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PREPARATION OF FRESH DEAD SEA TURTLE CARCASSES FOR AT-SEA DRIFT EXPERIMENTS

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Cover photograph: Fresh dead, cold stun green sea turtle carcass during preparation.
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Introduction

All populations of sea turtles that occur in United States (U.S.) waters are listed as threatened or endangered by the U.S. Endangered Species Act (ESA). Five of these six species have been documented in the Gulf of Mexico (GOM): loggerhead (*Caretta caretta*), Kemp's ridley (*Lepidochelys kempii*), green (*Chelonia mydas*), leatherback (*Dermochelys coriacea*) and hawksbill (*Eretmochelys imbricata*). Loggerhead and green sea turtles inhabiting the GOM are considered threatened while the Kemp's ridley, leatherback and hawksbill sea turtles are all listed as endangered throughout their entire range (National Oceanic and Atmospheric Administration *n. d.*). Interest in conservation and management of sea turtles has been prevalent for decades across the globe (Hamann et al. 2010). Interest in sea turtles inhabiting northern GOM (nGOM) waters increased after the *Deepwater Horizon* oil spill in 2010 with many research efforts being undertaken to better understand species' population dynamics (Lamont et al. 2012, Hart et al. 2013). One area of focus has been furthering our understanding of mortality, which primarily comes from sea turtle stranding data.

Sea turtle strandings refer to turtles that wash ashore onto beaches or are found floating after being killed or injured in the marine environment. Turtles that strand may be dead or alive, often injured or in a weakened state. Basic data such as species, location, size, weight, condition, presence/absence of tags and photographs are collected from stranded turtles whenever possible by trained members of the Sea Turtle Stranding and Salvage Network (STSSN). Some carcasses may also be salvaged for necropsy where additional information such as sex, health status, diet and potential cause of death can be determined. Important data such as these have bolstered our understanding of sea turtle life history (i.e. age estimates, growth rates and diet; Avens et al. 2012, Avens et al. 2013, Servis et al. 2015). Sea turtle stranding data are also often used as an indicator for at-sea mortality; however, these estimates may be greatly affected by factors such as time of year, geographic location, decomposition rate as well as oceanographic and atmospheric conditions (Hart et al. 2006, Epperly et al. 1996).

Many researchers utilize at-sea drifters, such as bottles and oranges, to study environmental factors affecting floating objects. Studies have combined results from at-sea drifters with sea turtle stranding data to evaluate and understand seasonal stranding trends and potential locations for at-sea mortalities in U.S. Atlantic and Mexican Pacific waters (Hart et al. 2006, Koch et al. 2013). To date, no research has been conducted in the GOM, which would likely encompass different environmental conditions. In Mississippi, located in the nGOM (Figure 1), the majority of strandings (80%) occur from March to June. There is typically a second small peak in stranding numbers in the fall (October) with few strandings reported throughout the rest of the year (Figure 2). These trends are prevalent across years and raise questions pertaining to the forces driving noted fluctuations. In order to understand seasonal stranding patterns, a comprehensive field study was conducted during the 2017 calendar year in state and federal waters off Mississippi using fresh dead, cold stun sea turtles and wooden effigies.

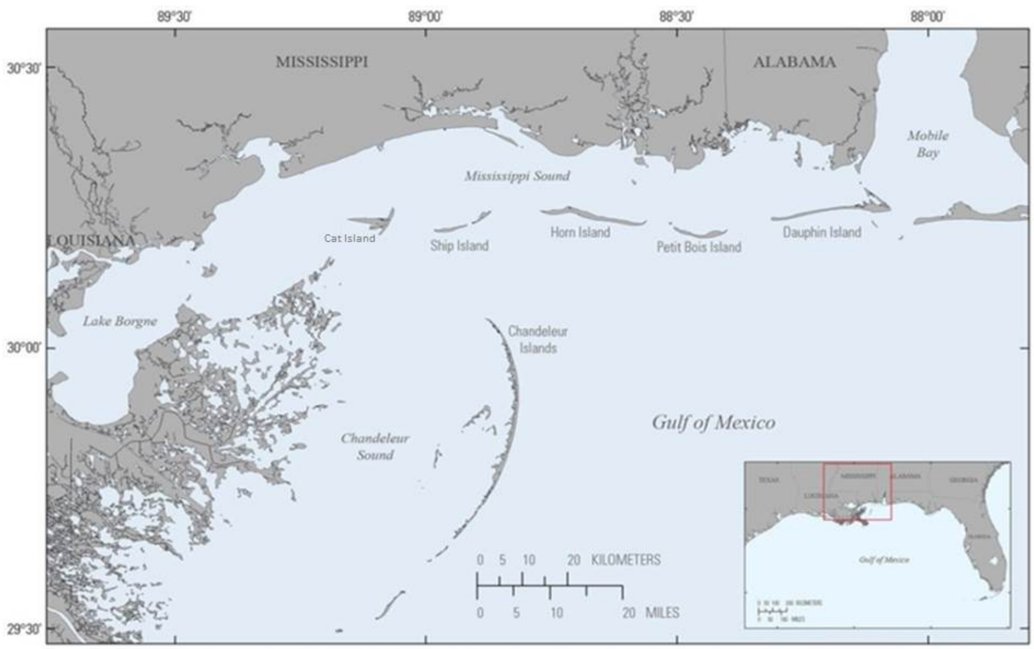

Figure 1. Map of coastal Mississippi and offshore waters in relation to the nGOM.

