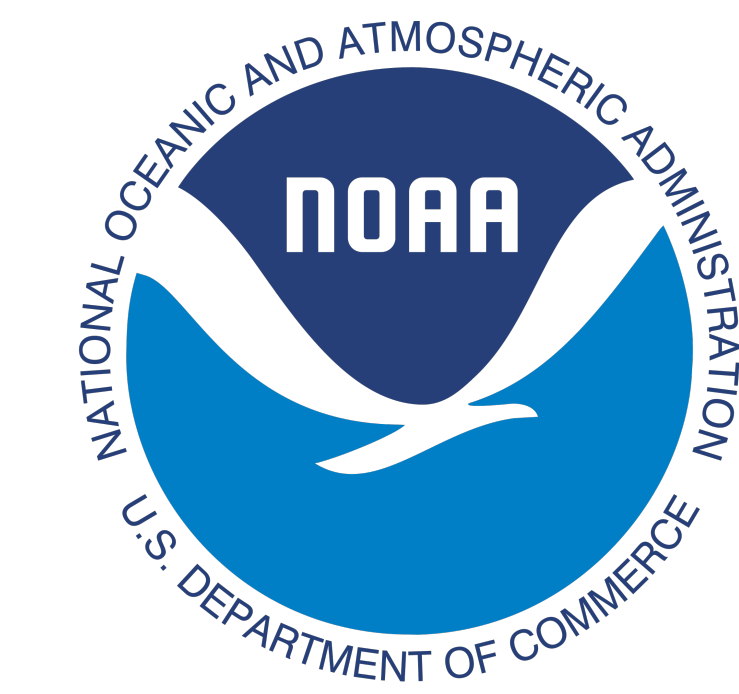




Quantifying Relatedness of Green Sea Turtles in the Hawaiian Islands

Mikayla Newbrey^{1*}, Jamie Adkins¹, Lisa Komoroske¹

¹ Department of Environmental Conservation, University of Massachusetts Amherst MA, United States,
Contact Information: mnewbrey@umass.edu



UMassAmherst

College of Natural Sciences
Center for Agriculture, Food,
and the Environment

Background Information

The Hawaiian green sea turtle (*Chelonia mydas*), listed as a threatened species under the Endangered Species Act, faces risks from climate change due to its temperature-based sex determination (TSD) and nesting behaviors. TSD is the process by which the sex of sea turtle hatchlings is determined, where nest conditions during incubation influence hatchling biology, including sex ratios. Since temperature variations alter sex ratios, assessing the population's genetic makeup is essential for future conservation efforts. Studying relatedness among adults on the same nesting beach in a season informs inbreeding risks that can harm genetic diversity and the population's overall health. This pilot investigation will provide insights into the Hawaiian sea turtles' genetic relationships amidst changing environmental conditions.

Research Questions

1. Can we determine the relatedness of adult turtles utilizing the same nesting beach in one season?
2. Can we correctly assign offspring to known mothers?



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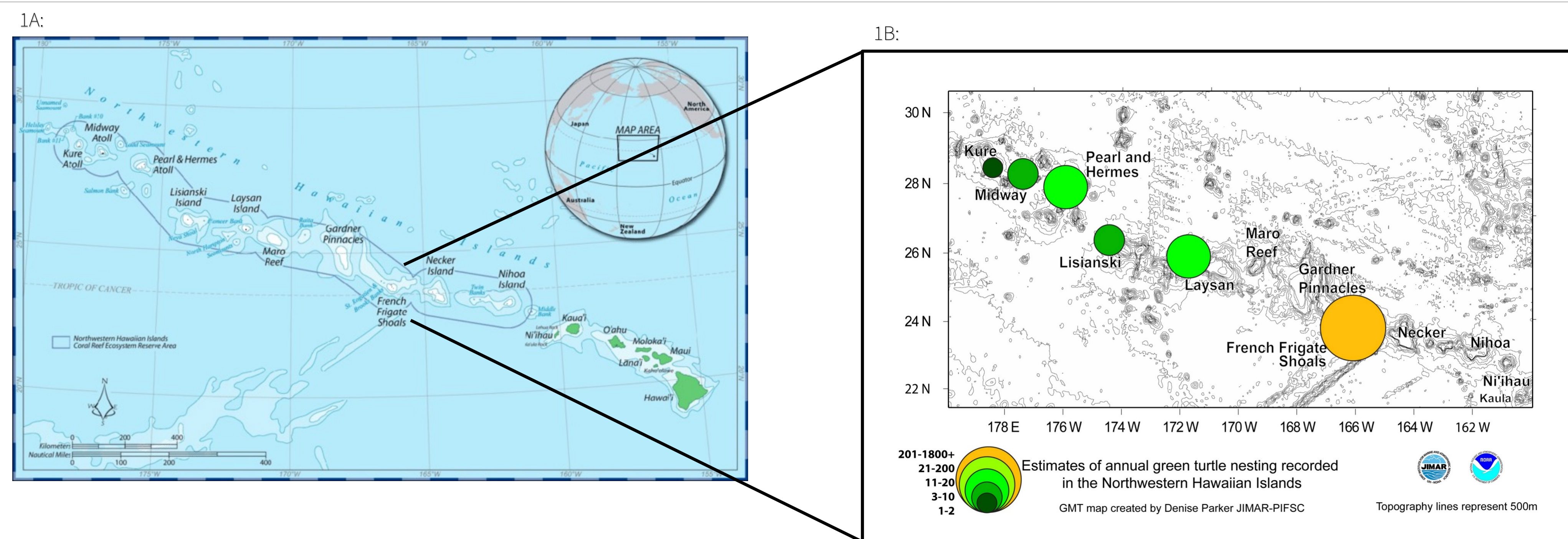


