moser *et al*. 2000b). If sturgeon are likely present in areas where agencies are using electrofishing gear to target other species, only low voltage direct current should be used if no alternative sampling method is available. While electrofishing likely reduces feeding and alters spawning behavior (Moser *et al.* 2000b), such sub-lethal effects may not be significantly different than effects caused by other capture methods. However, due to more effective and safer methods of capture, NMFS prohibits electrofishing to capture Gulf, green, Atlantic, or shortnose sturgeon.

Other Non-Lethal Sampling Gear

While fyke, hoop, and pound nets are not commonly used by researchers to capture sturgeon, they occasionally capture sturgeon as bycatch in several fisheries. Usually sturgeon captured as bycatch in these gear types are found in relatively good condition. Large numbers of sturgeon captured in fyke, hoop, and pound nets have been used by researchers in cooperation with these commercial fisheries in Canada. Because these nets are less stressful to sturgeon, they are an acceptable alternative to gillnets.

Set lines have also been used to effectively sample white, pallid, shovelnose, and lake sturgeon and are approved options for sampling Gulf, green and Atlantic sturgeon as well. Shortnose sturgeon are less likely to be taken on a set line because of their diets. The two concerns with set lines are predation and hooking mortality. If there are predators such as pinnipeds in the area, the set line should be monitored constantly and pulled if any predators are seen surfacing. The hooks can be swallowed, damaging organs such as the gills and stomach, if the hook sizes are too large or small for the targeted sturgeon life stage. Every effort should be made to limit and monitor adverse effects, including not using set lines in some locations if they cannot be fished without mortality.

Trawling
While gillnets and trammel nets are most commonly used for targeting adult and sub-adult sturgeon, they are not as effective as trawls at capturing young of the year juvenile sturgeon. In larger river systems such as the Mississippi and Missouri River, and more recently in Atlantic coastal rivers, researchers have successfully employed a modified "Missouri trawl" (Herzog *et al.* 2005) — a two-seam (i.e., standard) slingshot balloon trawl (Gutreuter *et al.* 1995) completely covered with heavy, delta-style mesh.

Trawls in general are limited by shallow water (less than 20 inches) and benthic obstacles. The location of trawling should be monitored using a sounding device and global positioning system to avoid snags and limit repeated disturbance of the same location. The tow rope should be quickly released from the boat if any debris is caught and the trawl unengaged to minimize damage to the substrate or catch. Ideally, a chase boat is recommended to assist with recovery of the cod end or assisting with snags, but if that is not possible, a buoy should be attached to a single 70 to 100 foot rope line fastened to the cod end of the trawl to assist retrieval if the trawl becomes snagged.

The footrope of a trawl should maintain contact with the substrate during conditions of heavy current, fast tow speeds, or undulating bottom surfaces (e.g., sand waves). The trawl should be operated attached to the boat with 100 to 200 foot towlines, the length dependent on water depth (i.e., deeper water required longer towlines as reported in Brabant and Nedelec 1979). The trawl should be manually deployed and retrieved by powering the boat in reverse (bow upstream) with continued movement downstream. A standard haul should be approximately 300 to 500 feet, lasting approximately 10 minutes, and towed at a range of three to five knots (Gutreuter *et al.* 1995).

Areas successful for trawling are characterized by a variety of habitat substrate including fine and course sands with mobile bedforms (sand dunes) and mudflats. Particularly productive areas are located at the mouths of tributaries entering a larger river. However, any large, straight river segment, devoid of benthic material that may entangle nets, can be successfully trawled.

D-Nets
When targeting eggs and early life stage (ELS) sturgeon, the two commonly used sampling methods are D-nets and artificial substrates. Both techniques can be non-lethal, but due to the risk of mortality, no more eggs and ELS sturgeon should be captured than are absolutely necessary. While not mandatory, in rivers with unknown spawning populations, adults can be tagged and tracked to document possible spawning runs and spawning areas prior to sampling for eggs (Kieffer and Kynard 1996). Otherwise, D-nets should be deployed well before the earliest time spawning would be expected. Due to the risks associated with capturing and impinging ELS sturgeon in the D-Nets, however, they should be checked at least every three hours to minimize incidental mortality (Boyd Kynard, USGS, pers. comm.). D-nets should also be equipped with flow meters to calculate filtered water volume when developing an index of abundance and spawning success (# ELS/ volume of water sampled) (Taubert 1980). If the purpose of the research is to verify the occurrence of spawning, nets should be checked every hour. As soon as

ELS are captured, sampling should be discontinued. If the purpose of the research is to verify duration of the spawning period, then additional samples may need to be taken, but the acceptable number of ELS fish to be captured would depend on the status of the sturgeon populations in the river.

Egg Mats

Artificial substrates consist of floor buffing pads or similar materials, approximately two feet in diameter (described in Fox *et al.* 2000) for the purpose of collecting eggs as they are deposited in the water column. These pads should be anchored to the river bottom in suspected spawning areas. No more pads should be fished than is necessary. If the researcher is unsure of the number of pads required to identify spawning areas and success, no more than 100 to 150 pads should be fished at once across several sites. Pads should be checked at least twice a week or more frequently if circumstances allow. The artificial substrates should be examined in the field for sturgeon eggs and only returned to the river if more samples are needed. If it is not necessary to remove the eggs from the mat, the mat can be returned to the river bottom allowing the eggs to incubate and hatch before being removed. For every artificial substrate that collects an egg, environmental conditions such as latitude, longitude, velocity, substrate type, depth, dissolved oxygen, etc. should be collected.

Other Methods of Egg Collection

There are other methods of sampling eggs and ELS, such as epibethic sleds, ichthyoplankton nets, and pump sampling. These methods are not considered as effective as the other described methods, though they are acceptable sampling methods.

Recommendations

General

- NMFS recommends capturing adult sturgeon while they are still in their winter staging areas, but does not recommend targeting sturgeon during their upstream spawning migration due to the risks of aborted spawning runs.

Water Temperature, Dissolved Oxygen, and Salinity

- When air temperatures are below freezing, handling procedures should be limited to less than two minutes to prevent exposure of a sturgeon's skin to freezing temperatures.
- NMFS recommends Gulf, Atlantic, and shortnose sturgeon are not captured or handled when DO concentrations are below 4.5 mg/L. Green sturgeon should not be captured or handled when DO concentrations are below 5 mg/L.
- NMFS recommends not sampling for Gulf, shortnose, or Atlantic sturgeon occur when temperatures exceed 28°C; while sampling for green sturgeon should not occur when temperatures exceed 25°C.
- NMFS recommends not sampling for Gulf, Atlantic, or shortnose sturgeon when the oxygen saturation is below 55% and not sampling green sturgeon when the oxygen saturation is below 58%.

Gillnets and Trammel Nets

- Due to disproportionately high reports of mortality using ten inch stretch mesh with Atlantic sturgeon, this size mesh should not be used to sample adult Atlantic or Gulf sturgeon.
- NMFS recommends no gill net fishing be conducted in the Sacramento River, California all year round to prevent interactions with listed salmonids and also to protect green sturgeon during their upstream migrations.
- NMFS also recommends that no gillnetting or trammel netting take place in the Feather River, California between October 31st and March 1st of each year to protect spawning salmonids.
- NMFS recommends net soak times should not exceed four hours in water temperature up to 19°C, should not exceed two hours between 19° and 23°C, and one hour for water temperature between 23° and 25°C.
- Gillnets should be used sparingly and carefully in waters where other listed species may be encountered. The researcher must contact NMFS or the USFWS when other listed species may be incidentally affected.

Electrofishing

- NMFS prohibits electrofishing to capture Gulf, green, Atlantic, or shortnose sturgeon.

Other Non-Lethal Sampling Gear

- Fyke, hoop, and pound nets are an acceptable alternative to gillnets for Gulf, green, Atlantic, and shortnose sturgeon.
- Set lines are approved options for sampling Gulf, green, and Atlantic sturgeon.

Trawling

- NMFS recommends trawling as safe, efficient sampling gear to target small juvenile Gulf, Atlantic, shortnose, and green sturgeon; however, small mesh gillnets and trammel nets are also acceptable.

D-Nets

- NMFS recommends D-nets and egg mats to sample rivers for eggs or ELS of Gulf, Atlantic, shortnose, or green sturgeon.
- Due to risks associated with capturing and impinging ELS sturgeon in D-Nets, they should be checked at least every three hours to minimize incidental mortality.

Egg Mats

- No more egg mats should be fished than is necessary. If the researcher is unsure of the number of pads required to identify spawning areas and success, no more than 100 to 150 pads should be fished at once.

Handling and Holding

Handling of sturgeon refers to the time period actual research activities are conducted on live fish and does not refer to the time a fish is held in live cars before and after research activities. Holding is the period of time a sturgeon is in possession but kept in live cars either waiting to be handled or recovered from handling prior to being released.

Proper Handling of Sturgeon

Improper handling can result in lethal or sub-lethal impacts to sturgeon. In some cases, sturgeon may display altered behavior after being released, for example, swimming towards the ocean rather than remaining in the river, or, in some instances, aborting spawning runs completely (Moser and Ross 1995, Schaffter 1997, Kelly *et al.* 2007, Benson *et al.* 2007, Moser and Lindley 2007). There are no other alternatives to handling sturgeon during research; however, the researcher's primary focus should be the well-being of the sturgeon.

NMFS strongly recommends standard handling procedures performed on all sturgeon captured including measuring, weighing, PIT tagging, and tissue sampling. The total time required to complete routine research procedures should not exceed 15 minutes. Additional procedures such as internal tagging, lavage, boroscoping, etc. will take more time for handling and recovery. However, only one additional discretionary procedure to the standard handling procedures should be performed on each sturgeon, thus minimizing handling time prior to release. For example, if a sturgeon is fitted with a telemetry tag, it should not also undergo gastric lavage. And when water temperatures are above 23°C for green sturgeon or 25°C for Gulf, shortnose, or Atlantic sturgeon, the extent of research should be limited to the standard handling procedures of measuring, weighing, PIT tagging, and tissue sampling.

Fish should be handled rapidly, but with care and kept in water to the maximum extent possible during handling. During handling procedures, each fish should be immersed in a continuous stream of ambient water passing over the sturgeon's gills. Many sturgeon researchers provide sturgeon with supplemental compressed oxygen, thereby reducing stress and ensuring DO does not fall below acceptable saturation levels.

Researchers should also attempt to support larger sturgeon in slings preventing struggle during transfer. Sturgeon should be weighed using hand held sling scales or a platform scale for larger sturgeon. Also, because sturgeon are sensitive to direct sunlight, they should be covered and kept moist.

Short-Term Holding

All captured sturgeon should be removed from the capture gear and immediately transferred to short-term holding. When multiple fish are captured, those not processed immediately should be held in a net pen or live car while waiting to be transferred by hand or sling to a processing station on board. Net pens measuring three feet wide, six feet long, and three feet deep can safely hold about 20 adult shortnose sturgeon or comparably sized juvenile Atlantic, Gulf or green sturgeon when temperatures are below

15°C (Doug Peterson, University of Georgia, pers. comm.). Larger net pens (8 feet long) are required for holding adult Atlantic, green, and Gulf sturgeon or they should be processed as quickly as possible (or scheduled first) instead of subjected to confined holding conditions. When water temperature is between 15° and 25°C, fewer fish should be held in the same enclosure because overcrowding animals amplifies short term stress, particularly at higher temperatures (Safi *et al.* 2006). If the fish are being held on-board a vessel in a holding tank, compressed oxygen should be added to increase DO in the water. If the researcher observes a visually stressed sturgeon, efforts should be made to revive the fish and release it in a healthy condition. In some cases, recovery can be achieved by allowing a sturgeon to rest in an appropriately sized net pen for several hours prior to release.

Sturgeon should never be held in gillnets if there isn't enough room to safely hold them in net pens. In some rivers with large populations of sturgeon, catches can exceed the number of fish that can possibly be held safely in live cars or net pens. In such cases, researchers should have multiple holding bins at their disposal. If more fish are captured than can be processed and released within two hours, those excess fish may need to be released to minimize stress or lethal injury.

When sturgeon are held on-board research vessels, they should be placed in flow through tanks where the total volume of water is replaced every 15 to 20 minutes. Traditionally, some species of sturgeon have been held for research purposes by tethering with ropes looped around tails to the sides of research vessels until they can be handled. In a study of lake sturgeon (Axelsen and Mauger 1993 cited in Dick *et al.* 2006), tethered fish experienced greater stress and higher mortality than sturgeon kept in uncrowded cages. Therefore, NMFS recommends only using on-board holding tanks or net pens large enough to hold a large sturgeon. NMFS does not recommend holding any sturgeon by tethering its caudal peduncle to the research vessel. However, while a rope should never be tied around the caudal peduncle, it may be necessary to use a rope placed under the sturgeon immediately posterior to the pectoral fins when moving large sturgeon from net pens onto the boat.

Following handling procedures, fish should be returned to the net pen for observation and to ensure full recovery prior to release. Total holding time in the net pens would be variable depending on water temperature and the condition of each fish, however, the maximum amount of time a fish should be held after removal from capture gear is approximately two hours, unless more time is needed to recover from the effects of an anesthetic or because prolonged holding would benefit a sturgeon. When water temperature is above 25°C for Gulf, shortnose, and Atlantic sturgeon, or 23°C for green sturgeon, they should be held for as little time as possible. Holding time includes the time to remove any other captured sturgeon, time to process other fish, and time necessary for recovery ensuring the safety of the fish.

Prior to release, sturgeon should be examined and, if necessary, recovered by holding fish upright and immersed in river water, gently moving the fish front to back, aiding freshwater passage over the gills to stimulate it. The fish should be released when

showing signs of vigor and able to swim away under its own power. A spotter should watch the fish, making sure it stays submerged and does not need additional recovery.

Recommendations

Proper Handling of Sturgeon

- NMFS strongly recommends standard handling procedures performed on all sturgeon captured including measuring, weighing, PIT tagging, and tissue sampling.
- Only one additional discretionary procedure to the standard handling procedures should be performed on each sturgeon, thus minimizing handling time prior to release.
- When water temperatures are above 23°C for green sturgeon or 25°C for Gulf, shortnose, or Atlantic sturgeon, the extent of research should be limited to the standard handling procedures of measuring, weighing, PIT tagging, and tissue sampling.
- During handling procedures, each fish should be immersed in a continuous stream of ambient water passing over the sturgeon's gills.
- Researchers should attempt to support larger sturgeon in slings preventing struggle during transfer.
- If the researcher observes a severely stressed sturgeon, efforts should be made to revive the fish and release it in a healthy condition.

Short-Term Holding

- Sturgeon should never be held in gillnets while waiting to be handled, but should instead be transferred to a net pen for holding.
- NMFS recommends only using on-board holding tanks or net pens large enough to hold a large sturgeon. NMFS does not recommend tethering sturgeon to the boat by its caudal peduncle.
- The maximum amount of time a fish should be held after removal from capture gear is approximately two hours, unless more time is needed to recover from the effects of an anesthetic or because prolonged holding would benefit a sturgeon.
- Adult Atlantic, green, and Gulf sturgeon over six feet in length should be processed as quickly as possible (or scheduled first) instead of subjected to confined holding conditions.

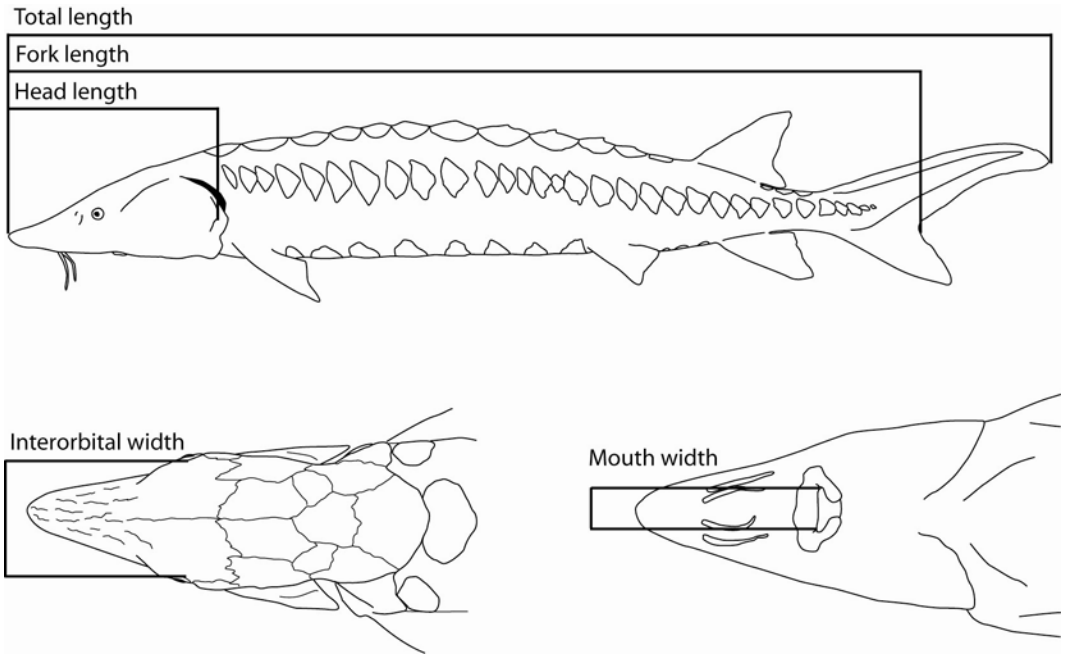
Standard Research Methods

Upon capturing a green, Gulf, shortnose, or Atlantic sturgeon, there are several research procedures strongly recommended on all sturgeon. First, the captured fish is to be measured. The sturgeon should also be weighed if possible. It can also be photographed, if possible. Then, their entire bodies should be scanned for previously inserted PIT tags; and, if none are found, one should be properly inserted. Finally, a small sample of the soft tissue of the pelvic fin should be removed for genetic identification.

