Leveraging 3rd Generation DNA Sequencing Technology to Explore the Role of Epigenetics in Round Scad Fish under Global Climate Stress

Ashley Felber, Owen Frank, Sophia Altman, Micah Ford, Dr. Alice Tarun* and Dr. Salvador Tarun Jr.
Department of Chemistry, State University of New York at Geneseo
*1 College Circle, Geneseo, NY 14454; * University at Buffalo, NY 14261*

Introduction

Abstract: ***Global climate change has been a recent pressing issue that has been seen to have environmental impacts on various ecosystems. Such environmental changes induce stress-related heritable traits without changes to genome coding, a concept known as epigenetics. DNA methylation plays a key role in these cellular responses to environmental stress. The Round Scad fish (Decapterus macarellus) is an affordable source of protein for common citizens in the Philippines, but is currently facing rapid decline both in population and average body size. The purpose of our study is to obtain genome sequence data for Round Scad fish and determine its phylogenetic relationship with other fish species. This data is critical for our long-term goal of exploring the patterns of DNA methylation in wild Round Scad to determine whether these changes are associated with epigenetic response to stress due to global climate change. Samples of fish DNA from the Philippines were collected and isolated. Using Nanopore MinION, a portable third generation DNA sequencing technology, we are able to obtain initial DNA sequences allowing us to identify a novel phylogenetic relationship between Round Scad and Atlantic Horse Mackerel. The high quality base-calling genomic data we are building will be used in the future to identify genes that are under global climate stress by extracting back DNA methylation patterns directly from these original data using MinION analytical pipelines.***

Significance

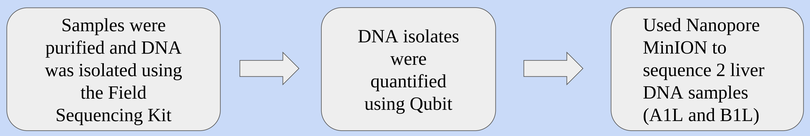
We anticipate that long term findings on the epigenetics of Round Scad under global climate stress will provide information critical to managing this economically important species and other marine fish facing similar environmental stressors. The portability of the MinION technology also affords its use directly in the field.

Long-term Research Question

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

Methods

Various body tissue samples were collected from wild Round Scad in the Philippines. Samples underwent further processing and analysis. Here, we used isolated genomic DNA from liver tissues of 2 fish samples (A1L and B1L).



The NCBI database, BLAST, was used to identify species with similar DNA sequences. A targeted BLAST was performed against Zebrafish and Atlantic Salmon to identify common genes.

Fig 1. Portable Nanopore MinION device used to obtain DNA sequences (University of Oxford, 2015).

Results

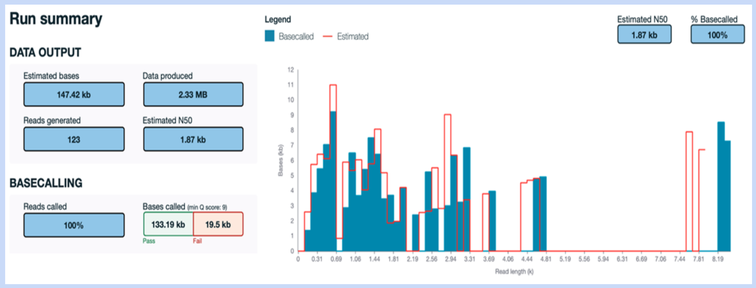


Fig 2. Output summary from A1L DNA sample using high accuracy basecalling. The high accuracy mode classifies the data as pass or fail based on a scoring parameter used by Nanopore. Size distribution of the A1L DNA sequencing data is shown above.