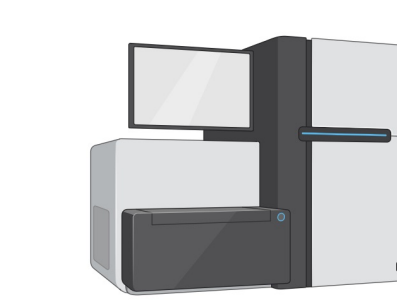
Figure 1:
A: A geographical representation of the Hawaiian islands¹.
B: A visual representation illustrating the estimated distribution of nesting sites for green sea turtles. The color gradient of each point corresponds to the estimated number of female nesters in the region of French Frigate Shoals, the collection area of the samples. Over 90% of the population's nesting activity is concentrated at the French Frigate Shoals location².

Methodology



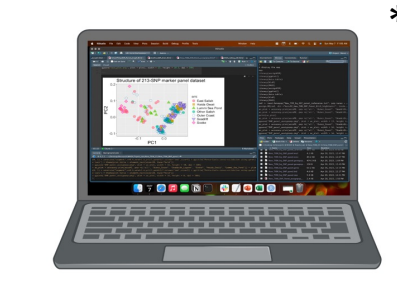
Sampling & Extraction

- Skin tissue samples were collected from the primary nesting habitat in French Frigate Shoals in one nesting season.
- DNA extraction was performed on the samples using a NucleoMag bead isolation protocol.
- DNA quantification was conducted using both the Qubit fluorometer and the Agilent Fragment Analyzer.



Library Design

- Two RAD libraries were created, utilizing 150 base pair end reads.
- The first library consisted of 96 samples, encompassing both adult and juvenile individuals collected from various locations across the Hawaiian Islands.
- The second library comprised an additional 96 samples, specifically including mother-offspring pairs for conducting parentage assignment tests.



Data Analysis

- Employed an existing custom script³ for demultiplexing the genomic reads.
- Generated summary statistics of sequenced data to quality check raw data
- Follow the proposed data analysis pipeline