Measuring

Standardized length measurements for all sturgeon should be taken from the snout to the fork in the tail (i.e., fork length – FL). The measuring device should be a solid ruler or board, so the measurement does not measure the curvature of the body. Additional length measurements should be taken at the researcher's discretion for total length (TL) or head length (Figure 1). While the heterocercal tail of larger fish may be damaged or shortened, the total length can still be obtained by pressing down the tail at the caudal peduncle and measuring to the tip of the tail. Girth measurements should also be taken at the widest part of the body. While not mandatory, measurements of the ratio of mouth width to interorbital width can also be obtained to differentiate between shortnose and Atlantic sturgeon (Dadswell *et al.* 1984). Interorbital width is measured as the distance between the lateral margins of the bony skull at the midpoint of the orbit and mouth width is measured as the distance between the left and right inside corners of the closed mouth (i.e., excluding the lips) (Figure 1).

Figure 1. Diagram of different types of measurements for sturgeons. Drawings by Eric Hilton, Virginia Institute of Marine Science.



Weighing
All captured sturgeon should be weighed if possible. Weights allow a better understanding of the conditioning of captured sturgeon during various seasons of the year or life span of the fish. For weighing sturgeon, animals should be supported with a sling or net and handling should be minimized throughout the procedure.

Boats used for researching green, Gulf, and Atlantic sturgeon should accommodate larger fish with scales available to safely weigh a 200 pound fish. When targeting shortnose sturgeon (or juvenile green, Gulf, or Atlantic sturgeon), hand-held sling scales are acceptable. When using a bench scale or platform scale to weigh large sturgeon, a five to six foot flat platform will be necessary to support the fish.

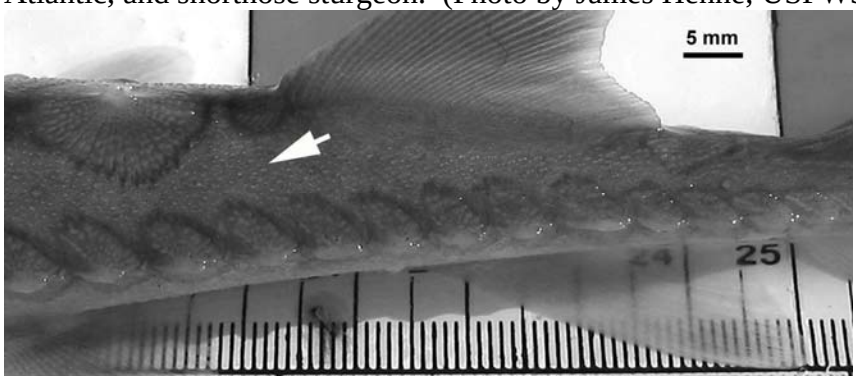
Photographing
When handling sturgeon, optional photography is often used to document the health of fish, research methods, and any identifying marks on the sturgeon potentially useful in the future. Although it is recommended to take as many pictures as needed, researchers should do so without interfering with other research activities.

PIT Tags
Every sturgeon should be scanned for PIT tags along its entire body surface ensuring it has not been previously tagged. Untagged sturgeon should then be appropriately PIT tagged (Figure 2) and the identifying number recorded. Each PIT tag consists of integrated circuitry and an antenna encapsulated in glass. PIT tags are "passive" because they contain no batteries; their internal code is activated and transmitted to the receiver when exposed to the transceiver's electromagnetic signal. The newest PIT tags, and those recommended by NMFS, use a frequency of 134.2 kHz.

Standardized PIT tag placement for Gulf, green, Atlantic, and shortnose sturgeon would enable subsequent researchers to locate prior PIT tags quickly and consistently. Sturgeon, are large fish growing a considerable amount from the time they're first PIT-tagged until they reach their adult size. If muscles grow over the PIT tag as they mature, the tag can become increasingly more difficult to read.

For this reason, NMFS strongly recommends PIT tag placement in all four sturgeon species to be located to the left of the spine, immediately anterior to the dorsal fin, and posterior to the dorsal scutes (Figure 2). This positioning would optimize PIT tag readability over the animal's lifetime as sturgeon experience the least new muscle growth in this location during their lifetimes (Berg 2004, Simpson and Fox 2006). After the tag is inserted, it should be scanned to ensure it is readable before the fish is released. If necessary, to ensure tag retention and prevent harm or mortality to small juvenile sturgeon of all species, the PIT tag can also be inserted at the widest dorsal position just to the left of the 4th dorsal scute.

Figure 2. Standardized location for PIT tagging all green, Gulf, Atlantic, and shortnose sturgeon. (Photo by James Henne, USFWS)



PIT tags have the highest reported retention rate of all identification tags, though they are not visible to the researcher or fisherman upon capture. Clugston (1996) found PIT tags implanted in gulf sturgeon have approximately a 90% retention rate. Musick and Hager (2007) tagging 445 Atlantic sturgeon reported a 99% retention rate of PIT tags after 96 hours. Smith *et al.* (1990) noted 100% retention after 60 days in wild shortnose sturgeon. In the Penobscot River, retention rates for PIT tags in Atlantic sturgeon were 93% after as much as 8.8 years (Gayle Zydlewski, University of Maine, pers. comm.). Nelson *et al.* (2007) report approximately 100% retention of PIT tags in recaptured white sturgeon.

Other researchers have had different results. Researchers with EDI Environmental Dynamics (2006) reported recapturing three white sturgeon, with 66% retention of PIT tags. DeHaan *et al.* (2008) recorded 51 to 95% retention when PIT-tagging juvenile pallid sturgeon, which is similar to rates observed by Henne *et al.* (unpublished).

As with all research procedures, there is a risk of injury or mortality either directly or indirectly related to PIT tagging. When PIT tags are inserted into animals having large body sizes relative to tag size, empirical studies generally conclude they have no adverse effect on the growth, survival, reproductive success, or behavior of individual animals (Brännäs *et al.* 1994, Elbin and Burger 1994, Keck 1994, Jemison *et al.* 1995, Clugston 1996, Skalski *et al.* 1998, Hockersmith *et al.* 2003). However, smaller sturgeon may experience mortality within the first 24 hours, usually as a result of inserting the tags too deeply or from pathogenic infection. When analyzing mortality of small sturgeon caused by PIT tags, Henne *et al.* (2008) found 11 and 14 mm tags inserted into shortnose sturgeon longer than 300 mm was safe. In this study, they found that when fish are under 300 mm, factors other than length, such as weight or condition, most influence the likelihood of mortality. Therefore, NMFS recommends only sturgeon over 300 mm should receive PIT tags.

A negative aspect of using PIT tags in sturgeon research is the difficulty for NOAA observers or non-researchers to detect tags in recaptured sturgeon without the benefit of a PIT tag reader. Rien *et al.* (1994) and Nelson *et al.* (2004) recommend removal of the second left lateral scute indicating the presence of a PIT tag in white sturgeon. This methodology has been subsequently used for green sturgeon as well. While removal of

scutes rarely results in bleeding, and is not considered deleterious, there are other, safer means for externally marking sturgeon. NMFS believes a standardized PIT tag location is less stressful to animals and is easily located. If an external mark is necessary, NMFS recommends using other external tags identified in this document. Those external tags are not only obvious to other researchers, but also to the general public for identifying recaptured animals to alert researchers of their recapture. NMFS therefore recommends using external tags to identify the presence of a PIT tag, if necessary, but researchers should not remove scutes from sturgeon for any reason.

Genetic Tissue Sampling

Tissue sampling is a common practice in fisheries science characterizing the genetic "uniqueness" and quantifying the level of genetic diversity within a population. NMFS strongly recommends genetic tissue samples be taken from every sturgeon captured unless, due to marks or tags, the researcher knows a genetic sample has already been obtained. Tissue samples should be a small (1.0 cm^2) fin-clip collected from soft pelvic fin tissues using a pair of sharp scissors. Tissue samples should be preserved in individually labeled vials containing 95% ethanol. There is no evidence that this procedure harms any species of sturgeon.

Recommendations

Strongly Recommended

- Researchers should measure all captured green, Gulf, Atlantic, and shortnose sturgeon. The sturgeon should also be weighed, if possible.
- Researchers should scan captured sturgeon for previously inserted PIT tags; and, if none are found, one should be properly inserted.
- Researchers should remove a small tissue sample by clipping the soft tissue of the pelvic fin.

Measuring

- Standardized length measurements for all sturgeon should be taken from the snout to the fork in the tail.
- NMFS recommends measuring the ratio of mouth width to interorbital width to differentiate shortnose and Atlantic sturgeon.

PIT Tags

- NMFS recommends PIT tag placement in all four sturgeon species to be located to the left of the spine, immediately anterior to the dorsal fin, and posterior to the dorsal scutes.
- NMFS recommends using 134.2 kHz PIT tags.
- If necessary, to ensure tag retention and prevent harm or mortality to small juvenile sturgeon of all species, the PIT tag can also be inserted at the widest dorsal position just to the left of the 4th dorsal scute.
- NMFS recommends only sturgeon over 300 mm should receive PIT tags.
- NMFS recommends using external tags to identify the presence of a PIT tag, if necessary, but researchers should not remove scutes from sturgeon for any reason.

Genetic Tissue Sampling

- NMFS strongly recommends genetic tissue samples be taken from every sturgeon captured unless, due to marks or tags, the researcher knows a genetic sample has already been obtained.
- Tissue samples from Gulf, green, Atlantic, and shortnose sturgeon should be archived at the NOAA/NOS Tissue Archive in Charleston, South Carolina. Proper certification, identity, and chain of custody of samples should be maintained during transfer of tissue samples.

Anesthetization

Anesthetics are physical or chemical agents preventing the initiation and conduction of nerve impulses (Summerfelt and Smith 1990). Therefore, the primary functions of anesthetics on ESA listed sturgeon are to immobilize the animal allowing precise, autorized procedures to be performed while blocking nerve impulses which might otherwise adversely affect the fish. This section, therefore, attempts to balance the risk of stress from invasive procedures with the risk posed by using an anesthetic, while also considering the risk of an unanesthetized sturgeon moving suddenly during a procedure resulting in trauma or hemorrhaging.

Invasive research activities can be stressful to fish, even if immobilized. The use of an anesthetic reduces the potential for short term stress response and risk of mortality during those procedures (Iwama *et al.* 1989, Small 2003, Wagner *et al.* 2003, Coyle *et al.* 2004, Roubach *et al.* 2005, Wanner *et al.* 2007). However, the use of some anesthetics have also proven to be stressors to fish (Iwama *et al.* 1989) as evidenced by the buildup of the cortisol hormone. NMFS recommends that noticeably stressed sturgeon should not be anesthetized.

Documented lethal or sub-lethal effects caused by improper dosage or exposure of anesthetics (Iwama *et al.* 1989, Summerfelt and Smith 1990) raises concerns whether it is acceptable to use anesthetic when handling listed Gulf, green, shortnose, or Atlantic sturgeon. In tests where anesthetics were not used during invasive procedures, cortisol levels were found significantly higher than when fish were anesthetized with tricaine methanesulfonate (MS-222) or clove oil (Wagner *et al.* 2003). Conversely, Wagner *et al.* (2003) found unanesthetized fish had lower cortisol levels than either of two anesthetized groups after one hour, demonstrating recovery of fish is more rapid without anesthetization. Nevertheless, in controlled studies when prolonged handling took place (30 minutes or more), Strange and Schreck (1978) documented fish had a higher survival rate when anesthetized.

Summerfelt and Smith (1990) and Bowser (2001) note a normal condition and six stages of anesthesia: light sedation, deep sedation, partial loss of equilibrium, total loss of equilibrium, loss of reflex reactivity, and asphyxia (Table 4). Light sedation occurs when there is a slight loss of reactivity, while deep sedation occurs when only the strongest external stimuli will elicit a response, but in both cases, the fish is able to maintain equilibrium. Partial loss of equilibrium is also characterized by partial loss of muscle tone and an increase in opercular movement, while total loss of equilibrium is characterized by total loss of muscle tone, the loss of spinal reflexes, and slow and steady opercular rate. The loss of reflex reactivity is when the fish losses all reflex response, but also when the heart rate becomes very slow and the opercular movements become slow and irregular. The final stage of anesthesia is a complete medullary collapse, when opercular movement ceases. Death is typically caused by an overdose or overexposure leading to eventual mortality.

Table 4. Stages of anesthesia (Summerfelt and Smith 1990).

Stage	Descriptor	Behavioral Response of Fish
0	Normal	Reactive to external stimuli; opercular rate and muscle tone normal
I	Light sedation	Slight loss of reactivity to external stimuli; opercular rate slightly decreased; equilibrium normal
II	Deep sedation	Total loss of reactivity to all but strong external stimuli; slight decrease in opercular rate; equilibrium normal
III	Partial loss of equilibrium	Partial loss of muscle tone; swimming erratic; increased opercular rate; reactivity only to strong tactile and vibration stimuli
IV	Total loss of equilibrium	Total loss of muscle tone and equilibrium; slow but regular opercular rate; loss of spinal reflexes
V	Loss of reflex reactivity	Total loss of reactivity; opercular movements slow and irregular; heart rate very slow; loss of all reflexes
VI	Medullary collapse (asphyxia)	Opercular movements cease; cardiac arrest usually follows quickly

The primary risks associated with anesthetizing sturgeon are overexposure and overdosing. Overexposure can occur when sturgeon are left in an anesthetic bath longer than necessary to achieve narcosis. Fish often have difficulty recovering with normal response time when overexposed, and sometimes will not respond for extended periods requiring continuous respiration to revive them. Overdosing can take place when the concentration of anesthetic is higher or more toxic than fish can tolerate. Both conditions often result in immediate or delayed mortality. As an anesthetic is applied, the sturgeon's opercular movement should be monitored closely. It should not be allowed to stop as this condition could result in blood hypoxia and high stress response, or even mortality of the anesthetized animal (Iwama *et al.* 1989).

There are various research activities commonly performed on sturgeon that present enough risk to the fish that they should only be done using anesthesia (Table 5). However, the same level of narcosis is not needed for each activity and therefore the researcher would not use the same concentrations of anesthetic. Physical restraint is not an appropriate substitute for anesthetization.

The rate at which anesthesia is induced in a fish is also important at minimizing stress. Prolonged induction generally leads to increased stress responses (e.g. prolonged thrashing during excited phase), while excessively rapid induction times (<1 minute) risks taking the fish beyond the surgical anesthesia plane because animals may skip typical behavioral signs characterizing stages of anesthesia. NMFS recommends initiating anesthesia gradually to reduce the risks of overdosing. NMFS also recommends monitoring the sturgeon during induction to avoid overexposure. If the desired stage of narcosis cannot be reached within 15 minutes (Summerfelt and Smith 1990), the sturgeon should be placed in freshwater to recover before being released.

Table 5: Procedures and stages of anesthesia.

Procedure	Stage of Anesthesia (see Table 4)
Internal tagging	III
Biopsy	III
Laparoscopy	IV
Gastric lavage	I
Boroscope	0 or I
Fin ray sectioning	II
Genetic fin clip	0
Blood sample	0
PIT tag	0
External tagging	0 but I is acceptable if necessary

Cold water species respond more rapidly and at lower doses to chemical anesthetics than do warm water species (Bowman *et al.* 2003, Coyle *et al.* 2004). Currently, it has not been demonstrated if shortnose, Atlantic, Gulf, or green sturgeon exhibit variable inter- or intra-species responses to chemical anesthetics with respect to temperature. As identified previously, however, larger green sturgeon grow more optimally at cooler temperatures than do shortnose or Atlantic sturgeon. This suggests green sturgeon are better adapted to cooler waters, may also be more likely to respond to lower levels of anesthetic than shortnose or Atlantic sturgeon. Correspondingly, Gulf sturgeon may need higher doses than the other species at cooler temperatures. Likewise, northern populations of shortnose and Atlantic sturgeon may be better adapted to cooler waters and respond differently to anesthesia.

Chemical Anesthetic

MS-222

A wide variety of chemical compounds have been utilized to anesthetize fish in fisheries research. However, tricaine methanesulfonate (MS-222) is the only anesthetic with a label for use with fish granted by the Food and Drug Administration (FDA) and as such, is the only chemical anesthetic recommended by NMFS for use on green, Gulf, Atlantic, and shortnose sturgeon.

MS-222 is absorbed rapidly through the gills and it prevents the generation and conduction of nerve impulses, with direct actions on the central nervous system and cardiovascular system. MS-222 is excreted in fish urine within 24 hours and tissue levels decline to near zero in the same amount of time (Coyle *et al.* 2004).

Proper dosing depends on the degree of anesthetization desired, the species and size of fish, water temperature and water hardness. In general, levels of MS-222 recommended do not typically exceed 100mg/L for salmonids or 250 mg/L for warm water fish (Coyle *et al.* 2004). To euthanize fish using MS-222, the recommended dosage varies from 150 to 500 mg/L for one minute or more depending on the species (DeTolla *et al.* 1995, Cho and Heath 2000, Callahan and Noga 2002, Borski and Hodson 2003).