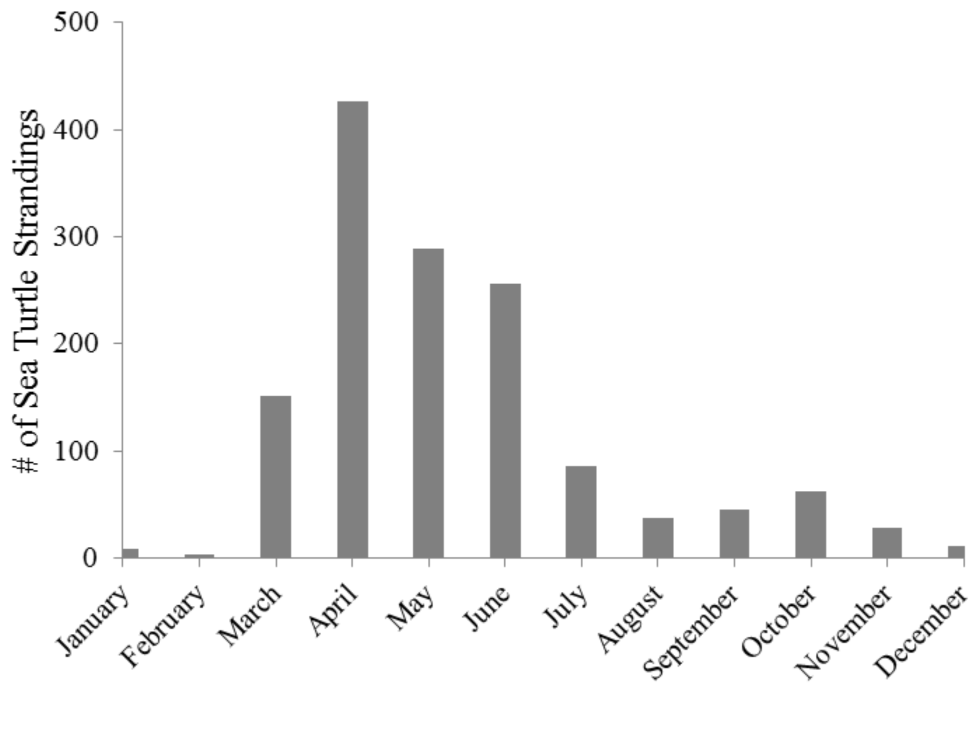

Figure 2. Number of sea turtle strandings in Mississippi from 2010-2017 by month (n = 1,410).

Real sea turtle carcasses were used for this study in order to mimic reality and determine how dead sea turtles float and drift in the water. This also allowed for gathering data on potential scavenging and the decomposition rate of carcasses, factors that may also affect whether a sea turtle carcass will strand. Twice a month from January to December 2017, 6–9 sea turtle carcasses and 3–6 neon colored wooden effigies (size 11.25” x 11.25” x 4.5”) were deployed at three sites in Mississippi state and adjacent federal waters (Figure 3). Small, 2" x 3" x 1", SPOT Trace® (SPOT) GPS satellite transmitters were vacuum sealed, placed in small plastic jars and attached to carcasses and effigies. The SPOT transmits locations every 10 min and is accurate to within a meter, therefore, their location was known throughout the deployment. All objects were monitored closely during deployment and were retrieved as soon as possible after beaching. Results of this study were presented by Reneker et al. (2018). This report documents the techniques used to prepare sea turtle carcasses for this study and provide evidence that cold-stun turtles make an excellent proxy for stranded sea turtles

Figure 3. Sea turtle carcass and effigy deployment.

Methods

Carcasses

Kemp’s ridley (n = 57) and green sea turtle carcasses (n = 125) were obtained from several cold-stun events in the GOM and U.S. Atlantic (Table 1). STSSN participants in North

Carolina, Massachusetts and Texas routinely conduct patrols of their respective beaches during cold-stun season in an effort to rescue as many turtles as possible. While some turtles are found alive, albeit in a lethargic state, others are found dead or die in transit to or at rehabilitation centers. Carcasses used in this study were frozen shortly after discovery and remained frozen until needed for a deployment. Carcasses averaged 27.7cm straight carapace length, notch to tip (SCLn-t) with Kemp's ridleys being smaller in comparison to the greens (Table 1).

Table 1. Summary of the number of sea turtle carcasses used during the study from GOM and U.S. Atlantic STSSN partners and size measurements (cm) straight carapace length, notch to tip (SCLn-t) by species.