Results

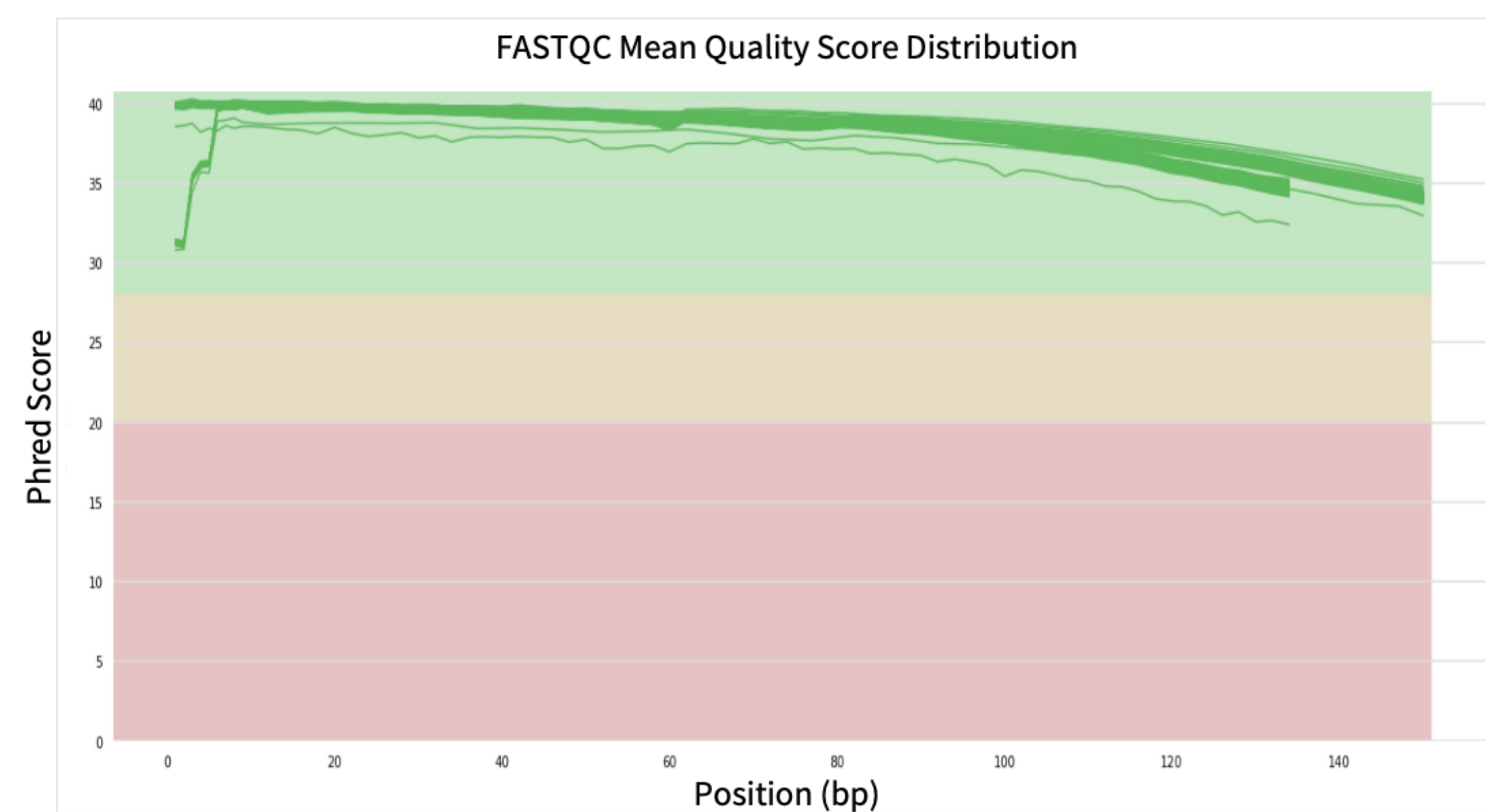


Figure 2: Mean Quality Score Distribution plot⁴ illustrating the average Phred quality scores for each base position across all reads in the sequencing dataset. The x-axis represents the position in base pairs along the sequenced reads. The y-axis represents the Phred quality scores, which indicate logarithmic probability of base call accuracy.