There are two methods commonly used by sturgeon researchers to anesthetize sturgeon. The first method incorporates a "knockout" initiation dose of MS-222 followed by a safer maintenance concentration (DeTolla *et al.* 1995, Callahan and Noga 2002, Thorsteinsson 2002, Borski and Hodson 2003). Alternately, researchers anesthetize sturgeon using the lowest possible dose of MS-222, raising it to achieve the desired stage of narcosis based on the procedure (Table 5). Neither method, when performed correctly, is safer than the other. However, more risk is associated with overdosing fish exposed to higher induction rates.

For most procedures, sturgeon should initially be lightly anesthetized with MS-222, and if needed, more should be added only to the level considered necessary to perform the appropriate procedures. MS-222 solutions are highly acidic, therefore the pH of the solution should be buffered to a neutral pH with equal amounts of sodium bicarbonate prior to use. In cooler water temperatures, either higher doses or longer exposure times may be necessary to achieve the proper narcosis because the absorption rate is lower at lower temperatures (Coyle *et al.* 2004). Additionally, because MS-222 is a hypoxic agent, the anesthetic container should be vigorously aerated to maintain DO levels equivalent to ambient river water.

Total loss of equilibrium (Stage IV) is the deepest level of narcosis acceptable for anesthetizing listed sturgeon. It may not be possible to reach this stage of narcosis by gradually increasing the dosage and instead, the researcher would need to begin with a high induction dose and then drop back to a maintenance dose. Because of the risks associated with this type of anesthetization, NMFS recommends inexperienced researchers first conduct this type of anesthesia in a laboratory using a heart rate monitor to prevent overdose. Only once a researcher has demonstrated the ability to consistently perform this type of anesthetization safely should they do this in the field.

When immersed in MS-222, sturgeon will initially experience rapid gill movement followed by marked reduced gill movement as the agent begins to have an effect. As gill movement slows, sturgeon will lose equilibrium and eventually turn upside down or float to the surface. At this stage, sturgeon should be watched closely to confirm continuous involuntary gill movement. If the procedure is brief, once the desired stage of anesthesia has been reached, sturgeon may be placed on a surgical cradle and the gills irrigated with fresh water to ensure respiration and to begin recovery as the procedure is quickly completed. After completing the procedure, the fish should be placed in a clean, anesthetic free recovery tank and observed until fully recovered. Once recovered, the sturgeon can be released.

Following is a review of the various concentrations and induction methods of MS-222 when anesthetizing Gulf, Atlantic, shortnose, and green sturgeon. Fleming *et al.* (2003a) suggested concentrations of MS-222 of up to 400 mg/L failed to adequately anesthetize Gulf sturgeon. These researchers concluded the anesthetic was potentially dangerous to the sturgeon. However, Hernandez-Divers *et al.* (2004) successfully anesthetized Gulf sturgeon submerging them in an initiating dose of 250 mg/L followed by a maintenance bath of 87.5 mg/L. Harris *et al.* (2005) anesthetized Gulf sturgeon using 160 mg/L MS-

222. Parkyn *et al.* (2006) anesthetized Gulf sturgeon using a single phase induction of 150 mg/L MS-222. Lankford *et al.* (2005) anesthetized green sturgeon placing them in concentrated baths of 350 mg/L of MS-222 followed by less concentrated doses of 150 mg/L. Kaufman *et al.* (2007) anesthetized green sturgeon using 350 mg/L removing them from the solution when anesthetized. However, Serge Doroshov, (University of California Davis, pers. comm.) regularly uses 100 mg/L when working on green sturgeon. Joe Cech (University of California Davis, pers. comm.) starts green sturgeon anesthesia in baths of 150 mg/L and then when respiration stops, places them in a second, less concentrated bath of 75 mg/L. The majority of shortnose and Atlantic sturgeon researchers interviewed for this document reported concentrations of MS-222 from 50 to 100 mg/L were sufficient to induce anesthesia for most invasive procedures (Boyd Kynard Permit #1549, Mark Collins Permit #1447, Michael Kennison Permit #1595, Doug Peterson Permit #10037, Haley 1998, Oakley and Hightower 2007, Savoy 2007). The USFWS' Biological Procedures and Protocols for Researchers and Managers Handling Pallid Sturgeon recommends using MS-222 at doses between 50 and 150 mg/L (USFWS 2008).

Induction and recovery times for chemical anesthetics vary based on the dosage level and duration the fish is under anesthesia. For rainbow trout in MS-222, Wagner *et al.* (2003) found induction takes two to three minutes at 60 mg/L with recovery taking 5 to 6 minutes. For Gulf sturgeon in MS-222, Hernandez-Divers *et al.* (2004), when initiating anesthesia at 250 mg/L, induction took 5 to 11 minutes before lowering the dosage to 87.5 mg/L, after which recovery took 3 to 13 minutes. For green sturgeon at 50 to 100 mg/L MS-222, induction and recovery both required 10 to 15 minutes at 18° to 21°C, but at cooler temperatures it took longer (Joel Van Eenannaam, University of California Davis, pers. comm.).

Sturgeon face several risks posed by MS-222, such as overdose, increased stress, or being released prior to recovering. Weakened fish are more susceptible to anesthetic shock and thus are more likely to be accidentally overdosed (Coyle *et al.* 2004). Even when anesthetized with MS-222, fish still experience elevated levels of plasma cortisol, indicating they are stressed either by handling or by additive stress of MS-222 (Coyle *et al.* 2004). After being handled under anesthesia, plasma cortisol levels increased 8 times over base in channel catfish (Small 2003) and nine times over base in rainbow trout (Wagner *et al.* 2003). Studies by Pirhonen and Schreck (2003) found fish anesthetized with MS-222 ate significantly less (15-20%) than control fish. If the dose of MS-222 is too high or the exposure is too long, recovery is longer if it occurs at all. Therefore, NMFS recommends monitoring sturgeon closely during recovery and taking protective measures if fish appear stressed and not recovering normally (e.g., providing supplementary DO and moving water across the gills until fully recovered).

Recovery is also influenced by the size and sexual condition of fish. Because MS-222 is fat soluble (Coyle *et al.* 2004) longer recovery times are experienced by larger sturgeon and gravid females. Holcomb *et al.* (2004) showed doses of 225 mg/L MS-222 had no effect to eggs or sperm of white sturgeon and could be used to harvest gametes. However, doses of 2,250 mg/L resulted in lower hatching success and doses of 22,500

mg/L resulted in complete loss of fertility. At the dosages typically used by researchers to anesthetize sturgeon, however, no impact to their eggs is expected.

Although the FDA permits the use of MS-222, it also requires a 21 day withdrawal period before an anesthetized fish can be consumed. This poses concerns for humans when non listed fish are released into the wild where they may be consumed. However, a 21 day withdrawal is not a consideration for threatened or endangered sturgeon, as taking or possessing them is prohibited by the ESA. Therefore, no external marks or tags are required for Gulf, green, Atlantic, or shortnose sturgeon following anesthetization with MS-222.

Clove Oil

Clove oil is approximately 90 to 95% eugenol with smaller portions of methyleugenol and isoeugenol and was initially experimented with as a substitute for MS-222 (Bowman *et al.* 2003). Showing promise as an anesthetic, it was marketed as AQUI-S (isoeugenol , 2-methoxy-4-propenylphenol) in an attempt to gain FDA approval. However, in 2007, the National Toxicology Program concluded exposure of male mice to isoeugenol resulted in clear evidence of cancer. As a result of its concern that isoeugenol's carcinogenic properties could be transmitted through the food web, the FDA's Center for Veterinary Medicine officially rescinded authorization for the "investigational food use" of AQUI-S under INAD 10-541 (AADAP 2008). Consequently, both NMFS and the FDA (2007) are concerned isoeugenol could have direct adverse effects to threatened and endangered aquatic species. NMFS does not authorize the use of clove oil or AQUI-S on Atlantic, green, Gulf, or shortnose sturgeon.

Physical Anesthetic

Electronarcosis

Electronarcosis (also referred to as electroanesthesia and galvanonarcosis) is a non-chemical method of anesthetization and, as such, does not require FDA approval. Researchers investigating the use of electricity to immobilize fish have used various methods and species of fishes. Alternating current (AC), rectified AC, constant direct current (CDC), and pulsed direct current (PDC) have all been tested (Hartley 1967, Walker *et al.* 1994, Barton and Dwyer 1997, Henyey *et al.* 2002). Some researchers leave the electricity on for the entire time the fish is immobilized (Gunstrom and Bethers 1985) while others apply a short burst of relatively high voltage resulting in immobization of the fish for several minutes after the electric current is discontinued (Sterritt *et al*. 1994). Much of what has been learned about electronarcosis is based on the same principles applied during electrofishing.

Fish exposed to electric current may show electrotaxis (forced swimming), electrotetanus (muscle contractions), or electronarcosis (muscle relaxation). AC causes tetanus (Henyey *et al.* 2002) and at higher voltages pulsed direct current causes tetanus, whereas constant direct current causes narcosis first, and then will eventually cause tetanus as the voltage is increased (Summerfelt and Smith, 1990). Typically, when researchers have studied electronarcosis, the electricity used was either AC or PDC, or was CDC of a sufficiently

high voltage that the fish were immobilized by electrotetanus. Further, most studies using AC and PDC reported adverse effects including some bruising, burning, hemorrhaging, and mortality (Tipping and Gilhuly 1996, Redman *et al*. 1998, Holliman and Reynolds 2002). Consequently, NMFS does not recommend using AC or PDC currents for inducing anesthesia in listed sturgeon. When using CDC, the risks to sturgeon are over-applying the direct current resulting in either tetany or cessation of opercular movement. These adverse affects can be avoided by monitoring the sturgeon and reducing the voltage depending on the fish's behavior.

Henyey *et al.* (2002) describe using low voltage CDC to induce electronarcosis (muscle relaxation) in shortnose sturgeon without any changes in swimming or feeding behavior, burns, bruising, or mortality after monitoring the fish for six weeks (Boyd Kynard, USGS, pers. comm.). All evidence indicates electronarcosis induced by the method described is similar to the condition induced by chemical anesthetics; nevertheless, more research is needed on the physiological mechanisms by which it works. NMFS recommends low voltage direct current electronarcosis as described by Henyey *et al.* (2002) as a viable alternative to chemical anesthesia.

Electronarcosis has been used successfully by Boyd Kynard (USGS, pers. comm.) to anesthtize shortnose sturgeon since the 1980s. Since 2004, USFWS researchers in Maryland have also followed the Henyey *et al.* (2002) protocol to anesthetize Atlantic and shortnose sturgeon on the Potomac River and Chesapeake Bay with no adverse affects reported (Mike Mangold, USFWS, pers. comm.). Researchers in South America have also followed these methods reporting similar success (Alves *et al.* 2007).

As described in Henyey *et al.* (2002), a tank is prepared by positioning positive cathode and negative anode plates at opposite ends. With the sturgeon oriented head towards the cathode, a CDC is applied quickly so the fish loses equilibrium and then the voltage is adjusted downward until the fish is relaxed and exhibiting strong opercula movement. In practice, when inducing electronarcosis, if gill ventilation becomes irregular or stops, the electric current should be decreased and the fish will recover steady ventilation immediately (Boyd Kynard, USGS, pers. comm.). The amperes should be set to the minimal level (0.01A). Depending on the individual sturgeon and water chemistry, about 0.3 to 0.5 volts per centimeter is recommended to immobilize sturgeon. Typically, sturgeon should be supported by a net so only half of the body either dorsal or ventral depending on the work being conducted, is out of the water. Under these conditions, the researcher will feel nothing while working in the water (Hartley 1967, Boyd Kynard, USGS, pers. comm.) but researchers with sensitive skin or hand abrasions are also encouraged to wear rubber gloves during the procedure.

Induction and recovery from electronarcosis both require less than 10 seconds because as soon as fish are placed in or removed from the electrical current, it is no longer anesthetized (Gunstrom and Bethers 1985, Summerfelt and Smith 1990, Henyey *et al.* 2002). Henyey *et al.* (2002) state electronarcosis is ideal for non-invasive research. The methods in Henyey *et al.* (2002) elicited narcosis, not tetany; and Boyd Kynard (USGS, pers. comm.) states narcosis is induced by blocking nerve impulses at the medulla

oblongata. Kynard and Lonsdale (1975) demonstrated electronarcosis and MS-222 yielded similar states of muscle relaxation and immobility.

Recommendations

General

- NMFS recommends that noticeably stressed sturgeon should not be anesthetized.
- Physical restraint is not an appropriate substitute for anesthetization in procedures requiring anesthesia.
- NMFS recommends initiating both chemical and physical anesthesia gradually to reduce the risks of overdosing.

Chemical Anesthetic

- Because of the risks associated with high initial induction doses followed by a lower maintenance dose of MS-222, NMFS recommends using this technique in a controlled environment such as a laboratory and also using a heart rate monitor to prevent overdosing.
- NMFS also recommends monitoring the sturgeon during induction to avoid overexposure and if the desired stage of narcosis cannot be reached within 15 minutes, the sturgeon should be placed in freshwater to recover before being released.
- Tricaine methanesulfonate (MS-222) is the only chemical anesthetic with a label for use on fish granted by the FDA and as such, is the only chemical anesthetic recommended by NMFS for use on green, Gulf, Atlantic, and shortnose sturgeon. Dosages of MS-222 should be between 50 and 150 mg/L as identified in the pallid sturgeon protocols (USFWS 2008) and by green, Gulf, Atlantic, and shortnose sturgeon researchers.
- NMFS recommends monitoring sturgeon closely during recovery and taking protective measures if fish appear stressed and not recovering normally (e.g., providing supplementary DO and moving water across the gills until fully recovered).
- A 21 day withdrawal, normally associated with the use of MS-222 on food fish, is not a consideration for threatened or endangered sturgeon, as taking or possessing them is prohibited by the ESA.
- NMFS does not authorize the use of clove oil and AQUI-S on Atlantic, green Gulf, or shortnose sturgeon.

Physical Anesthetic

- NMFS recommends low voltage direct current electronarcosis as described by Henyey *et al.* (2002) as a viable alternative to chemical anesthesia but does not recommend using AC or PDC currents for inducing anesthesia in listed sturgeon.

Tagging

Tagging is an essential function of sturgeon research, serving to identify unique information about a captured or recaptured animal. PIT tags, as discussed earlier, should be inserted in all Gulf, green, shortnose, and Atlantic sturgeon without a PIT tag. Determining the life history, morphology, behavior, movement, and physiology of sturgeons are all highly dependent on proper tagging methods. Because sturgeon can live for decades, it is essential tags be retained for extended periods. In addition, because sturgeon exhibit very rapid juvenile growth rates and can achieve very large sizes, tags must be retained even as the tag placement area changes size and shape. Moreover, sturgeon are adept at shedding external tags and can also extrude internal tags through the body wall (Kieffer and Kynard 1993). Consequently, sturgeon researchers should keep well informed on the effectiveness of tagging methods and the technology best suited for local conditions.

Tagging varies based on tag function, location, method, technology, retention rates, and size. Internal tags (acoustic or radio) are surgically implanted in sturgeon for tracking movements, whereas externally mounted tags can be used for tracking or identification. Despite lower retention rates for some external tags, there are situations where external tags are the only option, such as tracking pre-spawning females. External archival tags and satellite tags can also passively record water quality information or geographic position without arrays. Other types of external-identifier tags are useful when non-researchers are involved in research activities, such as studies relying on fishermen to return data from tags on marked fish.

Telemetry Tags

Acoustic tags outperform radio tags in deeper water (or saline water) where sturgeon spend a majority of their lives; however, acoustic tags have disadvantages associated with limited range and ineffectiveness in turbulent or turbid waters. Acoustic signals can be monitored by field crews using either mobile hydrophones or, more commonly, stationary hydrophone arrays. Because the stationary arrays are designed to passively capture the location of transmitted signals from near-by fish, many researchers are converting to acoustic tag technology, collecting data over a longer period of time and downloading it at later intervals (Reine 2005).

Radio transponders emit radio signals from transmitter antennae to the atmosphere where they can then be monitored by researchers with a receiving antenna. For highly migratory species such as sturgeon, researchers can locate and track fish at distances up to three kilometers via airplane. Radio signals are also effective in environments having more physical disruptions such as turbidity (Thorsteinsson 2002). Combined acoustic and radio transmitter (CART) tags provide the researcher the advantages of each transmitter type.

Implanting internal telemetry tags is stressful to sturgeon and should be done using anesthesia. To gain access to the abdominal cavity, a two to four centimeter incision is made between the 3rd and 4th ventral scute between the anal and pelvic fin slightly left or

right of the mid-ventral line. Internal tags should be coated with a biologically inert substance, soaked in alcohol and allowed to dry, and then pushed deeply into the abdominal cavity to prevent tags from rubbing against the incision (Kynard and Kieffer 1991). In studies by Kynard and Kieffer (1997) no tags were rejected from shortnose sturgeon when they were coated in biologically inert material but when uncoated tags were used, they were rejected 33% of the time. Of those rejected, sonic tags were expelled within two weeks, while the radio tags were rejected within 14 weeks (Kynard and Kieffer 1997). Collins *et al.* (2002) recorded no mortality using completely internal tags during a three month study on tagging methods. Due to slower recovery time at lower temperatures, internal tags should not be implanted when water temperatures are below 8°C (Moser *et al.* 2000a, Ream *et al.* 2003, Kieffer and Kynard *in press*). Also, due to increased stress at higher temperatures, incisions should not be made in sturgeon when water temperatures exceed 27°C (Moser *et al.* 2000a, Kieffer and Kynard *in press*).