	North Carolina	Massachusetts	Texas	Average SCLn-t	Minimum SCLn-t	Maximum SCLn-t
Green	123	0	2	28.4	23.6	38.9
Kemp's ridley	1	56	0	24.7	18.4	31.3

Carcass Preparation

Approximately 3–4 days before a scheduled deployment, sea turtle carcasses were removed from the freezer to begin preparations (Figure 4a). The number of carcasses varied by trip but was typically 9 carcasses (6 green and 3 Kemp's ridley); deploying 3 carcasses per site (2 green and 1 Kemp's ridley). As there were not enough turtles to use 9 for every trip, a few consisted of only 6 carcasses (3 green and 3 Kemp's ridley or 6 green; 2 carcasses per site). An effort was made to select carcasses of similar size (SCLn-t) for each trip and individual deployment for consistency. Turtles were first placed in water ranging from 10–32°C, depending on the time of year, for approximately 1 hour to thaw the flippers (Figure 4b). Once flippers were thawed, two flipper tags were attached for identification purposes and a ¼ inch hole was drilled between the two most posterior marginal scutes in order to attach the jar with satellite tag (Figure 5). After this initial preparatory work, carcasses were still in a semi-frozen state, presumably with a <0°C internal temperature.

Floating carcasses were a requirement for all of the deployments to simulate the natural process a sea turtle carcass goes through after mortality occurs. When a sea turtle dies, it typically sinks to the sea floor (negative buoyancy) and then floats to the surface when enough decomposition gases have built up to cause bloating, resulting in positive buoyancy (Epperly et al. 1996, Nero et al. 2013). Previous work by Cook et. al (2018) on the decomposition rate of sea turtle carcasses was used as a reference for preparing heated water baths. For example, a carcass kept in <30 cm of water at 20°C is estimated to float to the surface in approximately 83 hours compared to a carcass kept at 24°C which would likely float after only 33 hours.

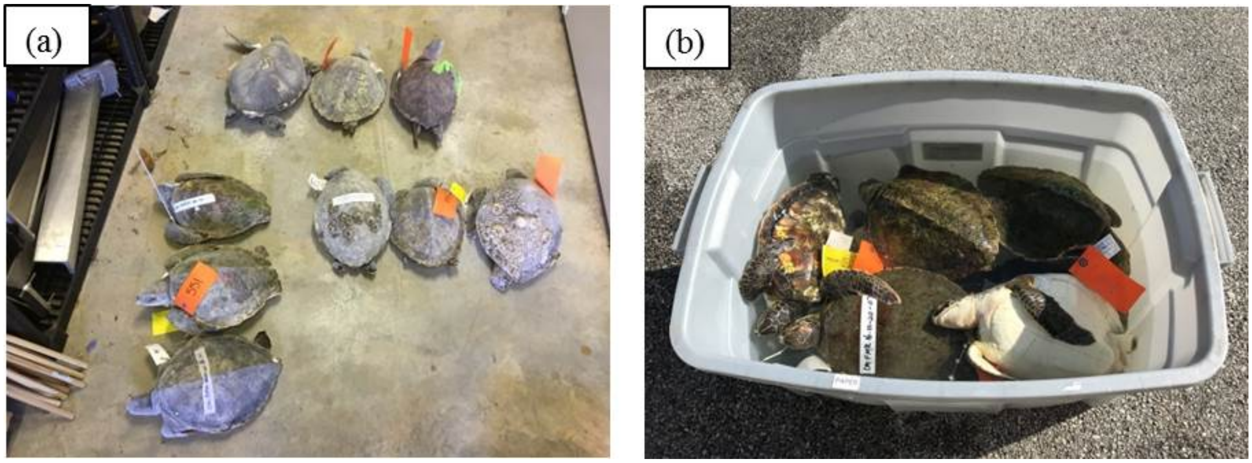

Figure 4. Sea turtle carcasses of similar SCL (a) removed from freezer and (b) thawing in ambient water temperature.

