From:	Britta Muiznieks
To:	<u>Mike Murray</u>
Subject:	Re: turtle question
Date:	09/14/2010 05:52 PM
Attachments:	2010 Egg Sampling Protocol.pdf
	DNA-SupportFWS.pdf

Mike-

If you look at the pie graph on the left it shows you the percentage of the eggs that were lost which is a relatively small percentage of all of our eggs. The pie graph on the right depicts our known losses which appears high but in reality it is not. We are taking one egg from each nest for the DNA study. At this point we have only received the results from the first seven eggs that we collected this season but should be getting back more results soon. I'm attaching FWS's letter of support and the egg sampling protocol.



2010 Egg Sampling Protocol.pdf DNA-SupportFWS.pdf

Britta Muiznieks Wildlife Biologist Cape Hatteras National Seashore

252-995-3740-**Office** 252-475-8348-**Cell** 252-995-6998-**FAX**

Mike Murray/CAHA/NPS

Mike Murray/CAHA/NPS To Britta Muiznieks/CAHA/NPS@NPS cc 09/14/2010 04:38 PM Subject turtle question

Britta,

On the WRC seaturtle.org website, the graphic for Losses indicates that a large number of eggs (or nests?) are lost to Research, both statewide and at CAHA? What does Research include and why are so many eggs lost to Research? (Just curious, since such high losses to Research doesn't make sense to me.)

http://www.seaturtle.org/nestdb/index.shtml?view=1

Thanks,

Mike Murray

0028001

Superintendent Cape Hatteras NS/ Wright Brothers NMem/ Ft. Raleigh NHS (w) 252-473-2111, ext. 148 (c) 252-216-5520 fax 252-473-2595

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United States Department of the Interior

FISH AND WILDLIFE SERVICE 6620 Southpoint Drive, South Suite 310 Jacksonville, Florida 32216-0912

March 15, 2010

Mr. Matthew H. Godfrey, PhD Biologist, NC Wildlife Resources Commission Sea Turtle Project 1507 Ann St. Beaufort, NC 28516

Dear Mr. Godfrey:

The U.S. Fish and Wildlife Service (Service) reviewed the proposal for the study focused on the genetic mark-recapture of the Northern Recovery Unit (GA, SC and NC) nesting female loggerheads, using DNA derived from freshly laid eggs. This letter serves as support for this research project. The proposed project will assist the Service in understanding loggerhead sea turtle (*Caretta caretta*) population dynamics. Information on clutch frequency, nesting beach fidelity, and interesting intervals for the Northern Recovery Unit will be assessed through this research as well.

The Service has determined that this project supports management needs and meets the actions plans identified in the Recovery Plan for the Northwest Atlantic Population of the Loggerhead sea turtle.

Please contact me at (904) 525-0661 if you have any questions.

Sincerely,

Jun Marce haur Asen

Ann Marie Lauritsen Fish and Wildlife Service Biologist

cc:

Mark Dodd-GA DNR DuBoise Griffin- SC DNR



Viable Egg Collection Protocol

- 1) Because of the extremely small amount of DNA present in the shells, it is necessary to change gloves between nests or wipe hands with hand sanitizer to prevent DNA cross contamination of samples from different females.
- 2) Pull a single egg from each nest either when the nest chamber is validated.
- 3) Open the egg and discard its contents. (The goal is to try to keep the yolk and associated embryonic disc and membranes OFF the egg shell to the extent possible. We are trying to avoid having the embryonic DNA contaminate the maternal DNA) Pinching a spot on the shell and opening the egg wide open like a bag of chips is the best way to avoid contamination. The egg contents should be discarded well away from the nest, ideally buried into wet sand. Eggs broken by predators (found the morning after oviposition) or while probing will work, but should be rinsed in the ocean first to cleanse any remaining embryonic tissue/yolk membranes.
- 4) Place the entire shell in a 30 ml conical tube containing 95% ethanol. (Tubes are flammable, so keep away from heat sources!)
- 5) Write the year, three-digit beach abbreviation (separate list) and nest number on the blue cap of the vial with a Sharpie.
- 6) While on the beach, try to keep the sample out of direct sunlight if possible.
- 7) Store the samples in a cool (room temperature is fine), dark place.

The goal is to collect a freshly laid (< 48 hours old ideally) eggshell from every nest on your beach. We need some sort of genetic sample from every nest laid- if a fresh egg wasn't collected because the nest was originally called a false crawl- then we can use a dead hatchling or embryo flipper collected at inventory. Each and every nest needs to be represented, with the order of preference being:

- 1) freshly laid egg
- 2) depredated eggshell (if available from ghost crab depredation, etc. and only if fresh egg wasn't taken)
- 3) dead hatchling tissue at inventory (fresher the better, only if fresh egg fails)
- 4) hatched egg shell at inventory (only if no dead hatchlings/embryos available and fresh egg wasn't collected)

Fresh eggs contain by far the best DNA compared to egg shells from various stages of incubation, so please make every effort to get a fresh egg from each and every nest. I will attempt to run all egg samples with sufficient lead time to identify any problem samples. In this case, we can use dead hatchling tissue as a back-up insurance sample. I will notify you of specific nests if this is the case, so don't worry about collecting anything extra if a fresh egg was collected unless you hear from me.

The ultimate goal of the project is to track site fidelity and clutch frequency of northern subpopulation loggerheads nesting from NC to the FL border. We do this by assigning nests to biopsied females or by matching multiple nests from unseen females. We have genetically "tagged" ~ 1000 nesting females in GA since 2005, and it will be interesting to see how many and where these turtles may show up! Getting a viable genetics sample from every nest is critical for producing accurate clutch frequency data with minimal bias.

Thanks so much for all of the help! This is obviously a massive undertaking with respect to scale, and the data we will produce is only as good as the sampling effort. So thank for participating! If you have any questions, concerns, or need additional supplies, please contact me:

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