Some researchers have experimented with an internal tag having external trailing antennae threaded through a permanent hole in the lateral wall of sturgeon. These tags, allowing for better transmission of radio frequencies, are known as Internal/External tags (I/E tag). However, depending on the surgical procedure used to anchor the trailing antennae at the exit point, certain harmful effects resulted from the chaffing and cutting of the trailing antenna. In one lake sturgeon I/E tagging study, Peterson and Bezold (2008) tagged both wild and hatchery raised fish, allowing them to recover for 14 to 21 days prior to release. In this study, wild fish experienced 9% mortality but hatchery-reared sturgeon experienced 90% mortality. In an I/E tagging study by Collins *et al.* (2002), laboratory sturgeon tagged in this manner endured large exit wounds resulting from the trailing antenna and eventually suffered 100% mortality. In the same study, internal telemetry tagging techniques and two methods of external tagging resulted in only one mortality.

More recent results documented by Kieffer and Kynard (*in press*) found trailing antennae did not appear deleterious to the health of shortnose sturgeon when designed to exit the body wall through holes drilled in lateral scutes. Five wild fish tagged in the Connecticut River with I/E tags exiting through the scute were tracked for a year. All fish were recaptured, but the exit holes in all scutes had become larger. Until these techniques are better documented, NMFS recommends I/E tagging should not be done on green, Gulf, shortnose, or Atlantic sturgeons.

Historically, external tags were easily shed. Collins *et al.* (2002) showed hatchery shortnose sturgeon were able to shed 100% of their external transmitters (9 cm long, 1.7 cm diameter) when attached with a wire through the dorsal fin. However, the same researcher reported no external transmitter tags lost when attached to a dart tag using heat shrunk plastic wrap. Counihan and Frost (1999) found no external tags were shed by juvenile white sturgeon after one to three weeks. Sutton and Benson (2003) reported a 14.4% shedding rate for external tags (2.1 – 4.0 cm), with 27% of the larger tags (3.4 - 4.0 cm) shed.

More recently, researchers have documented higher retention rates with the advent of newer, smaller external tags and better methods of attachment (Figure 3). These external tags range in size between 18 and 46 mm long and only 7 to 9 mm in diameter. Using 70 to 100 lb test monofilament line, Mike Randall and Ken Sulak (USGS, pers. comm.) described a method for attaching such tags bound externally to the dorsal fin using lightweight heat shrink electrical splice tubing and five minute, two-part epoxy. These researchers documented over 96% retention rates on Gulf sturgeon during 2005 to 2008 using the following method. Their method (Mike Randall and Ken Sulak, USGS, pers. comm.) is described as:

> About 25 cm of monofilament is centered in approximately 20 mm length of heat shrink. A small quantity of epoxy is added to the tag which is then seated into the heat shrink tubing. The tubing is then shrunk with a heat gun until snug. This also warms up the mono line enough to make right angle bends at the ends of the heat shrink tubing. A small amount of epoxy should extrude from each end of the heat shrink tubing making a smooth union. Once the attachment is cooled and the epoxy hardened, the tag should be re-checked and the tag's magnet affixed to the tag. A tape label with the identifying tag number is also wrapped around the monofilament. A hole is then made through the base of the sturgeon's dorsal fin with a PIT tag needle which is also used as a guide to thread the mono line through the dorsal fin. Similarly another hole is made through the dorsal fin anterior to the first hole and the aft monofilament line is passed through. As the transmitter tag is pulled snugly to fit within the crease at the base of the dorsal fin and the body, the two monofilaments ends are joined on the opposite side of the dorsal fin by a short length of steel leader. The external tag is then secured by threading the monofilament through crimps pre-fastened on the ends of the steel leader. As the monofilament lines are pulled with opposite pressure, the leader line crimps are compressed. Finally any trailing ends of the monofilament or leader are cut. The leader will eventually corrode freeing the external tag from the fish.

Figure 3: Location of external telemetry tag (USGS Southeast Ecological Science Center).

NMFS recommends acoustic telemetry tags for tracking the movements of Gulf, green, Atlantic, and shortnose sturgeon. NMFS would suggest tagging sturgeon externally, though both methods are acceptable.

External Identifier Tags

NMFS has authorized a variety of external-identifier tag designs and placement sites on shortnose sturgeon over the past 10 years. Some examples of external-identifier tags are: Carlin (Peterson) tags, coded wire tags, dart tags, disk anchors, double barb tag, elastomer, and Floy T-bar tags. Minimal research has been conducted on the effects of these types of tags on sturgeon species.

The need for researchers to identify sturgeon with external-identifier tags has been called into question by Bergman *et al.* (1992) as sturgeon can be uniquely recognized by PIT tags. Additionally, the effectiveness and retention of these external-identifier tags is uncertain (Bergman *et al.* 1992). However, using external identifier tags can be helpful for identifying wide ranging sturgeon, like the Gulf, Atlantic, and green sturgeons that can be captured in distant locations by other researchers or commercial fishermen. Shortnose sturgeon are less likely to travel great distances through the ocean and into different rivers; therefore, external identifier tags are not as beneficial for them. Consequently, NMFS recommends the use of external tags to assist with the identification of migratory sturgeon when that information will contribute to the species' recovery.

Smith *et al.* (1990) compared the effectiveness of dart tags with nylon T-bars, anchor tags, and Carlin tags in shortnose and Atlantic sturgeon. Carlin tags applied to scutes had low retention rates as did dart tags; however, they also noted the dart tags caused some tissue damage. Carlin tags applied at the dorsal fin and anchor tags inserted in the abdomen showed the best retention. Although anchor tags resulted in lesions and eventual breakdown of the body wall if fish entered brackish water prior to their wounds healing, Collins *et al.* (1994) found no significant difference in healing rates between fish tagged in freshwater or brackish water. Clugston (1996) also looked at T-bar anchor tags placed at the base of the pectoral fins, finding beyond two years, retention rates were about 60%. Collins *et al.* (1994) compared T-bar tags inserted near the dorsal fin, T-anchor tags implanted abdominally, dart tags attached near the dorsal fin, and disk anchor tags implanted abdominally. He found that T-anchor tags were most effective long-term (92%), but also noted that all of the insertion points healed slowly or not at all and, in many cases, lesions developed. Collins *et al.* (1994) also inserted coded wire tags into the sturgeons' snout and found a 100% retention after 62 days, but only 74% after two years, though the tags may not have been inserted deeply enough. Bordner *et al.* (1990) inserted coded wire tags deeply into the snouts of white sturgeon and found 100% retention after 180 days; and Isely and Fontenot (2000) also found that coded wire tags inserted near the dorsal fin have a 98% retention rate after 120 days.

Winter (1983) suggested the appropriate tag weight to body weight ratio for fish was 2% for the tag weight in air and 1.25% for the tag weight in water. Generally, heavier tags

reduce growth or affect the swimming ability of tagged fish. But, as noted by Brown *et al.* (1999), different species of fish are better able to respond to tag weight, handling higher ratios of tag weight to body weight. In a tag to body weight ratio study conducted on lake sturgeon, Sutton and Benson (2003) recommended tag weight in air not to exceed 1.25% of body weight. In a separate study by Counihan and Frost (1999), using the ratio of wet tag weight to sturgeon weight of less than 1.25%, they found the swimming performance of white sturgeon was affected. However, this effect was more attributed to the tag placement rather than the weight itself as external tags attached to the rear dorsal fin resulted in increased drag and unbalanced weight. Currently, NMFS is sponsoring directed research on a variety of sturgeon species to determine the appropriate tag to body weight ratio. However, until resolved, NMFS recommends not exceeding a tag to body weight ratio of 1.25% in water and 2% weight in air for all tags cumulatively.

Recommendations

General

- PIT tags are strongly recommended to be inserted in all Gulf, green, shortnose, and Atlantic sturgeon without a PIT tag.
- NMFS recommends not exceeding a tag to body weight ratio of 1.25% in water and 2% weight in air.

Telemetry Tags

- NMFS recommends I/E tagging should not be done on green, Gulf, shortnose, or Atlantic sturgeons.
- NMFS recommends acoustic telemetry tags for tracking the movements of Gulf, green, Atlantic, and shortnose sturgeon. NMFS would suggest tagging sturgeon externally, though both methods are acceptable.

External Identifier Tags

- When appropriate, NMFS recommends the use of external tags to assist with the identification of migratory sturgeon.

Gastric Lavage

The pulsed gastric lavage technique, demonstrated by Foster (1977) to sample diets of pickerel and largemouth bass, has not worked well for sturgeon species. This is largely due to the difficulty in navigating the lavage tube past the U-shaped bend of the alimentary canal in sturgeon, which begins after the pneumatic duct of the swim bladder joins the anterior end of the stomach (Figure 4, also see Haley 1998 and Brosse *et al.* 2002). Serious injury and mortality has occurred when lavaging sturgeon. Sprague *et al.* (1993), showed gastric lavage tubes positioned prior to the pneumatic duct filled and burst the swim bladder and when passed beyond the bend of the alimentary canal, those tubes were capable of puncturing the canal and stomach lining of an unrelaxed gut.

Haley (1998) modified the Foster (1977) protocols for gastric lavage to create a lavage technique appropriately safe and effective for use on sturgeon (Figure 4). Haley's (1998) technique has been modified a few times with different methods created for water delivery into the stomach through intramedic tubing. Murie and Parkyn (2000), Savoy and Benway (2004), and Collins *et al.* (2008) each used slight variations of the water delivery system, but essentially used the procedures described in Haley (1998) to safely lavage sturgeon. NMFS recommends researchers follow these methods, as described below, when conducting gastric lavage of Gulf, green Atlantic, or shortnose sturgeon.

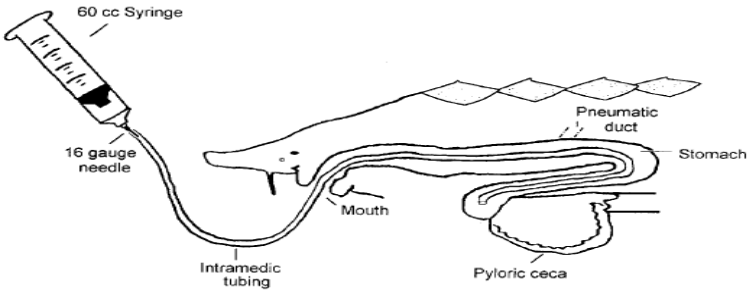


Figure 4: Depiction of the gastric lavage technique used by Haley *et al.* (1998).

First the sturgeon is anesthetized to the appropriate stage (Table 5, Stage I) causing the sturgeon's esophageal and gastric muscles to relax. The sturgeon is then placed ventrally head down on a stretcher or sling with an irrigation tube in its mouth to irrigate the gills during the procedure to ensure respiration. With water running over the gills, a fine mesh strainer is positioned under the sturgeon's mouth to capture the regurgitated contents of the stomach as it is lavaged. With the sturgeon correctly positioned, a soft, flexible intramedic tubing (typically polyethylene) is inserted into the mouth of the sturgeon and carefully directed down the alimentary canal past the pneumatic duct into the stomach region. At the point of resistance reached at the U-shaped bend of the stomach, the flexible tube is twisted ventrally and gently pushed further down the alimentary canal until the tube can be felt on the ventral surface of the fish. If the researcher is more conservative, the lavage procedure can begin once the tube reaches the point of resistance at the U-shaped bend in the stomach, as this method has been shown to be equally effective.

Once the tube is correctly positioned, the stomach contents are evacuated with injected pulses of water. Haley used a syringe to inject water into the stomach, flushing the contents into a strainer. Variations of Haley's technique have been used by other researchers to inject water using a garden sprayer holding a larger reservoir of water to administer the flushing, either timed (Savoy and Benway 2004) or manually (Collins *et al.* 2008). The contents are collected into an appropriately small meshed sieve, preserved in an alcohol filled container and the contents later identified in the laboratory.

In order to conduct gastric lavage procedures, researchers should have the following items:

- Garden sprayer or another appropriately sized water delivery device
- Intramedic tubing
- Means of anesthetization
- 500 micrometer sieve
- A sling or stretcher for holding the fish in the head down position
- Jars filled with alcohol for preserving gut content samples

Kamler and Pope (2001) and Shuman and Peters (2007) report Haley's (1998) protocols are more effective for smaller fish because the syringe can only deliver a small volume of water. Brosse *et al.* (2002), Nilo *et al.* (2006), Savoy (2007), and Collins *et al.* (2008) developed their methods to deliver larger volumes of water to effectively lavage larger Atlantic and shortnose sturgeon. These researchers used varying diameter tubes and depending on the size of the fish, flushing slightly less than a gallon of water into the sturgeon's stomach to completely evacuate its contents.

When gastric lavage was first used with sturgeon, there were serious perceived risks to the individual fish. Sprague *et al.* (1993) reported 33% mortality (4 of 12) of white sturgeon they attempted to lavage. Farr *et al.* (2001) practiced their technique on three dead green sturgeon but were unable to maneuver the tubing around the bend of the alimentary canal. In both methods, the swim bladder filled with water resulting in damage to the alimentary canal and stomach. Both of these studies however used a less flexible aquarium tubing, a factor which potentially prevented the tubing from bending with the stomach and reaching the ventral portion of the stomach near the pyloric caeca. To avoid adverse affects in future research, NMFS recommends practicing on non-listed or hatchery-reared sturgeon before attempting the procedure in the wild.

Several sturgeon researchers have also expressed concerns that delayed mortality and other risks associated with gastric lavage remains unknown and may not be worth the risks of data collection. The only way to adequately measure adverse affects is conducting gastric lavage on sturgeon in a laboratory setting and subsequently monitoring post-lavage survival, growth, and behavior. Brosse *et al.* (2002), Wanner (2006), and Mark Collins (South Carolina Department of Natural Resources, pers. comm.) practiced gastric lavage on captive fish with no delayed mortality prior to conducting lavage in the field. And in Collins *et al.* (2008), three Atlantic sturgeon were sacrificed to monitor adverse effects from lavage on wild fish. No adverse effects were

discovered. Brosse *et al.* (2002) reported all lavaged sturgeon were in poorer condition than control fish after 60 days due to weight loss. However, Collins *et al.* (2008) recaptured fish (over 70 days apart) and documented normal weight gains in the intervals between capture and re-lavage. Other researchers have reported successful gastric lavage work in the field with no immediate mortalities (Haley 1998, Brosse *et al.* 2002, Savoy and Benway 2004, Nilo *et al.* 2006, Guilbard *et al.* 2007, Nellis *et al.* 2007, Savoy 2007, Collins *et al.* 2008). Even if mortality is prevented by using appropriate lavage techniques on sturgeon, NMFS recognizes the potential risks to individual sturgeon from anesthesia, improper lavage technique, and individual sturgeon reacting negatively to the procedure.

Recommendations

- NMFS recommends researchers follow the methods presented in this document and Haley (1998) when conducting gastric lavage on Gulf, green, Atlantic, or shortnose sturgeon. Other documents detail acceptable ways to deliver larger volumes of water for adult Atlantic, Gulf, and green sturgeon.
- NMFS recommends using soft, flexible tubing (polyethylene tubing such as is used in hospitals) to maneuver the bend in the alimentary canal during gastric lavage procedures.
- NMFS recommends practicing on non-listed or hatchery-reared sturgeon before attempting the procedure in the wild.
- Sturgeon must be anesthetized to ensure relaxation of the gut walls to properly position gastric tubes during the procedure.

Sex Identification

The validation of techniques to accurately identify the sex and stage of maturation of sturgeon that leads to the conservation of Gulf, green, Atlantic, and shortnose sturgeon should be a priority. All sturgeon biologists should use safe and effective methods of sexual identification and maturity with the fewest adverse effects to the fish's health. Ideally, the sex of a sturgeon could be identified externally. A study by Vecsei *et al.* (2003) examined the urogenital openings of a variety of species of male and female sturgeon and was able to determine the sex correctly 82% of the time. However to date, the sample size is too small to be confident in the methods described.

Methods commonly used by sturgeon researchers to identify the sex and stage of gametogenesis of sturgeon include borescope (endoscope in the gonoduct), laparoscopy (endoscope through an incision in the ventral body wall), surgery and gonadal biopsy, ultrasound, and blood plasma. These techniques collect different information and have different success rates posing different risks to sturgeon.

The safest forms of sexual identification are methods not requiring anesthetic, like ultrasound (Moghim *et al.* 2002, Colombo *et al.* 2004, Wildhaber *et al.* 2006), borescoping (Kynard and Kieffer 2002), plasma lipophosphoprotein analysis (Craik and Harvey 1984), and plasma vitellogenin analysis (Wildhaber *et al.* 2006). However these methods are time or labor intensive, as with blood and plasma analyses where researchers may not receive the results of their analysis until weeks later.

Endoscopy

<u>Borescope</u>

Borescopic examination has proven an effective method for sexing sturgeon using fiber optic technology. Kynard and Kieffer (2002), Wildhaber and Bryan (2006), and Wildhaber *et al.* (2006) described the technique using a flexible borescope on shortnose, pallid, and shovelnose sturgeon where the head and body of the fish is examined under a lightly anesthetized condition. This procedure, lasting one to two minutes, is conducted with a flexible fiber optic endoscope (16cm long x 4mm diameter) inserted carefully through the urogenital opening and into place within the urogenital canal (Kynard and Kieffer 2002). Sampled females are verified by positively identifying eggs through the urogenital wall. Developed eggs are staged as either "early stage" or "late stage" individuals to identify potential spawners for the coming spring. This is done by carefully comparing the coloration and separation of oocytes viewed through the urogenital wall. Undeveloped eggs are often almond or cream-colored and sometimes indistinguishable from male testes, while mature eggs appear darker, separated, and well formed. It is noted that there are variations of this technique using a trocar to first pierce the genital canal to view and/or biopsy the gonads with an inserted fiber optic borescope; however, NMFS does not recommend this procedure on listed sturgeon.