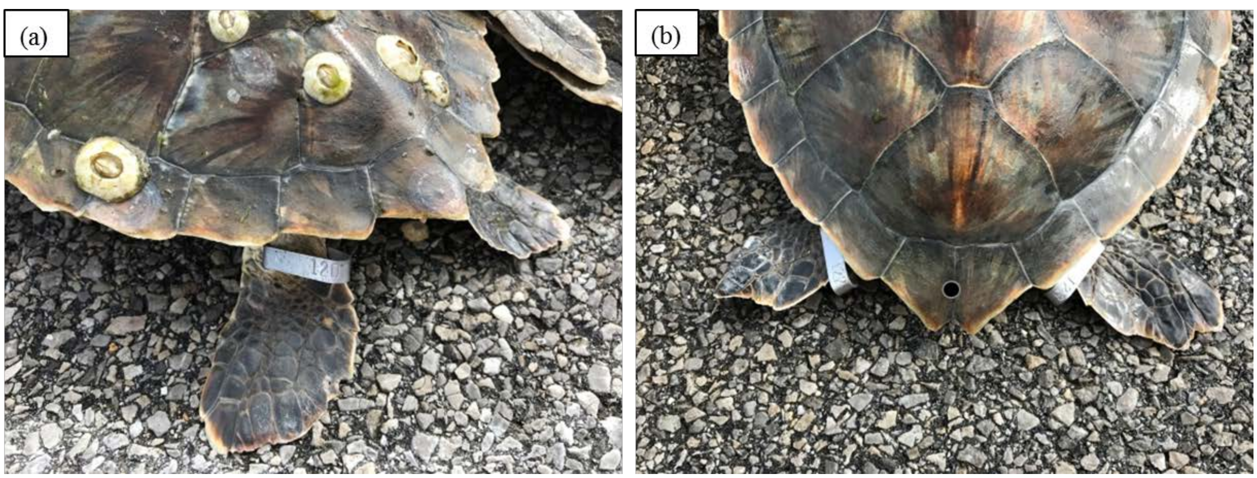

Figure 5. Sea turtle carcass (a) flipper tag identification and (b) ¼ inch drilled hole for attaching the jar with a satellite tag.

Large 120 quart coolers were used to bloat and float carcasses at controlled temperatures. AquaTop® 150 W aquarium heaters (Temperature Range: 20°C–34°C) were used to control the temperature of water baths. Kestrel® DROPs (Kestrel® Drop D1) were placed in the water and used to record temperature every 20 min. Kestrel® devices were Bluetooth enabled, allowing us to record water temperature every time the carcasses were checked. Over the course of carcass preparation, detailed notes were kept on the ambient air temperature and water temperature of all coolers. Additionally, buoyancy and the percent of carapace and limb exposure (Appendix 1, Figure 1) were recorded for all carcasses. The time was recorded for all carcass checks and notes were made if a turtle was negatively buoyant/sunk (on the bottom of the cooler), neutrally buoyant (floating but still submerged) or positively buoyant/floating (Figure 6a-c) and/or listing to one side. If any portion of the carcass began to float, the carcass was lifted to see how quickly it sank. Carcasses that sank slowly were likely to become positively buoyant soon. Often turtles were found floating during the first morning checks (7AM), so the exact float time was uncertain as it would have occurred sometime during the night or over a weekend.

If a carcass was already floating after being thawed and flipper tagged, it was placed and kept in chilled water (~14–18°C) until deployment. Sunken turtles were put in a cooler with an aquarium heater. This temperature varied but typically started at ~21°C and was raised incrementally to ~28°C for those turtles who had not yet floated 1 day prior to deployment. Once a turtle was found floating at the surface, it was moved to the chilled water cooler until deployment (Figure 6d). This slowed the decomposition process so carcasses would not reach a higher condition code than was desired.

