# Assessing the Use of MinION Nanopore DNA Sequencing in Obtaining High Quality Data from Zebrafish to Inform Round Scad Fish Epigenetics under Global Climate Stress

Ashley Felber, Sophie Altman, Mark Moroz, Molly O'Brien, Bryan DiLeo, Kristen Eichlinger, Dr. Alice Tarun, and Dr. Salvador Tarun Jr.

*Department of Chemistry, State University of New York at Geneseo*

*1 College Circle, Geneseo, NY 14454*

## Introduction

***Abstract: The issue of climate change has been gaining increased awareness and attention globally in recent years. It is having various impacts to ecosystems all over the world, causing many species to become environmentally stressed. Epigenetics is a concept that is being studied more prevalently regarding climate changes. Due to the changing environment, stress-induced heritable traits may appear without changes to the genomic code, known as epigenetic alterations. One such epigenetic alteration is DNA methylation, which occurs in cellular responses to environmental stress. One major source of affordable protein in the Philippines comes from the wild Round Scad fish, which has recently been facing rapid decline in both its population and body size. The purpose of our study is to explore the patterns of DNA methylation in wild Round Scad to determine whether these changes are associated with an epigenetic response to global climate stress. Samples of Round Scad DNA were collected and isolated from the Philippines. Using nanopore MinION, a portable third generation DNA sequencing technology, we are able to obtain high quality DNA sequences required for detection of methylation sites. However the DNA sequences are short, needing improvement. To facilitate our analysis we are sequencing the genome of the Zebrafish for comparison. Here, we shall report on the initial data collected. We anticipate that long term findings from this project will provide critical information to manage wild Round Scad and other marine fish facing similar environmental stressors.***

### Significance

We anticipate that long term findings on the epigenetics of Round Scad under global climate stress will provide information critical to managing this economically important species and other marine fish facing similar environmental stressors.

### Long-term Research Question

Does global climate stress cause an epigenetic response by altering the pattern of DNA methylation in wild Round Scad?

## Methods

In continuation from last year (2023), we used similar methods to extract, purify, isolate, and sequence DNA. However, we are using zebrafish instead of wild Round Scad samples in order to compare the output qualities.

Various tissue samples from zebrafish were extracted in order to obtain DNA samples. The highest quality sample underwent further processing and analysis, as seen in the steps below.

Zebrafish DNA sample was purified and isolated → Isolated DNA sample was quantified using Nanodrop → DNA library was prepped and Nanopore MinION was used to sequence the DNA

Nanopore MinKNOW was used to basecall the sequenced zebrafish DNA under the *super accuracy* quality threshold. Data output was then compared to the Round Scad basecalling output from last year under the *high accuracy* quality threshold.

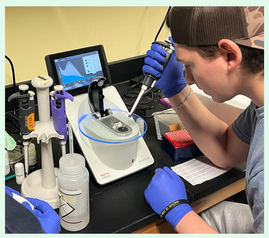

**Fig 1.** Portable Nanopore MinION device used for DNA sequencing (University of Oxford, 2015).

## Results

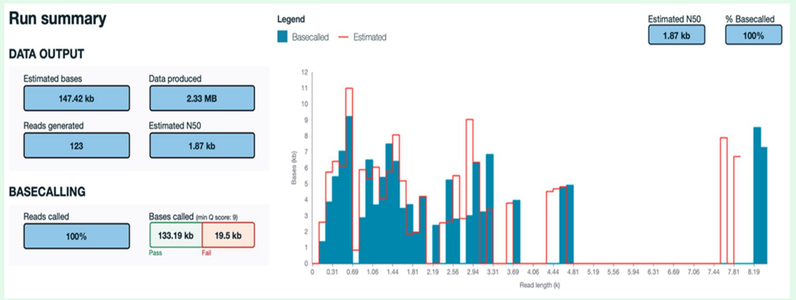


**Fig 2.** Output summary from wild Round Scad DNA sample generated last year (2023) using *high accuracy* basecalling. The high accuracy mode classifies the data as pass or fail based on a quality scoring parameter used by Nanopore. Size distribution of the DNA sequencing data is shown on the right.

