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. If you are relocating a nest, wait until you have moved the clutch to see if there are any broken eggs available on the bottom of the clutch before breaking open an egg. Do not use a spacer as they do not contain reliable maternal DNA.
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 or kept in a container. Eggs broken by predators (found the morning after oviposition) or while probing will work, but should be rinsed in the ocean first (you can gently use your fingers to scrub the shell) to cleanse any remaining embryonic tissue/yolk membranes. If the yolk is not disturbed, no need to wash the shell. Sand is not a problem.
4) Place the entire shell in a 50 ml conical tube containing 95% ethanol. (Tubes are flammable, so keep away from heat sources!)
5) Write the year (15 will suffice), three-digit beach abbreviation (separate list) and nest number on the orange cap of the vial with a Sharpie. If there isn't room there, please write on the white tab and cover with a piece of scotch tape. If you are on a beach with zones patrolled by multiple groups, please use whatever nest reference you will use to identify the nest in the seaturtle.org database.
6) If you are taking an egg shell that may have incubated for more than 3 days (inventory sample or depredated shell from an irregularly monitored beach), please mark the vial with an "I" so we know it may not be fresh.
7) While on the beach, try to keep the sample out of direct sunlight if possible.
8) Store the samples in a cool (room temperature is fine), dark place. A closet shelf or desk drawer is ideal.
9) Once the tube is in its final storage location, please ensure that the shell is covered by the ethanol.

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, undeveloped egg or a hatched shell collected at inventory. Each and every nest needs to be represented, with the order of preference being:

1) Broken (probing) or freshly depredated eggshell (the night nest was laid)
2) A freshly laid eggshell you have to break open
3) Dead hatchling/embryo at inventory (the fresher the better, only if fresh egg fails or was not collected)
4) Eggshell from an unhatched egg collected at inventory
5) Hatched egg shell at inventory (least desirable choice)- the bigger the shell piece, the better

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" more than 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:

Brian Shamblin
Cell (423) 595-0289
brian.shamblin@gmail.com