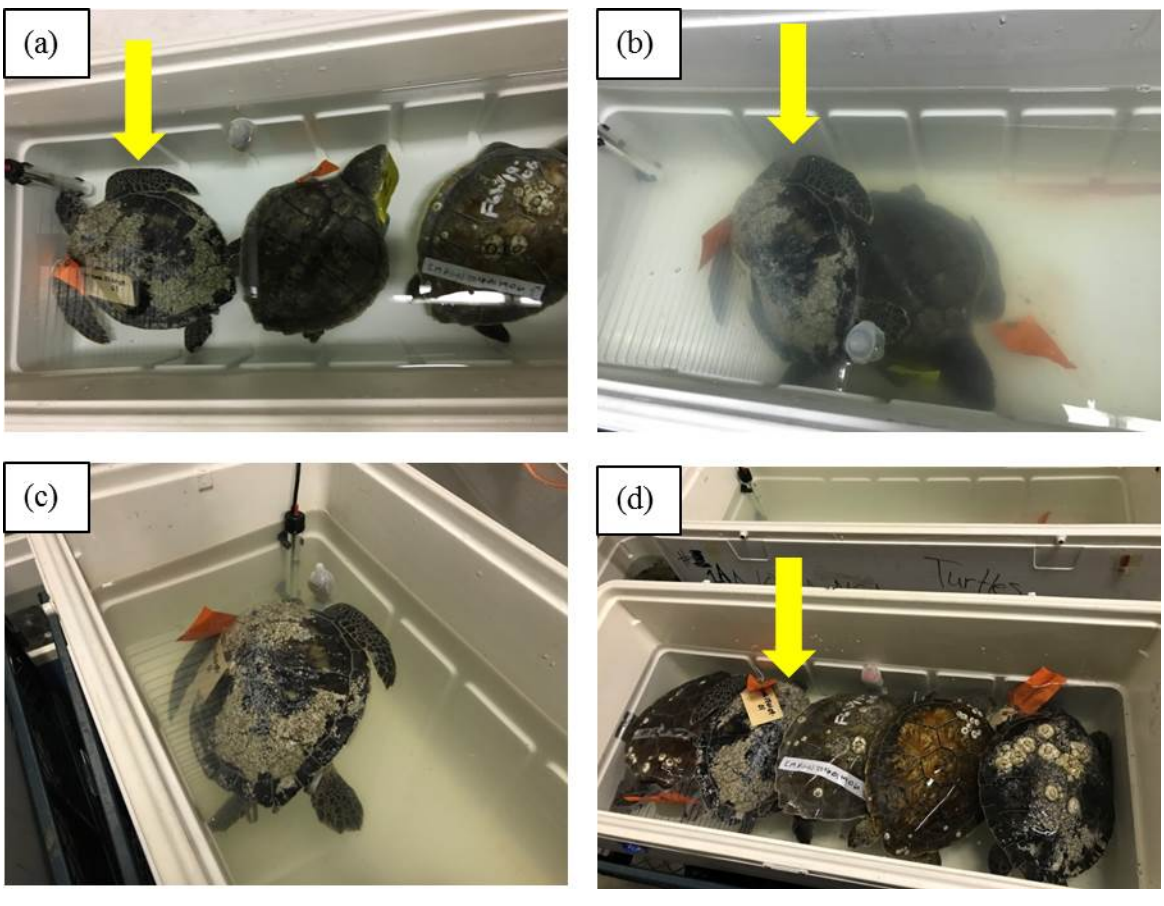

Figure 6. Progression of a green sea turtle carcass during preparation from 12/18/17 – 12/20/17 with aquarium heater set to 22°C. (a) 12/18 at 15:30 – carcass sank. (b) 12/20 at 12:00 – carcass is neutrally buoyant. (c) 12/20 at 16:00 – carcass floating at surface. (d) 12/20 at 16:00 – carcass placed in chilled water bath with other floating carcasses.

On the morning of every deployment, carcasses were individually photographed from multiple angles and the decomposition condition code was assessed (See Appendix A for list of condition codes). Along with condition code, notes were made on the percent carapace exposure above water, whether the head was/was not exposed (Appendix A, Figure 1) and if the carcass was listing to the right or left (Figure 7).

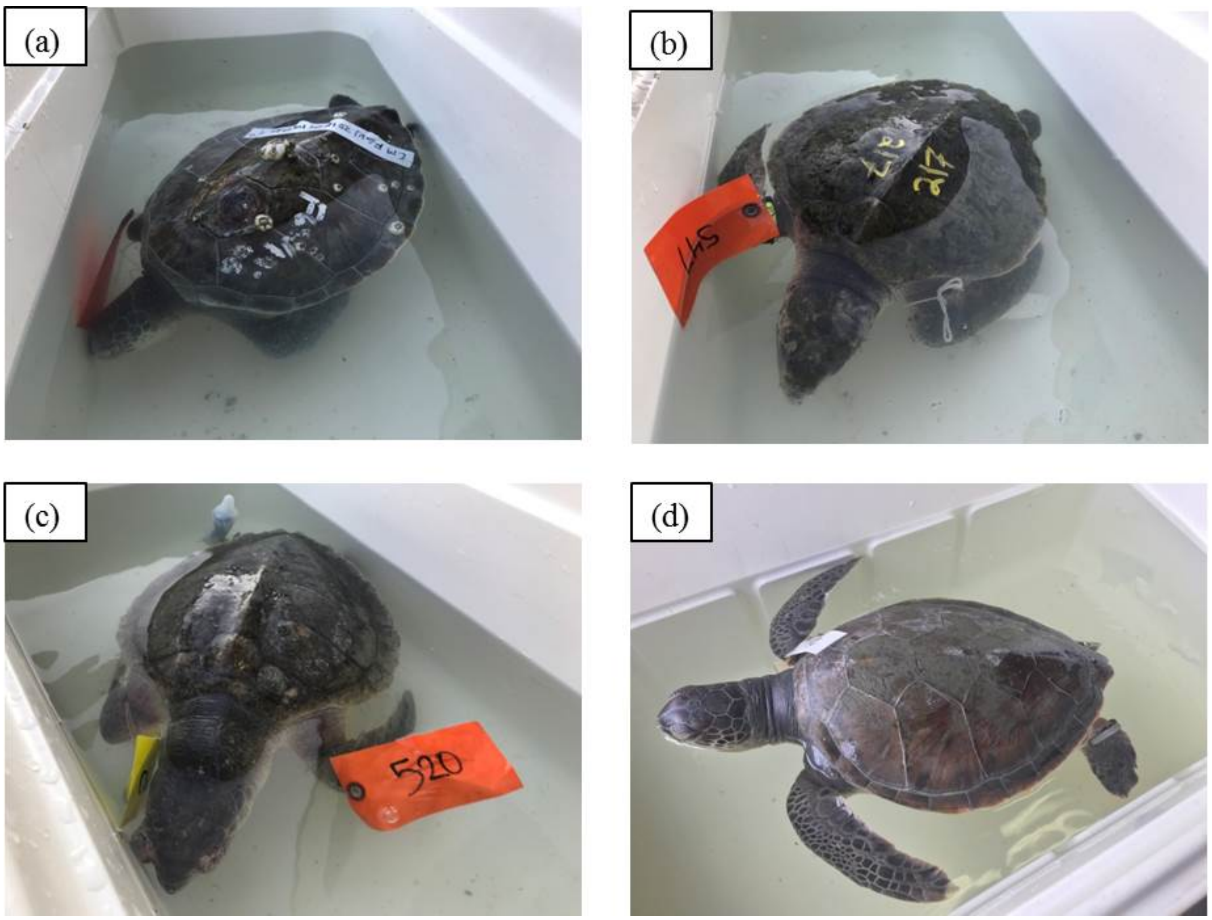

Figure 7. Sea turtle carcasses on the morning of a deployment (a) Green sea turtle carcass at condition code 2.1, 35% carapace exposed, head not exposed (b) Kemp's ridley carcass at condition code 2.1, 40% carapace exposed, head not exposed, listing left (c) Kemp's ridley carcass at condition code 2.2, 85% carapace exposed, head not exposed but neck bulging (d) Green sea turtle carcass at condition code 3.1, 100% carapace exposed, head fully exposed.