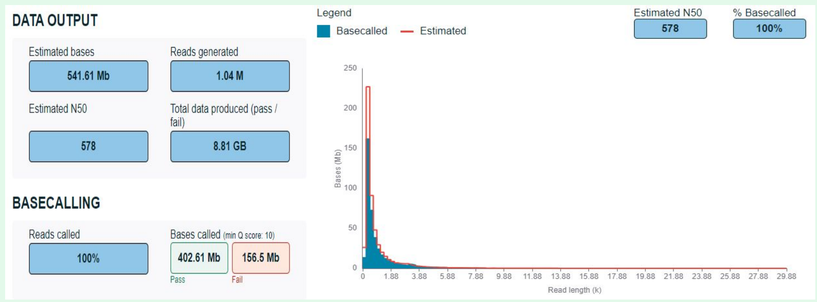


**Fig 3.** Output summary from zebrafish DNA sample using *super accuracy* basecalling. The super accuracy mode classifies the data as pass or fail based on a q-score of 10, which is the quality scoring parameter used by Nanopore. Size distribution of the DNA sequencing data is shown on right.

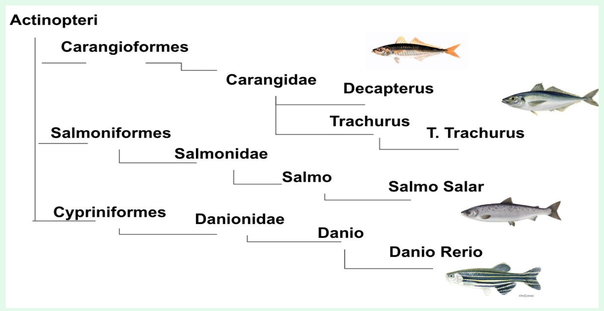


**Fig 4.** Phylogenetic tree showing genetic overlap between our Round Scad (*Decapterus*) DNA samples and three significant species. The common names for *T. trachurus, Salmo salar,* and *Danio rerio* are Atlantic horse mackerel, Atlantic salmon, and zebrafish, respectively.

## Discussion

- Zebrafish DNA was successfully sequenced using MinION
- Super accuracy basecalling successfully generated high-quality data outputs, which can be used to analyze DNA methylation patterns (Fig 3)
- A 3,022.8-fold increase in data was observed from last year's sequencing (Fig 2), with more consistent segment sizes

## Limitations and Future Work

- The sequenced Zebrafish DNA was not analyzed against a known Zebrafish genome standard due to software difficulties.
- Most high-quality sequences are short; a low N50 value indicates the data consists of many short DNA fragments (Fig 3).
- Only one basecalling method was used (super accuracy); in the future, using all three basecalling quality thresholds (fast, high accuracy, and super accuracy) will allow for more data to be analyzed, using only the high-quality outputs for DNA methylation analysis.
- Our next step is to use the Nanopore software to collect DNA methylation data on our sequenced sample (Fig 5). In the future, we also hope to apply these methods to our target fish species, wild Round Scad, in order to identify methylation patterns and any candidate genes that are under global climate stress.

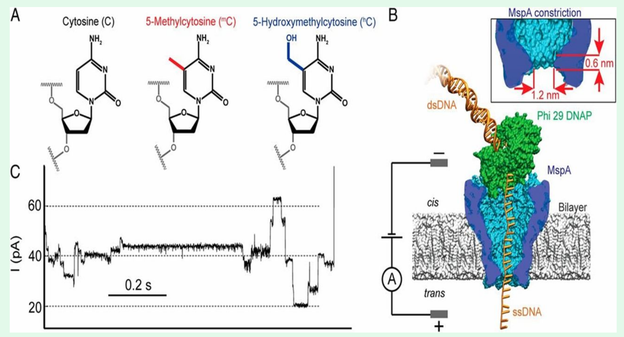


**Fig 5.** Methylcytosine and hydroxymethylcytosine detection on DNA bases with Nanopore sequencing (Laszlo et al., 2013).

## *Acknowledgements*

We want to thank the SUNY Geneseo Foundation and Geneseo Student Association for the **Sorrell Chesin '58 Award** and the **TRAC Grant** providing us funding for our research. We are grateful to **Dr. Salvador Tarun Jr.** and **Dr. Alice Tarun** for their assistance and mentoring for this research study.

## References

1. Laszlo, A. H. et al. Proceedings of the National Academy of Sciences (PNAS) (2013).
2. Mini DNA sequencer's data belies its size. University of Oxford (University of Oxford, 2015).

GENESEO
Department of Chemistry