INVENTORY OF PACIFIC SAMPLES STORED FOR BIOMONITORING BY THE NIST BIOLOGICAL AND ENVIRONMENTAL MONITORING AND ASSESSMENT OF SEA TURTLE TISSUES (BEMAST) PROJECT FROM 2011-2025*

Rachel M. Nakamoto[1], Jennifer M. Lynch[1], Katherine R. Shaw[2]

[1]Hawai'i Pacific University | Center for Marine Debris Research
[2]National Institute of Standards and Technology

The National Institute of Standards and Technology (NIST) began the Biological and Environmental Monitoring and Archival of Sea Turtle Tissues (BEMAST) project in 2011 as a long-term pollution trend monitoring project for sea turtles in the Pacific Ocean. Dedicated annual sample collections paused indefinitely in early 2025. This presentation will summarize the sample inventory from sea turtles that had stranded in Hawaii or were caught in the U.S.-based longline fishery in pelagic waters of the Pacific Ocean. Five out of the seven sea turtle species are represented. Thousands of tissue samples are curated and maintained, including ingested plastic, ingested prey, digestive fluids, scute, fat, liver, bile, follicles, blood, muscle, fibropapilloma lesions, blubber, and skin. These tissues were collected using standardized protocols to ensure minimal contamination of samples and are stored at liquid nitrogen vapor temperatures (~150°C) to preserve biological components and chemical contaminants for future analysis. Aliquots of cryo-homogenized tissues can be accessed through NIST for approved research projects related to pollution or health monitoring. This presentation will provide procedures for requesting aliquots, highlights from completed projects with the samples, and a complete list of sample providers and collaborators. Collaborations and funding are vital to the continuation of assessing sea turtle health and contaminants.

HEART RATE REDUCTION DURING EGG-LAYING IN LOGGERHEAD TURTLES REVEALED BY NON-INVASIVE MONITORING*

Tomoko Narazaki[1], Masanori Mori[2], Yoshimasa Matsuzawa[3], Ayaka Saito[4,5], Chihiro Kinoshita[1], Masanori Kurita[2], Kensuke Matsumiya[3], Hikari Okada[1], Kentaro Q. Sakamoto[4]

[1]Meijo University, Japan
[2]Port of Nagoya Public Aquarium, Nagoya Port Foundation, Japan
[3]Sea Turtle Association of Japan, Japan
[4]The University of Tokyo, Japan
[5]National Institute of Polar Research, Japan

Animals undergo significant physiological demands during reproductive processes, often accompanied by hormonal and neural changes. In sea turtles, nesting occurs on land, where they must support their own body weight without the buoyant assistance of seawater, potentially adding significant physical stress. Heart rate is an important physiological parameter reflecting an animal's internal state. To monitor changes in heart rate throughout nesting activities, non-invasive electrocardiogram (ECG) loggers (ECG400-DT, Little Leonardo Ltd.) and accelerometers (W2000-3MPD3GT, Little Leonardo Ltd.) were deployed on five gravid loggerhead turtles (*Caretta caretta*): two captive turtles nesting on an artificial beach at the Port of Nagoya Public Aquarium and three wild turtles nesting on a natural beach in Minabe, Japan. A set of loggers and electrode pads were attached to the carapace one or two nights prior to egg-laying, with the attachment procedure taking up to 1 hour. After deployment, all turtles promptly returned to the water. After egg-laying was completed, all instruments were retrieved either immediately before return to the sea

(wild turtles) or on the following morning (captive turtles). Upon landing on the beach, heart rate increased to 29.3 ± 5.5 bpm, approximately 1.5 times higher than just before landing, and remained elevated during most of the nesting phases. However, at the onset of egg-laying, heart rate significantly decreased to 16.9 ± 5.0 bpm. This bradycardia was accompanied by increased variability in heart rate intervals and elevated RMSSD (root mean square of successive differences), indicating dominance of parasympathetic nervous activity. Heart rate returned to elevated levels after egg-laying, suggesting a shift in autonomic balance. Our results demonstrate that nesting turtles maintain high heart rates during physically demanding terrestrial activities, except during the egg-laying phase, when parasympathetic regulation likely facilitates successful oviposition. External disturbances, such as artificial light, may disrupt parasympathetic regulation, potentially reducing egg-laying success.