Results

Of the 182 carcasses used for this study, 80 sank when initially placed into the heated water bath. An additional 30 carcasses were initially floating at the surface but later sank to the bottom of the cooler within a few hours. The remaining 72 turtles were floating immediately after being thawed and remained floating over the course of preparations (Table 2). Accumulated degree hours were calculated for all sunken carcasses. This is the sum of temperatures a carcass experienced from initially being placed in the water bath to time of first float. Turtles that were initially floating had no degree hours as they were at the surface from the beginning. Although the sample size was larger, green sea turtle carcasses tended to float approximately 120–385 hours before the Kemp's ridley carcasses (Table 2; Figure 8). The majority of Kemp's ridley carcasses (53%) never sank, limiting the sample size for determining degree hours to float. An example of the temporal environment, behavior and actions of a green sea turtle carcass during preparation is presented in Figure 9 and Table 3.

Table 2. Summary of the number of carcasses that were bloated and floated and the accumulated degree hours by species.

Species	# of Carcasses	Average Accumulated Degree Hours	Lowest Accumulated Degree Hours	Highest Accumulated Degree Hours
Sank				
Green	59	1195	686	1880
Kemp's ridley	21	1544	1001	1802
Total	80	1286	686	1880
Later Sank				
Green	25	1020	441	1460
Kemp's ridley	6	1139	1000	1474
Total	31	1043	441	1474
Initially Floating				
Green	41	0	0	0
Kemp's ridley	30	0	0	0
Total	71	0	0	0

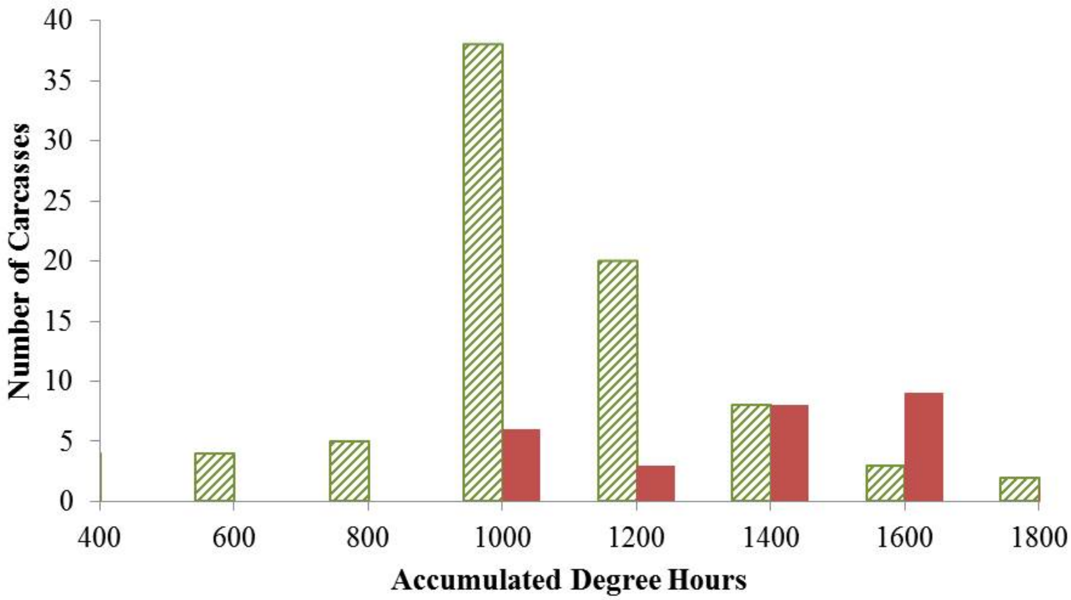

Figure 8. Summary of accumulated degree hours to first float for all carcasses that initially sank or later sank shortly after being placed in heated water baths. Green: green sea turtle carcasses (n = 84); Red: Kemp's ridley carcasses (n = 27).