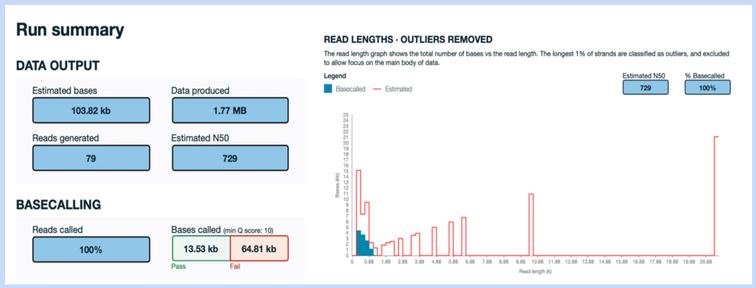


Fig 3. Output summary from B1L DNA sample using high accuracy basecalling. The high accuracy mode classifies the data as pass or fail based on a scoring parameter used by Nanopore. Size distribution of the B1L DNA sequencing data is shown above.

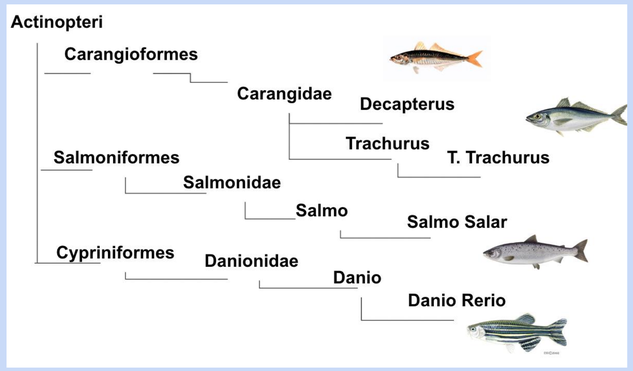


Fig 4. Phylogenetic tree showing genetic overlap between our Round Scad (*Decapterus*) DNA samples and three significant species. Common names for *T. trachurus, Salmo salar, and Danio rerio* are Atlantic horse mackerel, Atlantic salmon, and zebrafish, respectively.

Table 1. BLAST data summary identifying the most prevalent species match to A1L and B1L DNA sequences and largest common genes identified during the targeted BLAST.

Sample	Total Number of Base Sequences	Number of Sequences Matching *Trachurus Trachurus*	Matching Genes in Atlantic Salmon (% identity, e-value)	Matching Genes in Zebrafish (% identity, e-value)
A1L	102	82	LOC106601437 (glutamate receptor ionotropic, NMDA 2C-like isoform X2) (80%, 0.012) LOC106589633 (phospholipid phosphatase-related protein type 5-like) (85%, 1E-21)	Intergenic region of chromosome 7 (96%, 3E-77) Intergenic region of strain T5D chromosome 8 (92%, 4E-57)
B1L	22	14	COX3, ND3 (76%, 0.0)	COX3, ATP6, ND3 (74%, 0.0)

Discussion

- We successfully utilized Nanopore MinION to obtain initial sequencing data for Round Scad (Fig 2, 3). Both A1L and B1L samples were sequenced. With there being no previous genomic sequencing data for Round Scad, our data serves as a novel source for phylogenetic analysis.
- Initial alignment found Round Scad to match highly to *T. trachurus* (Atlantic Horse Mackerel).

Limitations / Future work

- Rapid Field Sequencing Kit gave low DNA yield. In the future, we will use a higher yield kit to obtain more purified DNA product. We originally chose the rapid kit because it was more time efficient and we wanted to show that it can be used in the field to obtain isolates, if necessary.
- The next step is to use the Nanopore software to collect DNA methylation data (Fig 5) on our sequenced DNA samples in order to identify candidate genes that are under any global climate stress

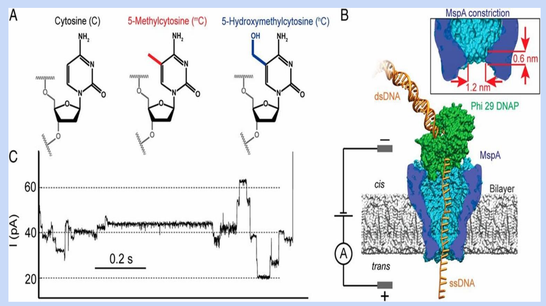


Fig 5. Methylcytosine and hydroxymethylcytosine detection with Nanopore sequencing (Laszlo et al., 2013).

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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).

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Department of Chemistry