HANDLING PROTOCOLS IN STRAGGLER SEA TURTLE HATCHLINGS: TRADITIONAL BLOOD ANALYSIS AND METABOLOMICS AS TOOLS FOR ASSESSING BEST CONSERVATION MANAGEMENT PRACTICES*

Theresa Sophie Nehring[1,2]

[1]*Universidade do Algarve*
[2]*Loggerhead Marinelife Center*

Sea turtle hatchlings are particularly vulnerable during their early life stages, with straggler hatchlings, those that remain in nests after the mass emergence, requiring temporary holding before nighttime release under current Florida Fish and Wildlife Conservation Commission (FWC) protocols. This study presents a comprehensive assessment of physiological and metabolic responses in sea turtle straggler hatchlings during mandatory holding periods of 5–12 hours, using a complementary multi-species approach. In green turtle hatchlings (*Chelonia mydas*; n=30 Experimental, n=20 Validation), morphometric measurements and blood analytes (including blood chemistry and hematology) were evaluated, with corticosterone analyzed in a subset of individuals (n=18 Experimental, n=11 Validation). Untargeted metabolomic profiling using ultra-high-performance liquid chromatography–high-resolution mass spectrometry (UHPLC-HRMS) was conducted for loggerhead hatchlings (*Caretta caretta*; n=9 Experimental, n=7 Validation). Hatchlings sampled both on intake and release (Experimental group) were compared to single-sampled controls (Validation group; sampled on release only) to distinguish holding effects from potential sampling artifacts. Hatchlings demonstrated measurable physiological responses during holding, including mass loss, declining body condition index, and metabolic changes. Blood chemistry revealed increased β-hydroxybutyrate concentrations, indicating lipid mobilization from residual yolk via ketogenic pathways, and declining lactate, suggesting recovery from emergence stress through lactate clearance and transition to aerobic metabolism. Hematologic analyses showed increased heterophil counts and heterophil:lymphocyte ratios exceeding reference intervals, indicating stress- and/or inflammation-mediated immune responses. Clinical assessment at intake further showed that baseline clinical condition significantly influenced physiological responses to holding: compromised hatchlings had higher heterophil and lymphocyte counts. Corticosterone concentrations remained stable and within ranges indicative of moderate stress. Metabolomic analysis identified significantly altered metabolites in loggerheads, with decreases in pseudouridine, β-pseudouridine, and lactic acid, alongside increases in 4-hydroxybenzaldehyde and 4-acetamidobutyrate, reflecting targeted biochemical adjustments rather than systemic disruption. The selective nature of these responses aligned with findings from traditional biomarkers, demonstrating activated adaptive and compensatory mechanisms across both species. Overall, hatchlings demonstrated physiological tolerance to short-term holding through physiologically adaptive mechanisms, while experiencing measurable energetic costs. This study validates current FWC protocols requiring nighttime release for hatchlings collected after 0900 AM and provides an evidence-based

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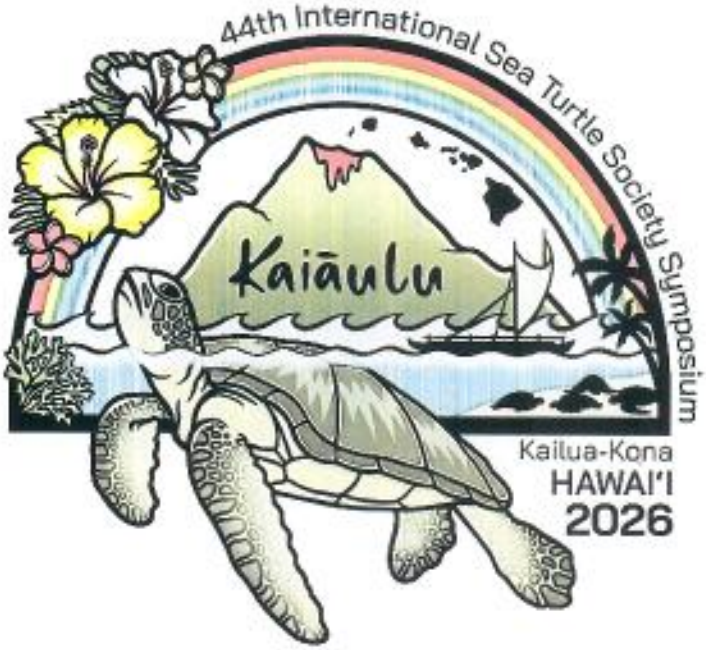

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