The above borescope is easily passed through the urogenital opening (average 7.6mm) of adult shortnose, juvenile Atlantic, and other sturgeon species, although there are no

similar morphological data for green sturgeon reported. Van Eenennaam *et al.* (2008) have suggested that the diameter of the urogenital canal of green sturgeon is smaller than other sturgeon species. The greatest potential for injury with this procedure, according to Kynard and Kieffer (2002), is internally at the juncture of the oviduct and urogenital canal, located approximately 9 to 20% of a sturgeon's body length from the vent, regardless of species. The borescope must be maneuvered carefully beyond the oviduct to clearly see and stage eggs. However, when using a 16-cm long borescope, the probe tip will reach beyond the oviduct in most sturgeon of one meter length or less. Further, Kynard and Kieffer (2002) reported repeated probing of the oviduct valve by 4-mm and smaller diameter probes did not penetrate the oviduct valve or damage the urogenital canal regardless of species or fish length. They concluded that careful use of a properly sized borescope would not harm reproductive structures and would be suitable for most sturgeon species.

Kynard and Kieffer (2002) examined 443 adults using a boroscope over six years. Of those viewed, 173 were identified as female and 270 were unidentified — either as females with immature eggs or identified as males. However, Wildhaber *et al.* (2006) was able to correctly identify 85% (93% accurate for males, 63% for females) of shovelnose and pallid sturgeon examined using a similar borescope. During their work, Wildhaber and Bryan (2006) and Wildhaber *et al.* (2006) did not document any injuries or mortalities associated with their borescope activities.

Borescopy requires less time than more invasive surgery, making it a safer alternative to laparoscopy (described below) for field use when handling large numbers of sturgeon under adverse conditions. However, the borescope has limited ability to distinguish between females with immature eggs and male fish as compared to laparoscopy or biopsy.

<u>Laparoscope</u>

Several sturgeon researchers have described using laparoscopic procedures in the lab and field to identify the sex and egg maturity of individual sturgeon. The method for laboratory laparoscopy is described thoroughly by Mohler (2003), Hernandez-Divers *et al.* (2004), and Matsche and Bakal (2008). As with borescopy, the sturgeon should be anesthetized and held in water as much as possible. An incision (approximately 4 mm) is made on the ventral (Hernandez-Divers *et al.* 2004, Wildhaber *et al.* 2006) side or between the lateral scutes (Conte *et al.* 1988) of the sturgeon and the endoscope is inserted through the incision and maneuvered internally to allow the researcher to view the gonads. In Hernandez-Divers *et al.* (2004), the body cavities were insufflated and the swim bladders collapsed, but NMFS recommends avoiding either of these procedures when conducting laparoscopy on Gulf, shortnose, Atlantics, or green sturgeon. Although NMFS considers laparoscopy a more invasive endoscopic procedure than boroscopy, it is a more reliable method for determining the sex and stage of maturity of sturgeons (Wildhaber *et al.* 2006) and therefore recommends laparoscopy as the endoscopic procedure of choice.

Hernandez-Divers *et al.* (2004) laparoscoped 17 Gulf sturgeon. During these procedures, seven fish were positively identified by endoscopy alone and the other 10 were identified by biopsy samples of the gonad tissue. Wildhaber and Bryan (2006) examining 34 pallid sturgeon with both ultrasound and endoscope, positively identified the sex of 100% of the fish. Wildhaber *et al.* (2006) found that laparoscopy could positively identify the sex of shovelnose sturgeon 93% of the time (93% for males, 92% for females).

Adverse effects were not reported in any of the papers discussing laparoscopy. Hernandez-Divers *et al.* (2004) reported 100% survival after extensive surgeries (45 minutes to an hour) for their 17 Gulf sturgeon. Unfortunately this work was conducted in a controlled laboratory setting by three surgeons and does not represent typical field research conditions. Additional research determining adverse effects associated with laparoscopic procedure still need to be documented, particularly on gravid females captured prior to initiating a spawning run. Several researchers have reported capturing sturgeon can may be related to abandoned spawning runs (Moser and Ross 1995, Kynard *et al.* 2007), but there have been no studies addressing the effects of anesthesia or laparoscopy on mature, late stage females still occupying their winter staging habitat prior to spawning.

Surgical Biopsy

Surgical biopsy and histological examination of a sturgeon's gonadal tissue is the most accurate while also the most invasive way to identify the sex and stage of maturity of a sturgeon (Van Eenennaam *et al.* 1996, Van Eenennaam and Doroshov 1998, Fox *et al.* 2000, Webb and Erickson 2007, Flynn and Benfey 2007). Chapman and Park (2005) conducted gonad biopsies on Gulf sturgeon by anesthetizing them and placing them in a sling on their backs. A two to four cm ventral incision was made, after which, a small gonadal biopsy was removed (Chapman and Park 2005, Webb and Erickson 2007). Surgical biopsy, usually removing about 1 cm^3 of tissue (Fox *et al.* 2000, Webb and Erickson 2007), lasts two to three minutes (Chapman and Park 2005). After biopsies are completed, the gonadal tissue is microscopically examined to verify the sex as well as the precise stage of maturation of sturgeon (Van Eenennaam *et al.* 1996, Van Eenennaam and Doroshov 1998).

As with other forms of surgery, the risks are minimized when performed in the laboratory but there is little to no information available on the extent of infection or delayed mortality. Although there is documentation of surgically sterilized sturgeon regenerating gonadal tissue, there is little information regarding the loss of reproductive potential due to the removal of small samples of gonadal tissue (Kersten *et al.* 2001, Hernandez-Divers *et al.* 2004). And, while it is known that the gonads deliver hormones to the fish that influence behavior (Hernandez-Divers *et al.* 2004), there have been no studies dealing with potential changes in behavior from small losses of gonadal tissue. Chapman and Park (2005) monitored Gulf sturgeon for 30 days following biopsy and reported no mortality.

In situations when knowing the stage of gametogenesis could lead to recovery of the listed species, laparoscopy or biopsy would be appropriate, but due to the increased risk

of these procedures, NMFS only recommends using these procedures in a laboratory setting. If there are situations when these methods would be more likely to contribute to the recovery of these species than other available methods, NMFS would recommend their use under limited circumstances. Gonadal biopsy should only be performed in the field opportunistically while a researcher is implanting an acoustic tag.

Ultrasound

One of the safest and least invasive methods of sexual identification is the use of ultrasound. These devices, although costly, allow researchers to observe the sex organs of sturgeon without surgical incision or sedation. Ultrasound is the technique with the most potential and is becoming more accurate as both technologies improve and readers become more experienced (Joel Van Eenennaam, University of California Davis, pers. comm.).

When conducting ultrasound analyses, the procedures described by Wildhaber *et al.* (2006), or slight variation of these techniques, appear to be the safest described in the literature. Sturgeon are placed in a prone position in a tank of water with their ventral surfaces exposed to air. The ultrasound transducer is coated with ultrasound gel and then covered in a protective plastic sheath to prevent any scratches to the ultrasound from the sturgeon's scutes. During scanning, output power, focus depth, and frame rate are kept constant. The transducer is maneuvered along the abdomen between the gills and the anus, keeping the wide end of the transducer facing the head and tail. The ultrasound cannot penetrate the hard calcium of the scutes, so there is no reason to attempt to ultrasound the sides or back of the sturgeon (Wildhaber *et al.* 2006).

Moghim *et al.* (2002) examined 249 anesthetized stellate sturgeon with ultrasound and then performed necropsies to verify the accuracy of the ultrasound. Overall, ultrasound was 97.2% accurate in determining sex with the procedure taking only 30 seconds to complete. Mature females were the easiest to identify (100%), followed by immature females (99.3%), mature males (96.5%), and then immature males (76.2%). Colombo *et al.* (2004) examined 51 euthanized shovelnose sturgeon and determined ultrasound was a viable method of sex identification. They were able to correctly identify the sex of sturgeon 88% of the time, though only 40% of post-spawned females were accurately identified. Excluding post-spawned female sturgeon, the ultrasound correctly identified the sex of sturgeon 94% of the time. Additionally, Wildhaber and Bryan (2006) accurately identified the sex of 100% of pallid and shovelnose sturgeon using ultrasound coupled with borescope. In another study, Wildhaber *et al.* (2006) correctly identified only 68% of fish in the field and 70% of fish in the laboratory. In both of these cases, males were more often correctly identified, which is similar to the results from Colombo *et al.* (2004) but opposite the findings from Moghim *et al.* (2002).

When performed without anesthesia, there are no risks associated with ultrasound examination of sturgeon. However, while ultrasound is able to identify gender, it is not a promising method for determining the stage of eggs. When working with listed Gulf, shortnose, Atlantic, and green sturgeon, NMFS generally recommends using ultrasound for instant sexual identification of fish in the field. This method is the least stressful and

comparably accurate to other available methods that provide immediate identification. Due to the expense of ultrasound technology, boroscoping shortnose, Gulf, and Atlantic sturgeon is an acceptable alternative. More research is needed to determine if boroscoping is safe for green sturgeon.

Blood Plasma

Potentially one of the most promising, most accurate, and least stressful procedures used to sex sturgeon is an analysis of blood plasma. Researchers have used vitellogenin or sex steroids such as testosterone, 11-ketotestosterone, and estradiol to assess the sex and stage of maturity for pallid, shovelnose, hybrid bester, and white sturgeon (Amiri *et al.* 1996, Webb *et al.* 2002, Wildhaber *et al.* 2006).

Blood samples are obtained from the caudal vein (Figure 5) and centrifuged to isolate the plasma where it is then analyzed by radioimmunoassay or frozen for later analysis. In initial studies, testosterone was used to discern sexual maturation (79% accuracy for males, 85% for females), as it is significantly elevated in mature male and female sturgeon (Webb *et al.* 2002). If testosterone indicates the sturgeon is maturing, estradiol levels of female white sturgeon exceed 2 ng/ml 93% of the time, while males and immature white sturgeon estradiol levels never exceed 2 ng/ml (Webb *et al.* 2002), resulting in reasonably accurate identification of immature males (72%), immature females (88%), mature males (96%), and mature females (98%). Later, researchers studied vitellogenin along with the sex steroids testosterone and estradiol (Wildhaber *et al.* 2006). At all stages of development, vitellogenin was significantly elevated in females when compared to males, predicting the sex of the sturgeon with over 99% accuracy. After sex determination, the same steps taken by Webb *et al.* (2002) can determine whether each gender of fish is sexually mature, as estradiol is significantly higher in maturing females and ketotestosterone is significantly higher in maturing males.

Figure 5: Blood collection from a shortnose sturgeon.
Photograph by J. Gibbons, SCDNR

Techniques for blood plasma analysis show promise in identifying sex and egg maturation of sturgeon, and should continue to be evaluated for use on Gulf, shortnose, Atlantic, and green sturgeon. However, this technique can only identify the sex and stage of maturity of a sturgeon after the sturgeon has been captured and released. Therefore

this technique is not useful if researchers only need to know the sex of a sturgeon to identify optimal fish for an acoustic tag. If the sex of the fish is not needed immediately, but rather for later population analyses, blood samples are the preferred method. Ultrasound would also be an acceptable method even if the results are not needed immediately. These methods are least stressful and highly accurate in this situation.

Recommendations

Endoscopy

- During borescope procedures, NMFS does not recommend using a trocar to first pierce the genital canal to view and/or biopsy the gonads.
- Althought NMFS considers laparoscopy a more invasive endoscopic procedure than boroscopy, it is a more reliable method for determining the sex and stage of maturity of sturgeons (Wildhaber *et al.* 2006) and therefore recommends laparoscopy as the endoscopic procedure of choice.

Gonadal Biopsy

- NMFS does not recommend the use of laparoscopy or biopsy on wild Gulf, green, Atlantic, or shortnose sturgeon, but does recommend their use on hatchery and laboratory sturgeon. However, if there are situations when these methods would be more likely to contribute to the recovery of these species than other available methods, NMFS would recommend their use under limited circumstances.
- Gonadal biopsy should only be performed in the field opportunistically while a researcher is implanting an acoustic tag.

Ultrasound

- NMFS generally recommends using ultrasound for instant sexual identification of fish in the field.

Blood Plasma

- Blood samples are the preferred method for determining the sex and stage of maturity of sturgeon when that information is not needed at the time of sampling.

Age Estimation

Age estimates of sturgeon populations help researchers and managers understand sturgeon growth rates, ages at maturity, mortality rates, productivity, longevity, and year class strength (Campana 2001, Paragamian and Beamesderfer 2003). Such knowledge is critical for designing appropriate fisheries management policies.

Bony structures form opaque and transparent age rings each year in most fish species in response to changes in temperature or other annual cycles. These rings, or annuli, are roughly correlated to sturgeon age. Unfortunately, most bony structures, such as clavicles, cleithra, opercles, and medial nuchals are not options for listed species of sturgeon because such sampling is lethal (Brennan and Cailliet 1989, Stevenson and Secor 1999, Jackson *et al.* 2007). Other structures such as dorsal scutes and pectoral fin spines, so named because of a dermal bone sheath (Feindeis 1997), are more viable options, but scutes are more difficult to read than fin spines (Huff 1975, Brennan and Cailliet 1989, Stevenson and Secor 1999, Jackson *et al.* 2007).

Pectoral fin spines are sampled by researchers similarly across the United States. The following methodology is therefore recommended for sampling pectoral fin spines of Gulf, green, Atlantic, and shortnose sturgeon (Figure 6).

> Using a hacksaw or bonesaw, two parallel cuts are made across the leading pectoral fin spine approximately 1-cm deep. The blade of the first cut is positioned no closer than 0.5-cm from the point of articulation of the flexible pectoral base to avoid an artery at this location (Rien and Beamesderfer 1994, Rossiter *et al.* 1995, Collins and Smith 1996). The second cut is made approximately 1-cm distally (Everett *et al.* 2003, Fleming *et al.* 2003b, Hurley *et al.* 2004, Hughes *et al.* 2005), where a pair of pliers can be used to remove the resulting fin spine section. The section is then placed in an envelope and air-dried for several days or weeks. Later it is cut into thin slices (usually about 0.5 to 2 mm thickness) typically using a jeweler's saw or a double bladed saw (Stevenson and Secor 1999, Everett *et al.* 2003, Fleming *et al.* 2003b, Hurley *et al.* 2004, Hughes *et al.* 2005, Johnson *et al.* 2005, Collins *et al.* 2008). The sections are then mounted onto the substrate of choice including clear glue, fingernail polish, cytosel, or thermoplastic cement. The cross-section detail of the fin spine annuli are then studied using stereoscopic readers.

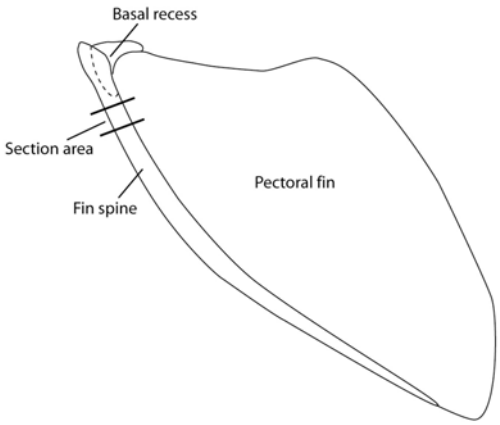


Figure 6: Diagram of the appropriate method for removing a small section of fin spine for age analysis.

Accuracy and Precision of Estimates

Accuracy and precision of the fin spine age estimates are concerns of fishery biologists and management agencies. Precision is a measurement of the distance between two reader's interpretations of the same fin spine sample, while accuracy is a measurement between the reader estimate of a sturgeon's age and the actual age (Beamish and MacFarlane 1983, Campana *et al.* 1995, Campana 2001, Hurley *et al.* 2004). To estimate precision, mark-recapture studies, oxytetracycline chemical marking studies, hatchery release studies, and in hatchery studies have been conducted to validate the age estimation process and also verify the assumption of one opaque and one translucent ring are formed each year (Clugston *et al.* 1990, Rien and Beamesderfer 1994, Campana *et al.* 1995, Rossiter *et al.* 1995, Stevenson and Secor 1999, Campana 2001, Paragamian and Beamesderfer 2003, LeBreton and Beamish 2004, Hurley *et al.* 2004, Jackson *et al.* 2007). Most studies of age estimates measure precision using at least two individual readings of the same slide. Subsequently, either the variability is recorded between readers, or the differences in reader's estimates are reconciled immediately after measurement.

Most age estimation studies suggest the results obtained should be used with caution because, while fin spines may provide the safest and most accurate estimation of age, they also consistently underestimate the actual age. The typical sources of error reported have been: 1) the rings are too close together or not clearly differentiated; 2) the original ring is difficult to identify; 3) the rings are missing within deteriorating sections; or 4) secondary fin spines, split rings, false rings, or spawning bands tend to create more or fewer rings than the actual age (Nakamoto 1995, Rossiter *et al.* 1995, Lai *et al.* 1996, Stevenson and Secor 1999, Farr *et al.* 2001, LeBreton and Beamish 2004, Whiteman *et al.* 2004). Moreover, fin spines from hatchery fish are often shaped differently, resulting in a more difficult age comparison control.