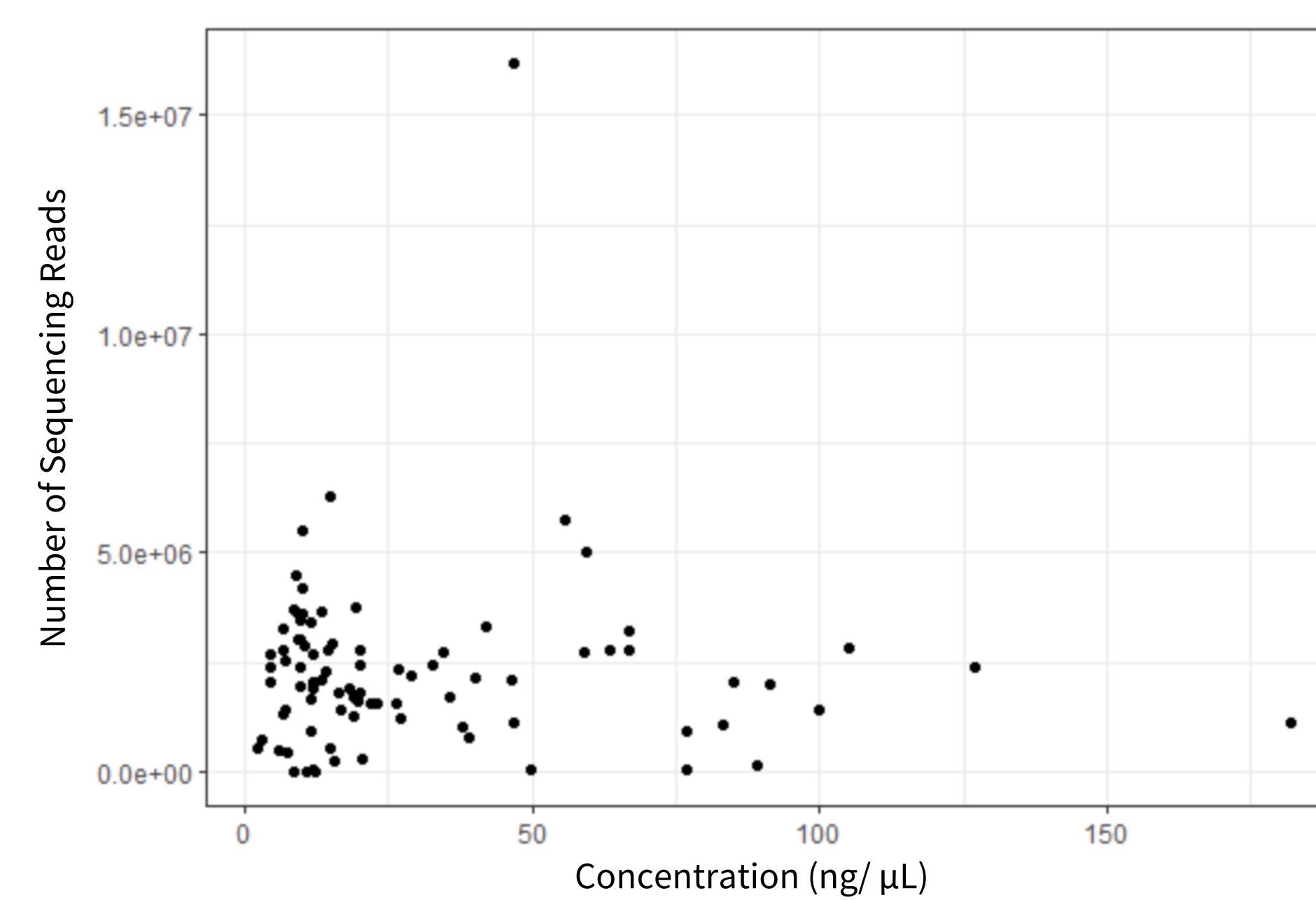
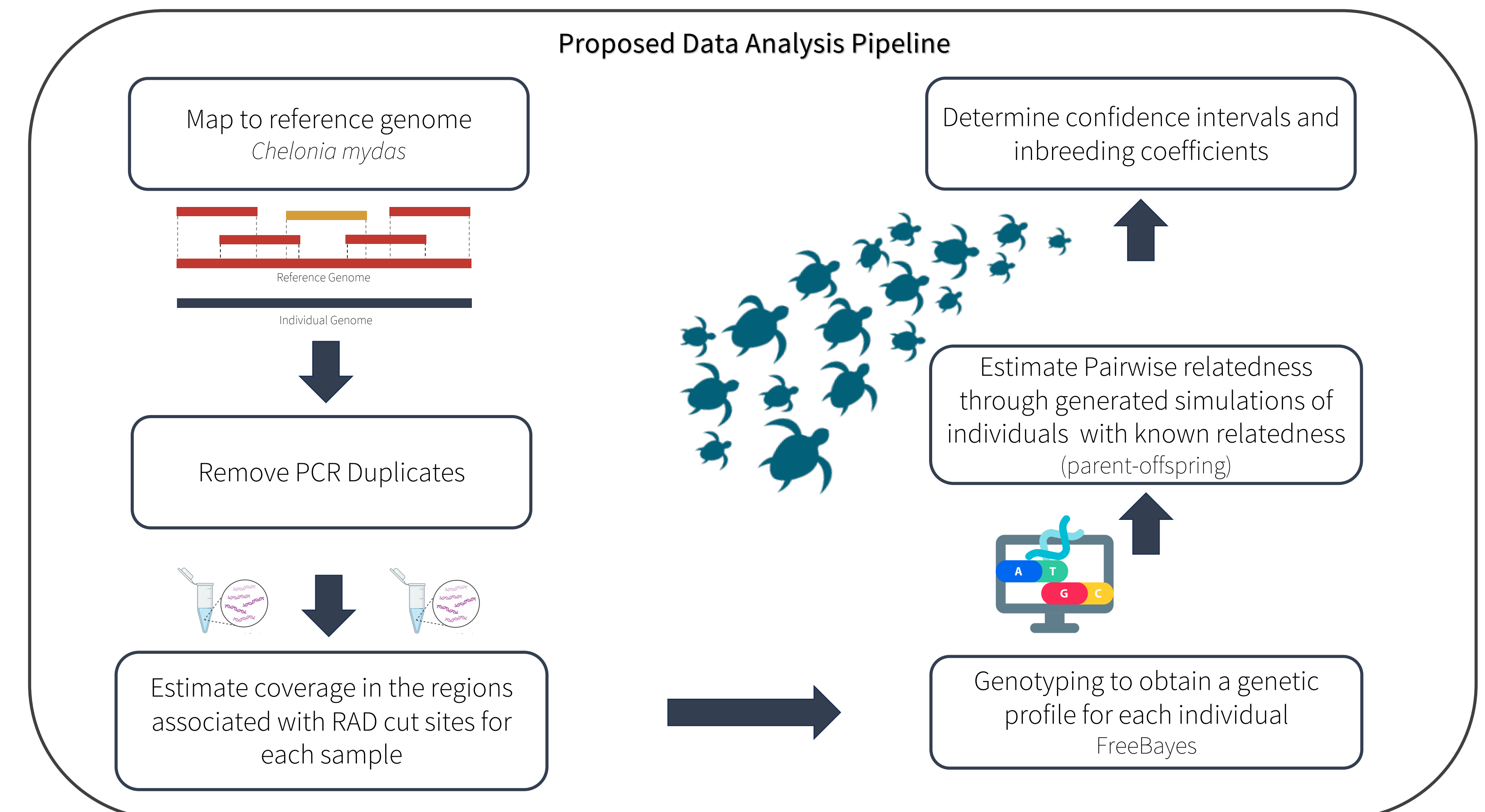