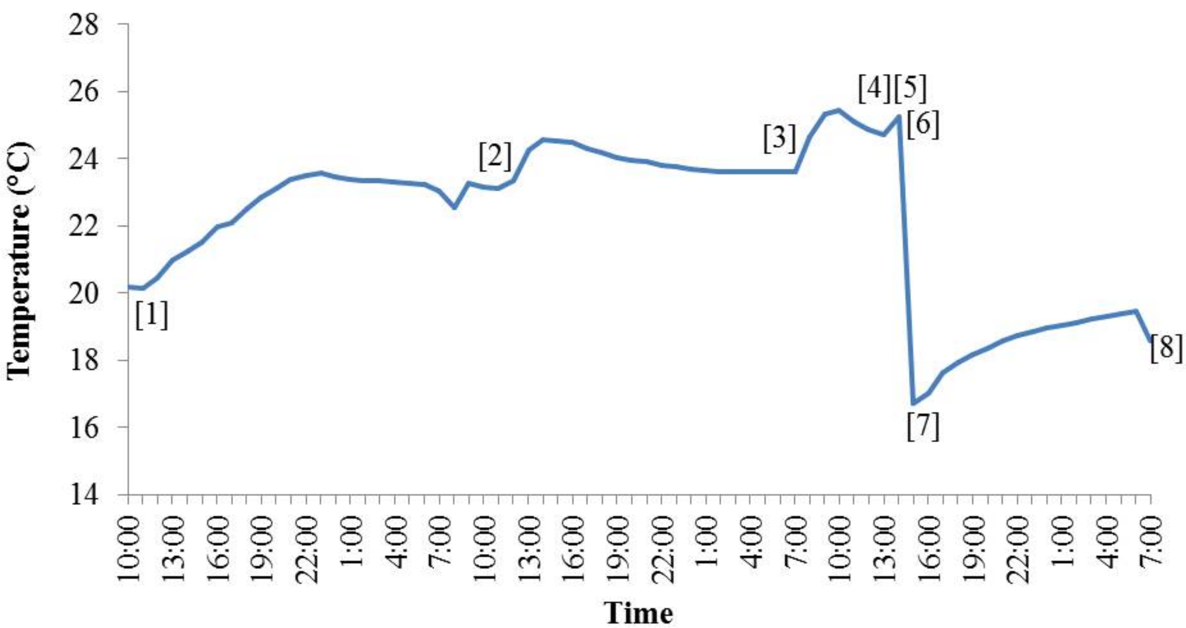

Figure 9. Temporal environment experienced by a green sea turtle carcass during preparation from 10/31/17 at 10:00AM to 11/3/17 at 7:00AM. See Table 3 for associated information.

Table 3. Observations of carcass behavior and actions performed during carcass preparation. See Figure 9 for associated graphical depiction.

Number from Graph	Date & Time	Carcass Behavior	Action Performed
1	10/31/17 10:00 AM	Sunk	Placed carcass in cooler set at 22°C
2	11/1/17 12:00 PM	Sunk	Increased water temperature to 24°C
3	11/2/17 7:30 AM	Sunk	Increased water temperature to 25°C
4	11/2/17 12:30 PM	Head buoyant only	Observation
5	11/2/17 2:00 PM	Carcass neutrally buoyant	Observation
6	11/2/17 3:30 PM	Floating	Observation
7	11/2/17 3:45 PM	Floating	Moved carcass to chilled cooler
8	11/3/17 7:00 AM	Floating	Removed carcass for deployment

Discussion

The purpose of this study was to document the preparation and effectiveness of using cold-stun sea turtle carcasses as a proxy for stranded sea turtles in research studies. Sea turtle cold-stun events occur annually throughout the U.S. and often result in a large number of fatalities within a very short period of time. Carcasses are typically in pristine condition, often classified as fresh dead, because many turtles strand alive and either die on the beach or at rehabilitation facilities. These mortalities provide an opportunity for researches to have access to a large number of fresh dead carcasses for use in research studies. This study determined that cold-stun turtles from different geographic regions make an excellent proxy for nGOM stranded sea turtles because they are often of similar size and species, and mimic drifting and decomposition rates of sea turtles killed at-sea.

When a sea turtle dies in the wild from an acute mortality event such as a vessel strike or forced submergence, it typically sinks to the sea floor before decomposition generates enough gases to float the turtle (Epperly et al. 1996). In addition, sea turtles that succumb to an illness or trauma while in rehabilitation are generally found on the bottom of their tanks (personal communication Lyndsey Howell, NOAA Fisheries). The cold-stunned carcasses used for this study were bloated and floated in a way that was similar to the natural progression observed after mortality occurs; however 39% of cold-stunned carcasses in our study never sank. It has been observed that sea turtles respond to cold-stuns by becoming buoyant and floating at the surface (Schwartz, 1978). Additionally, when cold-stunned turtles are recovered from the water by stranding responders, they are usually floating at the surface so it was not unusual to observe a high percent of our carcasses floating. Researchers should keep this observation in mind if planning to use cold-stunned sea turtle carcasses for research studies.

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Appendix A. Sea turtle condition codes and percent exposure guidelines.

Sea Turtle Condition Codes for Carcass Drift Study

Fresh dead (1): Carcass typically not floating, may have rigor mortis, eyes should be clear, no evidence of bloating, no odor.

***Early moderately decomposed (2.1):** Carcass barely floating, less than 50% of carapace exposed above the waterline, head typically below waterline, mild bloat, very little decomposition of skin, little to mild smell.