<u>Accuracy of Estimates</u>

The accuracy of fin spine estimates has been measured for Atlantic, pallid, shovelnose, white, lake, and Gulf sturgeon. Rossiter *et al.* (1995) and Stevenson and Secor (1999) monitored fish after one to three years between capture and found for lake and Atlantic

sturgeon respectively, growth rings did develop once a year. But LeBreton and Beamish (2000) determined only five of seven populations of lake sturgeon exhibited a series of one opaque and one translucent ring formed per year. This was also seen by Morrow *et al.* (1998) who documented two bands forming annually in shovelnose sturgeon fin spines during warmer years. Van Eenennaam *et al.* (1996) showed reader error of one to two years underestimation for Atlantic sturgeon at true ages ranging between 15 and 30 years. For shovelnose sturgeon and Atlantic sturgeon, age estimates underestimate actual age. The age underestimation was 1.6 years for fish under 15 years, 1.7 years for fish between 16 and 20 years, and 4.3 years for fish over 21 (Whiteman *et al.* 2004). A similar result was reported by Paragamian and Beamesderfer (2003) and also by Rien and Beamesderfer (1994) for white sturgeon, each finding age underestimation for white sturgeon under 60cm was over 70%, while the accuracy fell to below 60% for fish above 100cm. Moreover, using length to estimate age of sturgeon has proven unreliable. Clugston *et al.* (1990) recorded lengths of Gulf sturgeon after one year in the laboratory and then noted the inconsistent growth of fish in the wild during all months of the year. They concluded fish of similar sizes captured in the wild yield variable growth rates, suggesting length at age charts are flawed because growth is not constant among individuals. Paragamian and Beamesderfer (2003) provided additional evidence of invalid length at age charts using wild sturgeon. However, Peterson *et al.* (2000) and Schueller and Peterson (*in press*) demonstrated that juvenile sturgeon younger than three can be aged using length at age charts.

In the most extensive mark-recapture study to date, analyzing sturgeon at large over five years, Paragamian and Beamesderfer (2003) examined 760 marked (known age) white sturgeon recaptured up to 23 years later. They found ages were underestimated between 30 and 60%, depending on the time spent at large, meaning that age estimates were 1.5 to 2 times below the actual age of the fish. For marked-recaptured shortnose sturgeon, there was 96% accuracy between the readers' age estimates and the time the sturgeon spent at large. However, when using known-age fish, only 34% of the readers' estimates were accurate within one year (Collins *et al.* 2008). Also, when using multiple slides from the same fin spines of known-age hatchery fish, Hurley *et al.* (2004) reported only 28% of the estimates were correct, while 56% were within one year and 89% were within two years.

Precision of Estimates

As discussed previously, when measuring the precision of fin spine aging estimates, multiple readers estimate the age of identical sturgeon fin spines and then their results are compared to determine the variance between readers' estimates. Fleming *et al.* (2003b) studied 88 shortnose sturgeon fin spines where multiple readers were able to reach an agreement after consultation 100% of the time. Everett *et al.* (2003) analyzed shovelnose sturgeon using multiple readers and found the readers could not reach agreement on 26 of 736 (3.5%) of the samples when they attempted to reconcile measurements. Rossiter *et al.* (1995) also showed agreement between reader measurements while analyzing 20 lake sturgeon. They found high precision between readers for fish under 15 years old; however, for fish over 18 years old, reader agreement dropped to 80%. In the first two studies mentioned above, the readers reconciled measurements when there was a

disagreement in age estimation, while the latter study was conducted on only 20 samples without reconciliation of estimates.

While some studies have found general precision and agreement between readers, others were less successful. Van Eenennaam *et al.* (1996) showed multiple readers agreed on readings of Atlantic sturgeon fin spine samples approximately 33 to 40% of the time. Stevenson and Secor (1999) also evaluated reader agreement of Atlantic sturgeon fin spines and found no significant difference, but the disagreement error was approximately 1.2 years on average between readers. Nakamoto (1995) analyzed 154 green sturgeon fin spines and found readings from 34% differed by fewer than two years and 66% of the readings differed by fewer than five years. Rien and Beamesderfer (1994) measuring 935 white sturgeon fin spines twice, found only 37% agreement between readers and 68% agreement within one year. Jackson *et al.* (2007) found 80% of the time multiple readers estimated the age of shovelnose sturgeon within one year and 100% were estimated within two years. However Whiteman *et al.* (2004) found reader agreement on 234 shovelnose sturgeon samples of only 18% and within one year was still only 46%. NMFS recommends all sturgeon age estimates derived from fin spine analysis should test for precision between readers.

As discussed above, one major assumption for fin spine age estimation is each fin spine develops a ring each year; but there is evidence to suggest each fin spine may be different. Jackson *et al.* (2007) simultaneously removed both fin spines from shovelnose sturgeon and showed the spines from the same fish resulted in the same estimated age 36% of the time, within one year 66% of the time, and within two years 84% of the time. But this could be a result of how the fin spines are prepared, as measurements of 64 slides made from 16 pallid sturgeon fin spines resulted in only 25% agreement from the same spines (Hurley *et al.* 2004). Jackson *et al.* (2007) concluded the preparation of fin spines must be standardized so results can be reproducible.

Age Validation

Several researchers have suggested slow growth of adult and pre-spawn females may explain why some fin spine rings are closely spaced and become more closely spaced as fish get older (Beamish and MacFarlane 1983, Nakamoto 1995). It is thought the distance between rings is influenced by changes in food supply, metabolism, behavior, and environmental conditions as the sturgeon mature.

Accordingly, sturgeon researchers have begun to develop age estimate correction factors to validate age estimates of populations of different species. Bruch *et al.* (2009), while researching lake sturgeon, found growth increments on pectoral fin spine cross sections underestimated true age of fish older than 14 years and error increased with age, whereas otoliths accurately estimated true age up to at least 52 years. Increment formation in juvenile lake sturgeon pectoral fin spines was clearer and easier to interpret than otoliths. A power function developed by Bruch *et al.* (2009) provided a means for correcting existing age estimates obtained from lake sturgeon pectoral fin spines. For that reason, NMFS recommends using salvage specimens of Gulf, green, Atlantic, and shortnose sturgeon to establish age estimation correction factors.

Deleterious Effects of Fin Spine Sampling

Kohlhorst (1979) first reported potentially deleterious effects of fin spine removal from white sturgeon during a mark-recapture study where an incidence of mortality was recorded. The percentage mortality reported could have been magnified by a small sample size, but concern over this result triggered additional research in the laboratory.

Collins *et al.* (1995) and Collins and Smith (1996) monitored the effects of fin spine removal of juvenile shortnose and Atlantic sturgeon in a laboratory. Removing the entire leading fin spine from the base, a method not currently recommended for sampling fin-rays, they found wounds healed rapidly and that the remaining secondary pectoral fin spine grew in circumference until appearing very similar to the original fin spine. There were no significant differences for growth or survival between treatment and control sturgeon.

In other laboratory studies testing fin spine function, Wilga and Lauder (1999) found pectoral fins function by orienting the body vertically in the water column, but they are not used during locomotion. Following this study, Parsons *et al.* (2003) removed pectoral fin spines from shovelnose sturgeon placing them in tanks, where the current could then be increased to test their ability to hold position in a current. Without fin spines, treatment sturgeon were able to hold their position in a current as well as control sturgeon.

Most recently, while conducting mark-recapture surveys of Atlantic and shortnose sturgeon, Collins *et al.* (2008) discovered secondary fin spines had grown abnormally on older, mature Atlantic sturgeon after the leading fin spine had been taken months earlier. Concluding this regrowth could be due to slower growth of mature, adult fish and possibly become detrimental to the sturgeons' health, their team no longer samples fin spines from larger, adult sturgeon. Because of increased error in reading fin spines of older fish and evidence of abnormal regrowth, NMFS does not recommend taking fin spine samples from mature Gulf, shortnose, Atlantic, or green sturgeon.

Alternative Methods for Age Estimation

NMFS recommends developing newer, more accurate and precise methods of aging Gulf, green, Atlantic, and shortnose sturgeon. In recent years, Bruch et al. (2009) analyzed the use of radiocarbon bombing to estimate the ages of lake sturgeon using otolith cores. This is not a non-lethal technique, but if further testing indicates using other bony structures such as scutes for accurate and precise age estimates, this may become a useful method for age estimation. Likewise, telomeres have recently been used to estimate fish age. Hatakeyama *et al.* (2008), testing small teleost fish, found that telomere length shortens through the life of the fish and is inversely related to the length of the fish. However, no change in telomere length was noted for European sea bass between 12 and 94 months of age (Horn *et al.* 2009). Specific studies should be conducted on sturgeon to determine if telomere analysis could determine the age of sturgeon.

Recommendations

General

- NMFS recommends removing a 1cm portion of the pectoral fin spine from just above the point of articulation to estimate the age of Gulf, green, Atlantic, and shortnose sturgeon.

Accuracy and Precision of Estimates

- NMFS recommends fin spine derived age estimates be used with caution because they consistently underestimate the actual age.
- NMFS recommends all sturgeon age estimates derived from fin spine analysis should test for precision between readers.
- NMFS acknowledges the preparation of fin spines must be standardized so the results are reproducible and encourages future research to achieve this goal.

Age Validation

- NMFS does not recommend using lethal methods or length/age charts to estimate ages of Gulf, Atlantic, green, or shortnose sturgeon, except when working with juvenile sturgeon under three years of age.
- NMFS recommends using salvage specimens of Gulf, green, Atlantic, and shortnose sturgeon to establish age estimation correction factors.

Deleterious Effects of Fin Spine Sampling

- Because of increased error in reading fin spines of older fish and evidence of abnormal regrowth, NMFS does not recommend taking fin spine samples from mature Gulf, shortnose, Atlantic, or green sturgeon.

Alternative Methods for Age Estimation

- NMFS recommends developing newer, more accurate and precise methods of aging Gulf, green, Atlantic, and shortnose sturgeon.

Salvage Specimens

Dead or salvaged specimens can be invaluable for a number of basic and applied aspects of sturgeon biology and conservation. Scientific uses include, but are not limited to, morphology, genetics, histopathology, contaminants, age and growth, food habits, cryopreservation of sperm, and human impact/anthropogenic mortality. Educational uses of sturgeon collected include, but are not limited to, taxidermy, collection of hard parts (e.g., scutes, bones, and entire skeleton), necropsy, and development of sampling and necropsy procedures and manuals.

Although it is important to maintain salvaged specimens and their derivative tissues, making them available for future researchers and educators, listed sturgeon are protected and transfer of specimens must still be carefully documented under the ESA. Persons/laboratories receiving specimens must be authorized to possess listed species. All sturgeon research permits issued by NMFS currently include provisions for preserving incidental mortality resulting from research or found opportunistically.

If dead Gulf, green, shortnose, or Atlantic sturgeon are found or a researcher has a need for salvaged sturgeon or sturgeon parts, contact NMFS Headquarters in Silver Spring, Maryland at (301) 713-2289.

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References

AADAP (Aquatic Animal Drug Approval Partnership Program). 2008. AQUI-S use on food fish under IDAD 10-541 strictly prohibited. AADAP Newsletter 4-2:1.

Allen, P.J. and J.J. Cech, Jr. 2007. Age/size effects on juvenile green sturgeon, *Acipenser medirostris* , oxygen consumption, growth, and osmoregulation in saline environments. Environmental Biology of Fishes 79(3-4):211-229.

Allen, P.J., M. Nicholl, S. Cole, A. Vlazny, and J.J. Cech Jr. 2006. Growth of larval to juvenile green sturgeon in elevated temperature regimes. Transactions of the American Fisheries Society 135:89-96.

Altinok, I., S.M. Galli, and F.A. Chapman. 1998. Ionic and osmotic regulation capabilities in Gulf of Mexico sturgeon, *Acipenser oxyrinchus de sotoi*. Comparative Biochemistry and Physiology Part A 120:609-616.

Alves, C.B.M., L.G.M. da Silva, and A.L. Godinho. 2007. Radiotelemetry of a female jau, *Zungaro jahu* (Ihering, 1898) (Siluriformes: Pimelodidae), passed upstream of Funil Dam, Rio Grande, Brazil. Neotropical Ichthyology 5(2):229-232.

Amiri, B.K., M. Maebayashi, A. Hara, S. Adachi, and K. Yamauchi. 1996. Ovarian development and serum sex steroid and vitellogenin profiles in the female hybrid sturgeon, the bester. Journal of Fish Biology 48:1164-1178.

Axelsen, F., and Mauger, A. 1993. Sturgeon holding cage trials on Lake Opawica and Lake Lichen. Ministère de l'agriculture, des pêcheries et de l'alimentation du Québec. Direction de la recherche scientifique et technique Doc. rech. 93/06: iv + 42 p.

Balazik, M., S. Cameron, D. Clarke, C. Frederickson, S. Giordano, C. Hager, J. Lazar, S. McIninch, K. Reine, C. Dickerson, W. Shuart, A. Spells. 2009. Movements and habitat associations of Atlantic sturgeon in the James River estuary, Virginia. Oral Presentation Abstract, 139th Meeting of the American Fisheries Society, Nashville, Tennessee.

Barton, B.A. and W.P. Dwyer. 1997. Physiological stress effects of continuous- and pulsed-DC on electroshock on juvenile bull trout. Journal of Fish Biology 51:998-1008.

Beamish, R.J. and G.A. MacFarlane. 1983. The forgotten requirement for age validation in fisheries biolgy. Transactions of the American Fisheries Society 112(6):735-743.

Benson, R.L., S. Turo, and B.W. McCovey, Jr. 2007. Migration and movement patterns of green sturgeon (*Acipenser medirostris*) in the Klamath and Trinity Rivers, California, USA. Environmental Biology of Fishes 79:269-279.

Berg, J.J. 2004. Population assessment of the Gulf of Mexico sturgeon in the Yellow River, Florida. Thesis for a Masters of Science, University of Florida. 86p.

Bergman, P.K., F. Haw, H.L. Blankenship, and R.M. Buckley. 1992. Perspectives on design, use, and misuse of fish tags. Fisheries 17(4):20-25.

Bordner, C.E., S.I. Doroshov, D.E. Hinton, R.E. Pipkin, R.B. Fridley, and F. Haw. 1990. Evaluation of marking techniques for juvenile and adult white sturgeons reared in captivity. American Fisheries Society Symposium 7:293-303.

Borski, R.J. and R.G. Hodson. 2003. Fish research and the institutional animal care and use committee. ILAR Journal 44(4):286-294.

Bowman, M., J. Bowker, D. Carty, B. Johnson, and D. Erdahl. 2003. The efficacy and safety of AQUI-S as an anesthetic on freshwater fish. U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program, Bozeman, Montana. 3p.

Bowser, P.R. 2001. Anesthetic options for fish. In Gleed, R.D. and J.W. Ludders (Eds.), Recent Advances in Veterinary Anesthesia and Analgesia: Companion Animals. International Veterinary Information Service, Ithaca, New York.

Brabant, J. C. and C. Nedelec. 1979. Bottom trawls for small-scale fishing. Food and Agriculture Organization of the United Nations. Fisheries Technical Paper 189.

Brännäs, E., H. Lundqvist, E. Prentice, M. Schmitz, K. Brännäs, and B. Wiklund. 1994. Use of the passive integrated transponder (PIT) in a fish identification and monitoring system for fish behavioral studies. Transactions of the American Fisheries Society Symposium 123:395-401.

Brennan, J.S. and G.M. Cailliet. 1989. Comparative age-determination techniques for white sturgeon in California. Transactions of the American Fisheries Society 118:296-310.

Brosse, L., P. Dumont, M. Lepage, and E. Rochard. 2002. Evaluation of a gastric lavage method for sturgeons. North American Journal of Fisheries Management 22(3); 955-960.

Brown, R.S., S.J. Cooke, W.G. Anderson, and R.S. McKinley. 1999. Evidence to challenge the ‘‘2% rule’’ for biotelemetry. North American Journal of Fisheries Management 19:867–871.

Bruch, R.M., S.E. Campana, S.L. Davis-Foust, M.J. Hansen, J. Janssen. 2009. Lake sturgeon age validation using bomb radiocarbon and known-age fish. Transactions of the American Fisheries Society 138:361-372.

Callahan, H.A. and E.J. Noga. 2002. Tricaine dramatically reduces the ability to diagnose protozoa ectoparasite (*Ichthyobodo necator*) infections. Journal of Fish Diseases 25:433-437.

Campana, S.E. 2001. Review paper: accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biolgy 59:197-242.

Campana, S.E., M.C. Annand, and J.I. McMillan. 1995. Graphical and statistical methods for determining the consistency of age determinations. Transactions of the American Fisheries Society 124:131-138.

Campbell, J.G. and Goodman, L.R. 2004. Acute sensitivity of juvenile shortnose sturgeon to low dissolved oxygen concentrations. Transactions of the American Fisheries Society 133:772-776.

Cech, J.J., S.J. Mitchell, and T.E. Wragg. 1984. Comparative growth of juvenile white sturgeon and striped bass: effects of temperature and hypoxia. Estuaries 7:12–18.

Cech J.J., Jr., and C.E. Crocker. 2002. Physiology of sturgeon: effects of hypoxia and hypercapnia. Journal of Applied Ichthyology 18:320–324.

Cech, J.J., Jr. and S.I. Doroshov. 2004. Chapter 3: Environmental requirements, preferences, and tolerance limits of North American sturgeons. Pages 73-86 in G.T.O. Lebreton, F.W.H. Beamish, and R.S. McKinley (eds), *Sturgeons and Paddlefish of North America*. Netherlands: Kluwer Academic Publishers.

Chapman, F.A. and C. Park. 2005. Comparison of sutures used for wound closure in sturgeon following a gonadal biopsy. North American Journal of Aquaculture 67:98-101.