Figure 3: A scatterplot depicting the quality of raw RAD-Seq data. The x-axis represents a range of input DNA concentrations, measured in ng/μL. The y-axis displays the number of sequencing reads generated for the corresponding input concentration. There is no discernible pattern in the number of sequencing reads across the various input concentrations.

Proposed Data Analysis Pipeline



References

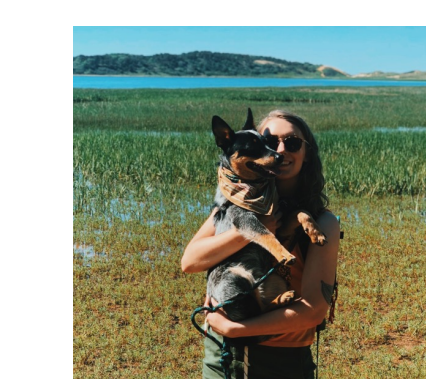
1. NOAA. (2015). National Coral Reef Monitoring Program Socioeconomic Monitoring Component Summary Findings for Hawai'i, 2015. NOAA Technical Memorandum CRCP 30. doi:10.7289/57TM-CRCP-30. (National Oceanic and Atmospheric Administration)
2. Balazs, G. H., et al. (2015). Map guide to marine turtle nesting and basking in the Hawaiian Islands. NOAA-PIFSC Marine Turtle Research Program.
3. Reference-guided RAD analysis workflow, Jamie Adkins, 2021. <https://hackmd.io/@jastott/155i4T1>
4. MultiQC: 10.1093/bioinformatics/btw354

Next Steps

We will map the sequenced reads onto the *Chelonia mydas* reference genome. Any PCR duplicates in the aligned reads will be removed, ensuring read depths are accurate and alleles are not overrepresented. The data will be prepared for input into the *related* R package, with a focus on quantifying the relatedness levels among adult individuals nesting in the same area and validating parent-offspring assignments. We hope that these data will also benefit the conservation management teams that we work with by providing information for ongoing population viability analyses.

Acknowledgements

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About the Presenter

Mikayla is a first-generation college student currently studying biology with a focus on genetics and ecology at the University of Massachusetts Amherst. She works as an undergraduate researcher in Dr. Lisa Komoroske's Molecular Ecology Lab, where she specializes in using molecular techniques to better understand local adaptation in response to climate change. Prior to her enrollment at UMass Amherst, Mikayla earned her associates degree at Cape Cod Community College where she completed two internships at the Woods Hole Oceanographic Institution.