***Late moderately decomposed (2.2):** Carcass floating well, 50% to <90% of carapace exposed above the waterline, head partially exposed, moderate bloat, bulging eyes, scutes and skin may be beginning to slough, moderate smell.

Early Severely decomposed (3.1): Carcass reaches maximum floatation, 90-100 % of carapace exposed above the waterline, head and neck severely distended and visible above waterline, mouth often open, severe bloat, skin on head and limbs tight, possibly a few loose scutes, carcass has not de-gassed and all structures are intact, strong smell.

Mid Severely decomposed (3.2): De-gassing begins and carcass begins to sink, less than 90% of carapace exposed above the waterline, head and neck limp and beginning to sink, post severe bloat, scutes and skin sloughing and disarticulation beginning, skin and limbs are becoming loose, foul smell.

Late severely decomposed (3.3): Carcass sank, or if floating sinks when disturbed or flipped over due to holes in the body cavity, carcass is completely degassed, severe decomposition and sloughing of scutes and skin, disarticulation of joints and carapace, foul smell.

Dried Carcass (4): Completely desiccated, only dry skin and bones, little to no smell.

Bones (5): Carcass has decomposed to bones, no soft tissue remaining, little to no smell.

*Target condition codes of carcasses used for deployment

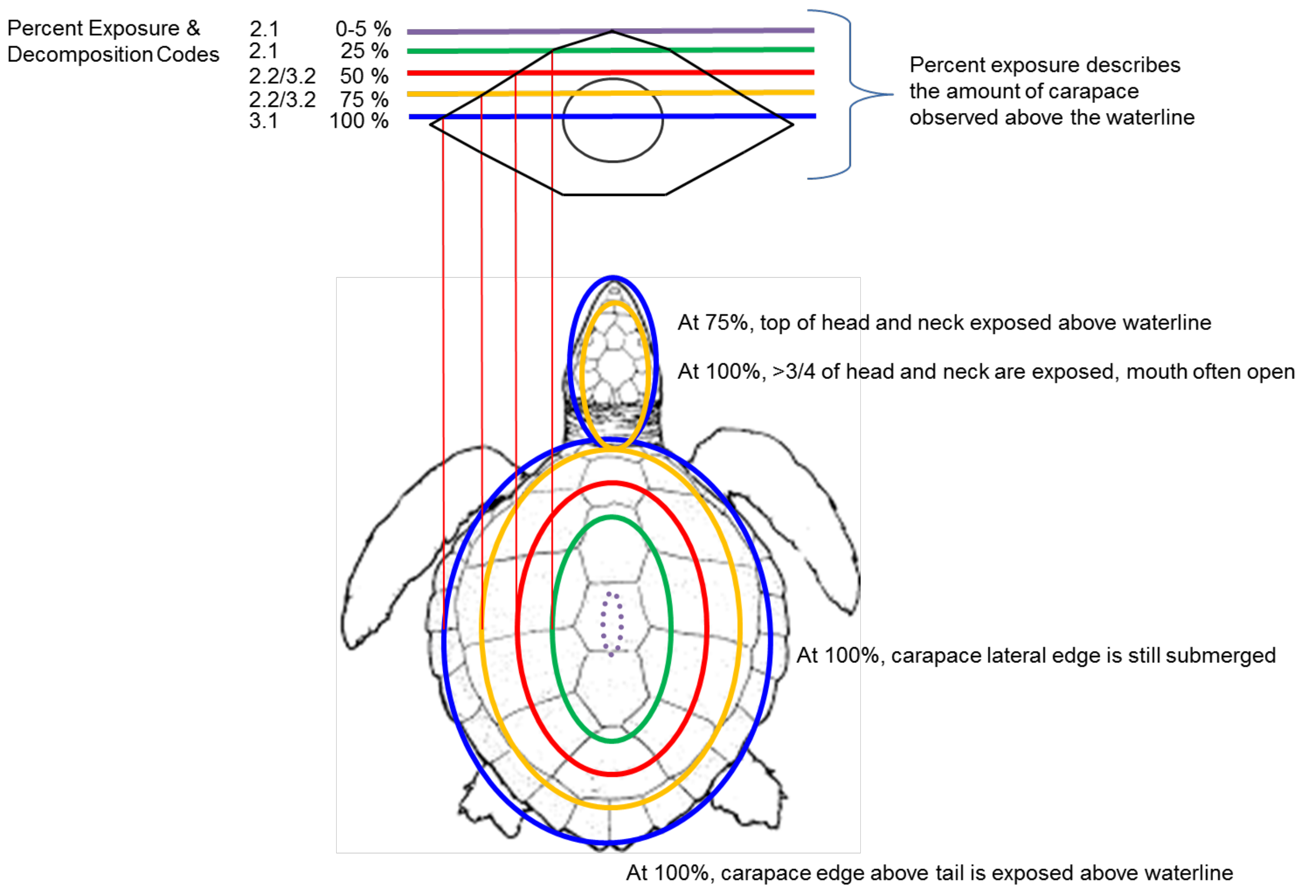

Figure 1. Sea turtle carcass percent exposure and associated decomposition codes.