Cho, G.K. and D.D. Heath. 2000. Comparison of tricaine methanesulphonate (MS222) and clove oil anaesthesia effects on the physiology of juvenile Chinook salmon *Oncorhynchus tshawytscha* (Walbaum). Aquaculture Research 31:537-546.

Clugston, J.P. 1996. Retention of T-bar anchor tags and passive integrated transponder tags by Gulf sturgeons. North American Journal of Fisheries Management 16:682–685.

Clugston, J.P., W.T. Mason, and A.M. Foster. 1990. Laboratory reared Gulf of Mexico sturgeon give clue to age of wild stock. Information Bulletin, U.S. Department of Interior, Fish and Wildlife Service. 2p.

Collins, M.R., C. Norwood, and A. Rourk. 2008. Shortnose and Atlantic sturgeon age-growth, status, diet, and genetics. Final Report to National Fish and Wildlife Foundation. South Carolina Department of Natural Resources, Charleston, South Carolina, 2006-0087-009. 41p.

Collins, M.R., D.W. Cooke, T.I.J. Smith, W.C. Post, D.C. Russ, and D.C. Walling. 2002. Evaluation of four methods of transmitter attachment on shortnose sturgeon, *Acipenser brevirostrum*. Journal of Applied Ichthyology 18(2002):491-494.

Collins, M.R. and T.I.J. Smith. 1996. Sturgeon fin ray removal is nondeleterious. North American Journal of Fisheries Management 16:939-941.

Collins, M.R. 1995. Report to the USFWS: Evaluation of the effects of pectoral spine removal on shortnose sturgeon. Final Report, South Carolina Department of Natural Resources, Charleston, South Carolina.

Collins, M.R., T.I.J. Smith, and L.D. Heyward. 1994. Effectiveness of six methods for marking juvenile shortnose sturgeon. Progressive Fish Culturist 56:250–254.

Colombo, R.E., P.S. Wills, and J.E. Garvey. 2004. Use of ultrasound imagery to determine sex of shovelnose sturgeon. North American Journal of Fisheries Management 24:322-326.

Conte, F.S., S.I. Doroshov, P.B. Lutes, and E.M. Strange. 1988. Hatchery manual for the white sturgeon (*Acipenser transmontanus*). University of California Press, Publication 322, Oakland.

Counihan, T.D., and C.N. Frost. 1999. Influence of externally attached transmitters on the swimming performance of juvenile white sturgeon. Transactions of the American Fisheries Society 128:965–970.

Coyle, S.D., R.M. Durborow, and J.H. Tidwell. 2004. Anesthetics in aquaculture. Southeastern Regional Aquaculture Center. SRAC Publication Number 3900, November 2004. 6p.

Craik, J.C.A. and S. M. Harvey. 1984. A biochemical method for distinguishing between the sexes of fishes by the presence of yolk protein in the blood. Journal of Fish Biology 25:293-303.

Dadswell, M.J., B.D. Taubert, T.S. Squiers, D. Marchette, and J. Buckley. 1984. Synopsis of biological data on shortnose sturgeon, *Acipenser brevirostrum* LeSueur, 1818. NOAA Technical Report-14. 53pp.

DeHaan, P.W., G.R. Jordan, and W.R. Ardren. 2008. Use of genetic tags to identify captive-bred pallid sturgeon (*Scaphirhynchus albus*) in the wild: improving abundance estimates for an endangered species. Conservation Genetics 9(3):691-697.

DeTolla, L.J., S. Srinivas, B.R. Whitaker, C. Andrews, B. Hecker, A.S. Kane, and R. Reimschuessel. 1995. Guidelines for the care and use of fish in research. ILAR Journal 37(4):159-173.

DeVries, R.J. 2006. Population dynamics, movements, and spawning habitat of the shortnose sturgeon, *Acipenser brevirostrum*, in the Altamaha River. Master's Thesis, University of Georgia. 103 p.

Dick, T.A., S.R. Jarvis, C.D. Sawatzky, and D.B. Stewart. 2006. The lake sturgeon, *Acipenser fluvescens* (Chondrostei: Ascipenseridae): an annotated bibliography. Canadian Technical Report of Fisheries and Aquatic Sciences 2671, Winnipeg, Manitoba. 257p.

EDI Environmental Dynamics. 2006. 2005 assessment of juvenile white sturgeon (*Acipenser transmontanus*) abundance and distribution in the Nechako River; development of an index of juvenile recruitment. Prince George, British Columbia. 33p.

Elbin, S.B. and J. Burger. 1994. Implantable microchips for individual identification in wild and captive populations. Wildlife Society Bulletin 22:677-683.

Everett, S.R., D.L. Scarnecchia, G.J. Power, and C.J. Williams. 2003. Comparison of age and growth of shovelnose sturgeons in the Missouri and Yellowstone Rivers. North American Journal of Fisheries Management 23:230-240

Farr, R.A., M.L. Hughes, and T.A. Rien. 2001. Green sturgeon population characteristics in Oregon. Oregon Department of Fish and Wildlife, Annual Progress Report, Project Number F-178-R. 31p.

Feindeis, E.K. 1997. Osteology and phylogenetic interrelationships of sturgeons (Acipenseridae). Environmental Biology of Fishes 48:73-126.

Fernandez, S.J. 2008. Population demography, distribution, and movement patterns of Atlantic and shortnose sturgeons in the Penobscot River estuary, Maine. Master's Thesis, University of Maine. 100 p.

Fleming, G.J., D.J. Heard, R.F. Floyd, and A. Riggs. 2003a. Evaluation of propofol and medetomidine-ketamine for short term immobilization of Gulf of Mexico sturgeon (*Acipenser oxyrinchus* De Soti). Journal of Zoo and Wildlife Medicine 34(2):153-158.

Fleming, J.E., T.D. Bryce, and J.P. Kirk. 2003b. Age, growth, and status of shortnose sturgeon in the lower Ogeechee River, Georgia. Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 57:80-91.

Flynn, S.R. and T.J. Benfey. 2007. Sex differentiation and aspects of gametogenesis in shortnose sturgeon *Acipenser brevirostrum* Lineaus. Journal of Fish Biology 70(4):1027-1044.

Food and Drug Administration. 2007. Guidance for industry: concerns related to the use of clove oil as an anesthetic for fish. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Veterinary Medicine. 3p.

Foster, J.R. 1977. Pulsed gastric lavage: An efficient method of removing stomach contents of live fish. The Progressive Fish Culturalist 39(4): 166-169.

Fox, D.A., J.E. Hightower, and F.M. Parauka. 2000. Gulf sturgeon spawning migration and habitat in the Choctawhatchee River System, Alabama-Florida. Transactions of the American Fisheries Society 129:811-826.

Guilbard, F., J. Munro, P. Dumont, D. Hatin, and R. Fortin. 2007. Feeding ecology of Atlantic sturgeon and lake sturgeon co-occurring in the St. Lawrence estuarine transition zone. American Fisheries Society Symposium 56: 85-104.

Gunstrom, G. K. and M. Bethers. 1985. Electrical anesthesia for handling large salmonids. Progressive Fish-Culturist 47:67–69.

Gutreuter S., R.W. Burkhardt, and K. Lubinski. 1995. Long Term Resource Monitoring Program Procedures: Fish Monitoring. LTRMP Technical Report 95-P002-1. National Biological Service, Environmental Management Technical Center, Onalaska, Wisconsin.

Haley, N. 1998. A gastric lavage technique for characterizing diets of sturgeons. North American Journal of Fisheries Management 18: 978-981.

Harris, J.E., D.C. Parkyn, and D.J. Murie. 2005. Distribution of Gulf of Mexico sturgeon in relation to benthic invertebrate prey resources and environmental parameters in the Suwannee River estuary, Florida. Transactions of the American Fisheries Society 134:975-990.

Hartley, W.G. 1967. Electronarcosis of fish for handling. Pages 251-255 in R. Vibert (Ed.), Fishing with Electricity: its application to biology and management.

Hatakeyama, H., K.I. Nakamura, N. Izumiyama-Shimomura, A. Ishii, S. Tsuchida, K. Takubo, and N. Ishikawa. 2008. The teleost *Oryzias latipes* shows telomere shortening with age despite considerable telomerase activity throughout life. Mechanisms of Aging and Development 129(9):550-557.

Henne, J.P. R.L. Crumpton, K.M. Ware, and J. Fleming. 2008. Guidelines for marking and tagging juvenile endangered shortnose sturgeon, *Acipenser brevirostrum*. Aquaculture America 2008, meeting abstract.

Henyey, E., B. Kynard, and P. Zhuang. 2002. Use of electronarcosis to immobilize juvenile lake and shortnose sturgeons for handling and the effects on their behavior. Journal of Applied Ichthyology 18:502-504.

Hernandez-Divers, S.J., R.S. Bakal, B.H. Hickson, C.A. Rawlings, H.G. Wilson, M. Radlinsky, S.M. Hernandez-Divers, and S.R. Dover. 2004. Endoscopic sex determination and gonadal manipulation of Gulf of Mexico sturgeon (*Acipenser oxyrinchus* De Sotoi). Journal of Zoo and Wildlife Medicine 35(4):459-470.

Herzog, D.P. V.A. Barko, J.S. Scheibe, R.A. Hrabik, and D.E. Ostendorf. 2005. Efficacy of a benthic trawl for sampling small bodied fishes in large river systems. North American Journal of Fisheries Management 25:594-603.

Hockersmith, E.E., W.D. Muir, S.G. Smith, B.P. Sandford, R.W. Perry, N.S. Adams, and D.W. Rondorf. 2003. Comparison of migration rate and survival between radio-tagged and PIT-tagged migrating yearling chinook salmon in the Snake and Columbia rivers. North American Journal of Fisheries Management 23:404-413.

Holcomb, M., J. Woolsey, J.G. Cloud, and R.L. Ingermann. 2004. Effects of clove oil, tricaine, and CO2 on gamete quality in steelhead and white sturgeon. North American Journal of Aquaculture 66:228-233.

Holliman, F.M. and J.B. Reynolds. 2002. Electroshock-induced injury in juvenile white sturgeon. North American Journal of Fisheries Management 22:494-499.

Horn, T., N.J. Gemmell, B.C. Robertson, and C.R. Bridges. 2009. Telomere length change in European sea bass (*Dicentrarchus labrax*). Australian Journal of Zoology 56(3):207-210.

Huff, J.A. 1975. Life history of Gulf of Mexico sturgeon, *Acipenser oxyrynchus desotoi*, in Suwannee River, Florida. Florida Marine Research Publications Number 16. 37p.

Hughes, T.C., C.E. Lowie, and J.M. Haynes. 2005. Age, growth, relative abundance, and SCUBA capture of a new or recovering spawning population of lake sturgeon in the lower Niagara River, New York. North American Journal of Fisheries Management 25:1263-1272.

Hurley, K.L., R.J. Sheehan, and R.C. Heidinger. 2004. Accuracy and precision of age estimates for pallid sturgeon from pectoral fin rays. North American Journal of Fisheries Management 24:715-718.

Isely, J.J. and Q.C. Fontenot. 2000. Retention of coded wire tags in juvenile shortnose sturgeon. North American Journal of Fisheries Management 20:1040-1043.

Iwama, G.K., J.C. McGeer, and M.P. Pawluk. 1989. The effects of five fish anesthetics on acid-base balance, hematocrit, blood gases, cortisol, and adrenaline in rainbow trout. Canadian Journal of Zoology 67:2065-2073.

Jackson, N.D., J.E. Garvey, and R.E. Colombo. 2007. Comparing aging precision of calcified structures in shovelnose sturgeon. Journal of Applied Ichthyology 23(4):525-528.

Jemison, S.C., L.A. Bishop, P.G. May, and T.M. Farrell. 1995. The impact of PIT-tags on growth and movement of the rattlesnake, *Sistrurus miliarus*. Journal of Herpetology 29(1):129-132.

Jenkins, W.E., T.I.J. Smith, L. Heyward, and D.M. Knott. 1993. Tolerance of shortnose sturgeon, *Acipenser brevirostrum*, juveniles to different salinity and dissolved oxygen concentrations. Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 47:476-484.

Johnson, J.H., J.E. McKenna, D.S. Dropkin, and W.D. Andrews. 2005. A novel approach to fitting the Von Bertalanffy relationship to a mixed stock of Atlantic sturgeon harvested off the New Jersey coast. Northeastern Naturalist 12(2):195-202.

Kamler, J.F. and K.L. Pope. 2001. Nonlethal methods of examining fish stomach contents. Reviews of Fisheries Science 9(1):1-11.

Kaufman, R.C., A.G. Houck, and J.J. Cech, Jr. 2007. Effects of temperature and dissolved oxygen on green sturgeon blood-oxygen equilibria. Environmental Biology of Fishes 79:201-210.

Keck, M.B. 1994. Test for detrimental effects of PIT tags in neonatal snakes. Copeia 1994:226-228.

Kelly, J.T., A.P. Klimley, and C.E. Crocker. 2007. Movements of green sturgeon, *Acipenser medirostris*, in the San Francisco Bay estuary, California. Environmental Biology of Fishes 79:281-295.

Kersten, C.A., M. Krisfalusi, J.E. Parsons, and J.G. Cloud. 2001. Gonadal regeneration in masculinized female or steroid-treated rainbow trout (*Oncorhynchus mykiss*). Journal of Experimental Zoology 290:396–401.

Kieffer M. and B. Kynard. 1993. Annual movements of shortnose and Atlantic sturgeons in the Merrimack River, Massachusetts. Transactions of the American Fisheries Society 122:1088-1103.

Kieffer, M.C. and B. Kynard. 1996. Spawning of the shortnose sturgeon in the Merrimack River, Massachusetts. Transactions of the American Fisheries Society 125:179-186.

Kieffer, M. and B. Kynard. In Press. Long-term evaluation of external and internal telemetry tagged shortnose sturgeon. Chapter 11 in Life history and behavior of Connecticut River shortnose sturgeon and other sturgeons. Special Publication 4, World Sturgeon Conservation Society.

Kohlhorst, D.W. 1979. Effect of first pectoral fin ray removal on survival and estimated harvest rate of while sturgeon in the Sacramento-San Joaquin estuary. California Fish and Game 65:173-177.

Kynard, B. and E. Lonsdale. 1975. Experimental study of galvanonarcosis for rainbow trout (*Salmo gairdneri*) immobilization. Journal of the Fisheries Research Board of Canada 32:300–302.

Kynard, B. and M. Kieffer. 1991. Protocol: techniques for implanting telemetry tags inside sturgeon. Conte Anadromous Fish Research Center, Turner Falls, Massachusetts.

Kynard, B. 1997. Life history, latitudinal patterns and status of shortnose sturgeon, *Acipenser brevirostrum*. Environmental Biology of Fishes 48(1-4):319-334.

Kynard, B. and M. Kieffer. 1997. Rejection of internal telemetry transmitters by shortnose sturgeon. Page 54 in Boehlert, G.W. (ed.), Application of acoustic and archival tags to assess estuarine, nearshore, and offshore habitat utilization and movement by salmonids. U.S. Department of Commerce, NOAA-TM-NMFS-SWFSC-236.

Kynard, B. and M. Kieffer. 2002. Use of a borescope to determine the sex and egg maturity stage of sturgeons and the effect of borescope use on reproductive structures. Journal of Applied Ichthyology 18:505-508.

Kynard, B., M. Breece, M. Atcheson, M. Kieffer, and M. Mangold. 2007. Status of shortnose sturgeon in the Potomac River. Prepared for the National Park Service. Washington, D.C. 52p.

Lai, H.L., V.F. Gallucci, D.R. Gunderson, and R.F. Donnelly. 1996. Age determination in fisheries: methods and applications to stock assessment. Pages 82-178 in Gallucci, V.F., S.B. Saila, D.J. Gustafson, and B.J. Rothschild (eds.), Stock Assessment: Quantitative Methods and Applications for Small-Scale Fisheries.

Lankford, S.E., T.E. Adams, R.A. Miller, and J.J. Cech, Jr. 2005. The cost of chronic stress: impacts of a nonhabituating stress response on metabolic variables and swimming performance in sturgeon. Physiological and Biochemical Zoology 78(4):599-609.

LeBreton, G.T.O. and F.W.H. Beamish. 2004. Growth, bioenergetics and age. Pages 195-216 in LeBreton, G.T.O., F.W.H. Beamish, and R.S. McKinley (eds.), Sturgeons and Paddlefish of North America.

LeBreton, G.T.O. and F.W.H. Beamish. 2000. Suitability of lake sturgeon growth rings in chronology development. Transactions of the American Fisheries Society 129:1018-1030.

Mason Jr., W.T. and J.P. Clugston. 1993. Foods of the gulf sturgeon in the Suwannee River, Florida. Transactions of the American Fisheries Society 122:378-385.

Matsche, M., and R. Bakal. 2008. General and reproductive health assessments of shortnose sturgeon with application to Atlantic sturgeon: anesthesia, phlebotomy, and laparoscopy. Maryland Department of Natural Resources. Oxford, Maryland. 27 p.

Mayfield, R.B. and J.J. Cech, Jr. 2004. Temperature effects on green sturgeon bioenergetics. Transactions of the American Fisheries Society 133:961-970.

Moghim, M., A.R. Vajhi, A. Veshkini, and M. Masoudifard. 2002. Determination of sex and maturity in *Acipenser stellatus* by using ultrasonography. Journal of Applied Ichthyology 18:325–328.

