# Creating a Molecular Aging Method for Brook Trout (*Salvelinus fontinalis*) with Methylation Data

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## Introduction

Age is a crucial parameter for fisheries management and conservation. The primary method of aging fish requires counting individual layers of calcareous deposition on otoliths.[1]

**Aging data is used for:[1]**
- Constructing age classes
- Growth, mortality, and fecundity
- Estimating population size
- Setting catch limits

**Drawbacks of Current Aging Methods:[2]**
- Time consuming
- Expensive
- Fatal for fish
- Prone to error

**A new method of aging is required that is cheaper, faster, non-fatal, and more accurate/precise.**

**Methylation:** A type of epigenetic mark which involves the addition of a methyl group to a cytosine base, or CpG site.[1]

## Objective

The goal of the project is to construct a clock that successfully ages brook trout (*Salvelinus fontinalis*) using machine learning to identify age associated methylated sites.

Figure 1: Image of Brook trout

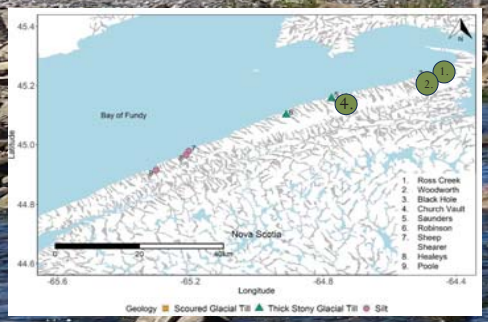


Figure 2: Map of North Mountain region of Nova Scotia and sampling locations.

## Sampling/ Study Site

27 brook trout were sampled with backpack electrofishing in three streams in the north mountain region of Nova Scotia. The length, fin clips, scales, and otoliths were retrieved from each fish. The fish were aged with both scales and otoliths. The brook trout in the study system are short-lived and have a three-year lifespan. Brook trout are a member of the salmonid family, which are culturally, economically, and biologically significant.

## Methods

### Pipeline of Epigenetic Clock Construction

Sampling of brook trout via backpack electrofishing → Whole genome methylation sequencing of 27 individuals

**Clock Construction:**

Pre-feature selection of CpG's: KSelectBest: **2000 sites** → Pre-feature selection of 11 sites with **Boruta** → **Elastic net** regression for clock construction

**Testing clock: MAE (mean absolute error)**
The mean of the total sum of errors

## Impact of Pre-Feature Selection

The model with no pre-feature selection had no changes to MSE across penalty coefficients (Figure 3). The model which utilized pre-feature selection had a smaller MSE and error compared to the model which did not(Figures 3, 4). The graphs demonstrate that the pre-feature selection method is effective at selecting a model with a smaller cross-validated error.

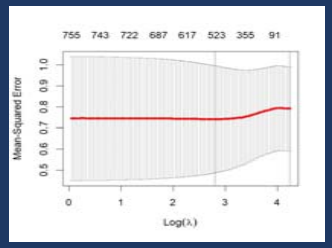


Figure 3: Mean squared error of non-preselection model against the log penalty coefficient (lambda) and number of sites in the model.

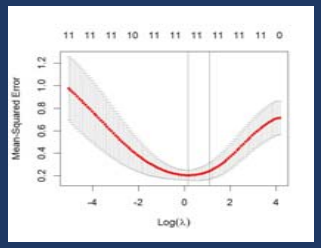


Figure 4: Mean squared error of pre-selection model against the log penalty coefficient (lambda) and number of sites in the model.

## Results:

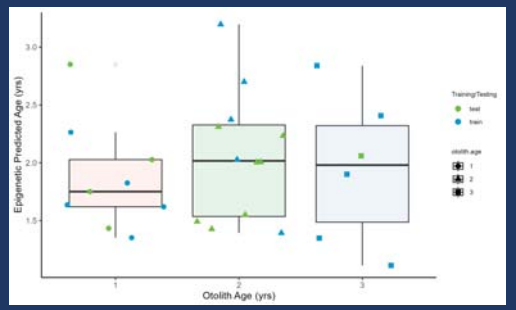


Figure 5: Boxplot of otolith age and epigenetic age (years) with testing and training datasets.

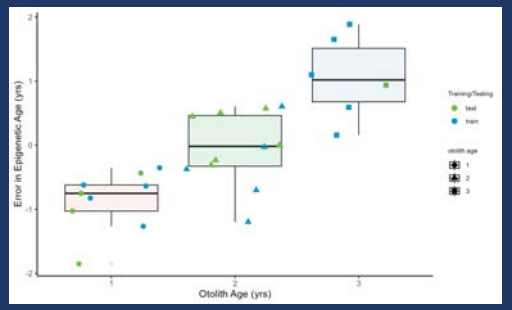


Figure 6: Boxplot of otolith age and the error in epigenetic age (years) with testing and training datasets.

## Discussion

An epigenetic clock was created to age brook trout with an absolute mean error of 0.7 years. The model struggled to accurately predict age one's and three's (Figure 5). The model tended to overestimate age one's and underestimate age three's (Figure 6). Pre-feature selection was employed to improve the accuracy of the clock and prevent model over-fitting, but was not able to completely overcome the challenges of aging short-lived, wild caught fish species. Future directions of the work include sequencing more fish to aid in model training, and continue to test different modelling techniques for improved accuracy.

**Acknowledgements:**
I would like to thank the Ruzzante lab: Daniel Ruzzante, Ellie Weise, Cait Nemeczeck, James Kho, and NSERC for funding.

**References**

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3.Katz, J., Moyle, P. B., Quiñones, R. M., Israel, J., & Purdy, S. (2013). Impending extinction of salmon, steelhead, and trout (Salmonidae) in California. Environmental Biology of Fishes, 96, 1169-1186.