Mohler, J.W. 2003. Culture manual for the Atlantic sturgeon, *Acipencer oxyrinchus oxyrinchus*. U.S. Fish and Wildlife Service, Region 5, Hadley, Massachusetts. http://www.fws.gov/northeast/fisherycenter/pdfs/ASTCultureManual.pdf

Morrow, J.V., J.P. Kirk, K.J. Killgore, and S.G. George. 1998. Age, growth**,** and mortality of shovelnose surgeon in the Lower Mississippi River. North American Journal of Fisheries Management 18:725-730.

Moser, M.L. and S.T. Lindley. 2007. Use of Washington estuaries by subadult and adult green sturgeon. Environmental Biology of Fishes 79:243-253.

Moser, M.L., M. Bain, M.R. Collins, N. Haley, B. Kynard, J.C. O'Herron II, G. Rogers, and T.S. Squiers. 2000a. A protocol for use of shortnose and Atlantic sturgeon. NOAA Technical Memorandum NMFS-OPR-18.

Moser, M.L., J. Conway, T. Thorpe, and J.R. Hall. 2000b. Effects of recreational electrofishing on sturgeon habitat in the Cape Fear River drainage. North Carolina Sea Grant 99-EP-06. 36p.

Moser, M.L. and S.W. Ross. 1995. Habitat use and movements of shortnose and Atlantic sturgeons in the lower Cape Fear River, North Carolina. Transactions of the American Fisheries Society 124:225–234.

Murie, D.J., and D.C. Parkyn. 2000. Development and implementation of a non-lethal method for collection of stomach contents from sturgeon related to diel feeding periodicity. Report to the Flordia Marine Research Institute, Florida Fish and Wildlife Conservation Commission, St. Petersburg, FL.

Musick, J.A. and C.H. Hager. 2007. Atlantic sturgeon, *Acipenser oxyrinchus*, restoration in Chesapeake Bay. Final Report to National Marine Fisheries Service. Grant Number NA06NMF4050068. 30p.

Nakamoto, R.J., T.T. Kisanuki, and G.H. Goldsmith. 1995. Age and growth of Klamath River green sturgeon (*Acipenser medirostrum*). U.S. Forest Service and U.S. Fish and Wildlife Service, funded by U.S. Fish and Wildlife Service. Project Number 93-FP-13. 27p.

Nellis, P., J. Munro, D. Hatin, G. Desrosiers, R.D. Simons, and F. Guilbard. 2007. Macrobenthos assemblages in the St. Lawrence estuarine transition zone and their potential as food for Atlantic sturgeon and lake sturgeon. American Fisheries Society Symposium 56: 105-128.

Nelson, T.C., W.J. Gazey, K.K. English, and N.L. Rosenau. 2004. Status of white sturgeon in the lower Frazier River: report on the findings of the lower Frazier River white sturgeon monitoring and assessment program, 1999-2004. LGL, Limited, Environmental Research Associates. Vancouver, British Columbia. 97p.

Nelson, T.C., W.J. Gazey, and K.K. English. 2007. Status of white sturgeon in the lower Frazier River: report on the findings of the lower Frazier River white sturgeon

monitoring and assessment program, 2006. Frasier River Sturgeon Conservation Society. Vancouver, British Columbia. 63p.

Niklitshek, E.J. and D.H. Secor. 2009a. Dissolved oxygen, temperature and salinity effects on the ecophysiology and survival of juvenile Atlantic sturgeon in estuarine waters: I. Laboratory results. Journal of Experimental Marine Biology and Ecology IN PRESS.

Niklitshek, E.J. and D.H. Secor. 2009b. Dissolved oxygen, temperature and salinity effects on the ecophysiology and survival of juvenile Atlantic sturgeon in estuarine waters: II. model development and testing. Journal of Experimental Marine Biology and Ecology IN PRESS.

Niklitshek, E.J. and D.H. Secor. 2005. Modeling spatial and temporal variation of suitable nursery habitats for Atlantic sturgeon in the Chesapeake Bay. Estuarine, Coastal and Shelf Science 64:135-148.

Niklitschek, E.J. 2001. Bioenergetics modeling and assessment of suitable habitat for juveniles Atlantic and shortnose sturgeon in the Chesapeake Bay. University of Maryland Ph.D. dissertation. 158p.

Nilo, P., S. Tremblay, A Bolon, J. Dodson, P. Dumont, and R. Fortin. 2006. Feeding ecology of juvenile lake sturgeon in the St. Lawrence River system. Transactions of the American Fisheries Society 135:1044-1055.

Paragamian, V.L. and R.C.P. Beamesderfer. 2003. Growth estimates from tagged white sturgeon suggest that ages from fin rays underestimate true age in the Kootenai River, USA and Canada. Transactions of the American Fisheries Society 132:895-903.

Parkyn, D.C., D.J. Murie, D.E. Colle, and J.D. Holloway. 2006. Post-release survival and riverine movements of Gulf of Mexico sturgeon (*Acipenser oxyrinchus desotoi* Acipenseriformes) following induced spawning. Journal of Applied Ichthyology 22:1-7.

Parsons, G.R., J.J. Hoover, and K.J. Killgore. 2003. Effect of pectoral fin ray removal on station-holding ability of shovelnose sturgeon. North American Journal of Fisheries Management 23:742-747.

Peterson, D.L. and J.D. Bezold. 2008. Comparing hatchery-reared (naïve) and wild-caught (naturalized) juvenile lake sturgeon used in a radio telemetry study in the Coosa River system, Georgia-Alabama. Manuscript.

Pirhonen, J. and C.B. Schreck. 2003. Effects of anesthesia with MS-222, clove oil, and CO2 on feed intack and plasma cortisol on steelhead trout (*Oncorhynchus mykiss*). Aquaculture 220:507-514.

Ream, R.A., J.A. Theroit, and G.N. Somero. 2003. Influences of thermal acclimation and acute temperature change on the motility of epithelial wound-healing cells (keratocytes) of tropical, temperate and Antarctic fish. The Journal of Experimental Biology 206:4539-4551.

Redman, S.D., J.R. Meinertz, M.P. Gaikowski. 1998. Effects of immobilization by electricity and MS-222 on brown trout broodstock and their progeny. Progressive Fish Culturist 60:44–49.

Reine, K. 2005. An overview of tagging and tracking technologies for freshwater and marine fishes. DOER Technical Notes Collection, ERDC TN-DOER-E18, U.S. Army Engineer Research and Development Center, Vicksburg, Mississippi.

Rien, T.A. and R.C. Beamesderfer. 1994. Accuracy and precision of white sturgeon age estimates from pectoral fin rays. Transactions of the American Fisheries Society 123:255-265.

Rien, T.A., R.C.P. Beamesderfer, and C.A. Foster. 1994. Retention, recognition, and effects on survival of several tags and marks for white sturgeon. California Fish and Game 80(4):161-170.

Rossiter, A., D.L.G. Noakes, and F.W.H. Beamish. 1995. Validation of age estimation for the lake sturgeon. Transactions of the American Fisheries Society 124:777-781.

Roubach, R. L.C. Gomes, F.A.L. Fonseca, and A.L. Val. 2005. Eugenol as an efficacious anaesthetic for tambaqui, *Collosoma macropomum* (Culvier). Aquaculture Research 36:1056-1061.

Safi, S., H.V. Roodsari, and M.R. Ahmadi. 2006. The effect of levamisole hydrochloride on survival of Persian sturgeon, *Acipenser persicus*, fry. Journal of Applied Ichthyology 22(suppl. 1):226-230.

Sardella, B.A., E. Sanmarti, and D. Kultz. 2008. The acute temperature tolerance of green sturgeon (Acipenser medirostrum) and the effect of environmental salinity. Journal of Experimental Zoology 309A:477-483.

Savoy, T.F. and J. Benway. 2004. Food habits of shortnose sturgeon collected in the lower Connecticut River from 2000 through 2002. American Fisheries Society Monograph 9:353-360.

Savoy, T. 2007. Prey eaten by Atlantic sturgeon in Connecticut waters. American Fisheries Society Symposium 56: 157-165.

Schaffter, R.G. 1997. White sturgeon spawning migrations and location of spawning habitat in the Sacramento River, California. California Fish Game 83:1–20.

Schueller, P. and D.L. Peterson. In Press. Abundance and recruitment of juvenile Atlantic sturgeon in the Altamaha River, Georgia. Transactions of the American Fisheries Society.

Secor, D.H. and T.E. Gunderson. 1998. Effects of hypoxia and temperature on survival, growth, and respiration of juvenile Atlantic sturgeon, *Acipenser oxyrinchus*. Fishery Bulletin 96:603-613.

Secor, D.H. and E.J. Niklitschek. 2002. Sensitivity of sturgeons to environmental hypoxia: a review of physiological and ecological evidence. Pages 61-78 in The Proceedings on the Sixth International Symposium on Fish Physiology, Toxicology, and Water Quality. La Paz, B.C.S. Mexico, January 22-26, 2001.

Shuman, D.A. and E.J. Peters. 2006. Evaluation of pulsed gastric levage on the survival of captive shovelnose sturgeon. Journal of Applied Ichthyology 23: 521-524.

Simpson, P.C. and D.A. Fox. 2006. Atlantic sturgeon in the Delaware River: contemporary population status and identification of spawning areas. Completion Report: Award NA05NMF4051093. 41p.

Skalski, J., S. Smith, R. Iwamoto, J. Williams, and A. Hoffmann. 1998. Use of passive integrated transponder tags to estimate survival of migrant juvenile salmonids in the Snake and Columbia rivers. Canadian Journal of Fisheries and Aquatic Sciences 55:1484-1493.

Small, B.C. 2003. Anesthetic efficacy of metomidate and comparison of plasma cortisol responses to tricaine methanesulfonate, quinaldine and clove oil anesthetized channel catfish (*Ictalurus punctatus*). Aquaculture 218:177-185.

Smith, T.I.J., S.D. Lamprecht, and J.W. Hall. 1990. Evaluation of tagging techniques for shortnose sturgeon and Atlantic sturgeon. American Fisheries Society Symposium 7:134-141.

Sprague, C.R., L.G. Beckman, and S.D. Duke. 1993. Prey Selection by Juvenile White Sturgeon in Reservoirs of the Columbia River. Pages 229–243 in R.C. Beamesderfer and A.A. Nigro (eds.), Status and Habitat Requirement of the White Sturgeon Populations in the Columbia River Downstream from McNary Dam, volume 2. Final Report of Oregon Department of Fish and Wildlife to Bonneville Power Administration, Portland, Oregon.

Sterritt, D.A., S.T. Elliott, A.E. Schmidt. 1994. Electrical anesthesia for immobilizing adult coho salmon in freshwater. North American Journal of Fisheries Mangement 14:453–456.

Stevenson, J.T. and D.H. Secor. 1999. Age determination and growth of Hudson River Atlantic sturgeon, *Acipenser oxyrinchus*. Fisheries Bulletin 97:153-166.

Strange, R.J. and C.B. Schreck. 1978. Anesthetic and handling stress on survival and cortisol concentration in yearling Chinook salmon (*Oncorhynchus tshawytscha*). Journal of the Fisheries Research Board of Canada 35:345-349.

Sulak, K.J. and J.P. Clugston. 1999. Recent advances in life history of Gulf of Mexico sturgeon, Acipenser oxyrinchus desotoi, in the Suwannee River, Florida, USA: a synopsis. Journal of Applied Ichthyology 15:116-128.

Sulak, K.J. and J.P. Clugston. 1998. Early life history stages of Gulf sturgeon in the Suwannee River, Florida. Transactions of the American Fisheries Society 127:758-771.

Sulak, K.J. and M. Randall. 2002. Understanding sturgeon life history: enigmas, myths, and insights from scientific studies. Journal of Applied Ichthyology 18:519-528.

Summerfelt, R.C. and L.S. Smith. 1990. Anesthesia, surgery, and related techniques. Pages 213 - 272 *in* C.B. Schreck and P.B. Moyle (eds.) Methods for fish biology. American Fisheries Society, Bethesda, Maryland.

Sutton, T.M. and A.C. Benson. 2003. Influence of external transmitter shape and size on tag retention and growth of juvenile lake sturgeon. Transactions of the American Fisheries Society 132:1257-1263.

Taubert, B.D. 1980. Reproduction of shortnose sturgeon (*Acipenser brevirostrum*) in Holyoke Pool, Connecticut River, Massachusetts. Copeia 1980:114-117.

Thorsteinsson, V. 2002. Tagging methods for stock assessment and research in fisheries. Report of Concerted Action FAIR CT.96.1394 (CATAG), Reykjavik, Iceland. Marine Research Institute Technical Report (79).

Tipping, J.M. and G.J. Gilhuly. 1996. Survival of electroanesthetized adult steelhead and eggs of fall chinook salmon. North American Journal of Fisheries Management 16:469–472.

USFWS (U.S. Fish and Wildlife Service). 2008. Biological procedures and protocols for researchers and managers handling pallid sturgeon. U.S. Fish and Wildlife Service, Billings, Montana.

Van Eenennaam, J.P., S.I. Doroshov, G.P. Moberg, J.G. Watson, D.S. Moore, and J. Linares. 1996. Reproductive conditions of the Atlantic sturgeon (*Acipenser oxyrinchus*) in the Hudson River. Estuaries 19(4):769-777.

Van Eenennaam, J.P. and S.I. Doroshov. 1998. Effects of age and body size on gonadal development of Atlantic sturgeon. Journal of Fish Biology 53:624-637.

Van Eenennaam, J.P., J. Linares-Casenave, X. Deng, and S.I. Doroshov. 2005. Effect of incubation temperature on green sturgeon embryos, *Acipenser medirostris*. Environmental Biology of Fishes 72:145-154.

Van Eenennaam, J.P., J. Linares-Casenave, J.B. Muguet, and S.I. Doroshov. 2008. Induced spawning, artificial fertilization, and egg incubation techniques for green sturgeon. North American Journal of Aquaculture 70:434-445.

Vecsei, P., M.K. Litvak, D.LG. Noakes, T. Rien, and M. Hochleithner. 2003. A noninvasive technique for determining sex of live North American sturgeon. Environmental Biology of Fishes 68:333-338.

Wagner, G.N., T.D. Singer, and R.S. McKinley. 2003. The ability of clove oil and MS-222 to minimize handling stress in rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquaculture Research 34:1139-1146.

Waldman, J.R., C. Grunwald, J. Stabile, and I. Wirgin. 2002. Impacts of life history and biogeography on the genetic stock structure of Atlantic sturgeon, *Acipenser oxyrinchus oxyrinchus*, Gulf sturgeon, *A. oxyrinchus desotoi*, and shortnose sturgeon, *A. brevirostrum*. Journal of Applied Ichthyology 18:509-518.

Walker, M.K., E.A. Yanke, and W.H. Gingerich. 1994. Use of electronarcosis to immobilize juvenile and adult northern pike. The Progressive Fish-Culturist 56:237-243.

Wang, Y.L., F.P. Binkowski, and S.I. Doroshov. 1985. Effect of temperature on early development of white and lake sturgeon, *Acipenser transmontanus* and *A. fulvescens*. Environmental Biology of Fishes 14(1):43-50.

Wanner, G.A., R.A. Klumb, W.J. Stancill, and G.R. Jordan. 2007. Habitat use and movement of adult pallid sturgeon in the Missouri River downstream of Fort Randall Dam, South Dakota and Nebraska. Proceedings of the South Dakota Academy of Science 86(2007):21-33.

Wanner, G.A. 2006. Evaluation of a Gastric Lavage Method on Juvenile Pallid Sturgeon. North American Journal of Fisheries Management 26: 587-591.

Webb, M.A.H. and D.L. Erickson. 2007. Reproductive structure of the adult green sturgeon, *Acipenser medirostris*, population in the Rogue River, Oregon. Environmental Biology of Fishes 79:305-314.

Webb, M.A.H., G.W. Feist, E.P. Foster, C.B. Schreck, and M.S. Fitzpatrick. 2002. Potential classification of sex and stage of gonadal maturity of wild white sturgeon using blood plasma indicators. Transactions of the American Fisheries Society 131:132–142.

Wehrly, K.E. 1995. The effect of temperature on the growth of juvenile lake sturgeon, *Acipenser fulvescens.* Michigan Department of Natural Resources, Fisheries Division, Research Report 2004, Lansing.

Whiteman, K.W., V.H. Travnichek, M.L. Wildhaber, A. DeLonay, D. Papoulias, and D. Tillett. 2004. Age estimation for shovelnose sturgeon: a cautionary note based on annulus formation in pectoral fin rays. North American Journal of Fisheries Management 24:731-734.

Wildhaber, M.L., D.M. Papoulias, A.J. DeLonay, D.E. Tillitt, J.L. Bryan, and M.L. Annis. 2006. Development of methods to determine the reproductive status of pallid

sturgeon in the Missouri River. U.S. Geological Survey final report to the U.S. Fish and Wildlife Service. 88p.

Wildhaber, M.L. and J.L. Bryan. 2006. Ultrasonic and endoscopic examination of lower Missouri and lower Mississippi pallid sturgeon to determine sex and 2006 broodstock potential. U.S. Geological Survey report to U.S. Army Corps of Engineers. 12p.

Wilga, C.D. and G.V. Lauder. 1999. Locomotion in sturgeon: function of the pectoral fins. The Journal of Experimental Biology 202:2413-2432.

Winter. J.D. 1983. Underwater biotelemetry. Pages 371–395 in L.A. Nielsen and D.L. Johnson (eds.) Fisheries techniques. American Fisheries Society, Bethesda, Maryland.

Ziegeweid, J.R., C.A. Jennings, D.L. Peterson. 2007. Thermal maxima for juvenile shortnose sturgeon acclimated to different temperatures. Environmental Biology of Fishes 82(3):299-307.