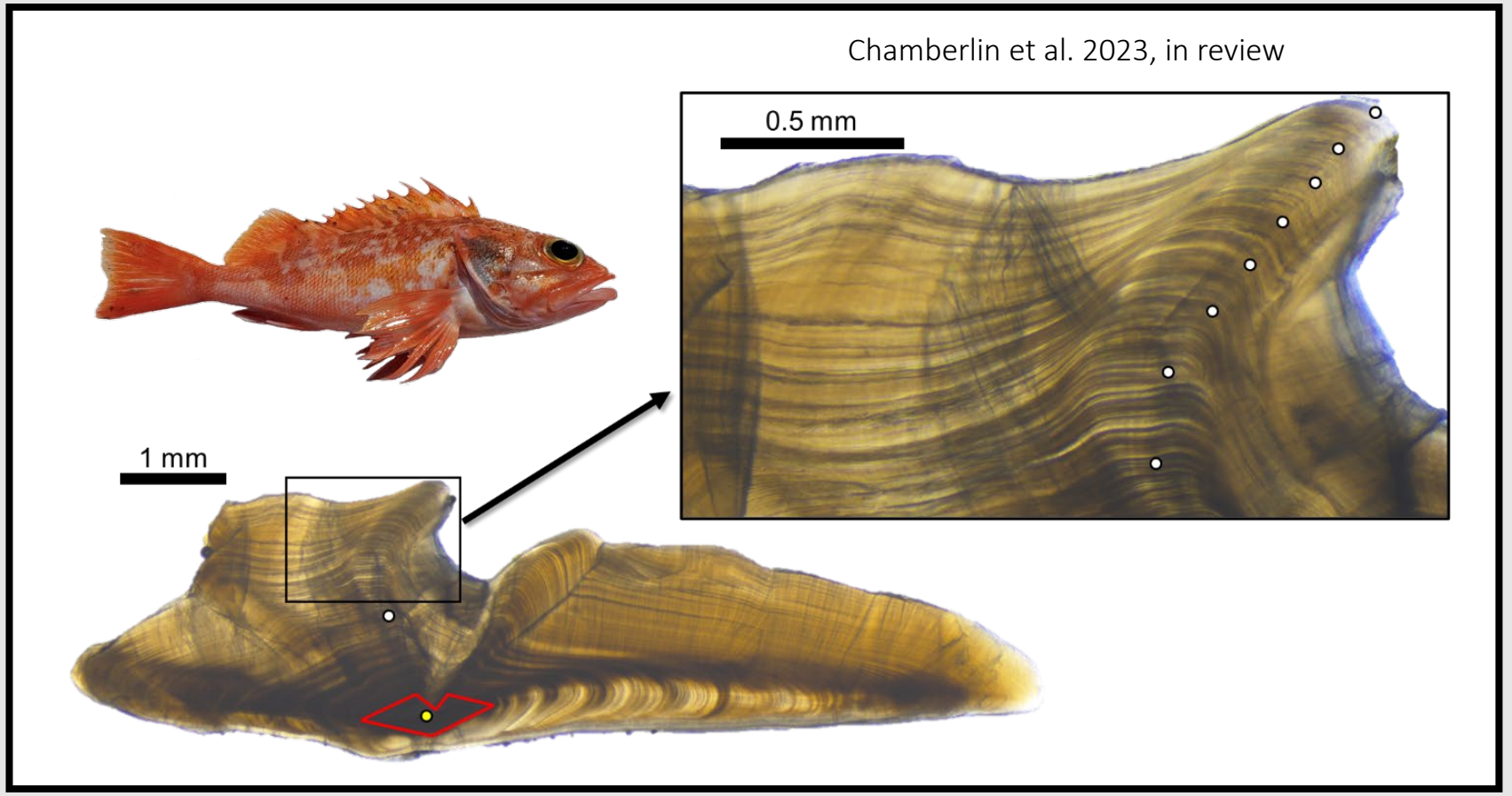
Epigenetic Age Estimation in a Deepwater Scorpionfish, Blackbelly Rosefish

D.N. Weber[1*], A.T. Fields[1], D.W. Chamberlin[2], W.F. Patterson III[2], D.S. Portnoy[1]

[1]Marine Genomics Laboratory, Texas A&M University–Corpus Christi
[2]Fisheries & Aquatic Sciences, University of Florida

Deepwater fishes can be difficult to age using traditional techniques

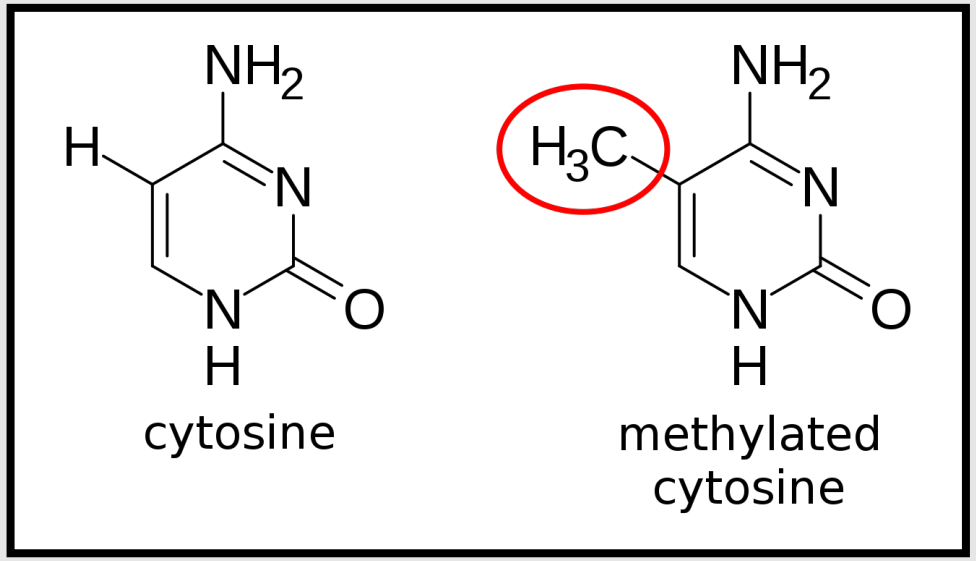
- Slow growth rates
- Long life spans
- Constant environment



→ Alternative age estimation method?

What is Epigenetics?

Mechanisms that affect gene expression without altering DNA sequence



DNA Methylation

- Addition of CH_3 to cytosine, often at CpG sites
- Typically acts to repress transcription
- Changes in DNA methylation at select CpG sites correlate with age

→ Epigenetic clocks!

Epigenetic Clock History

2019: European seabass

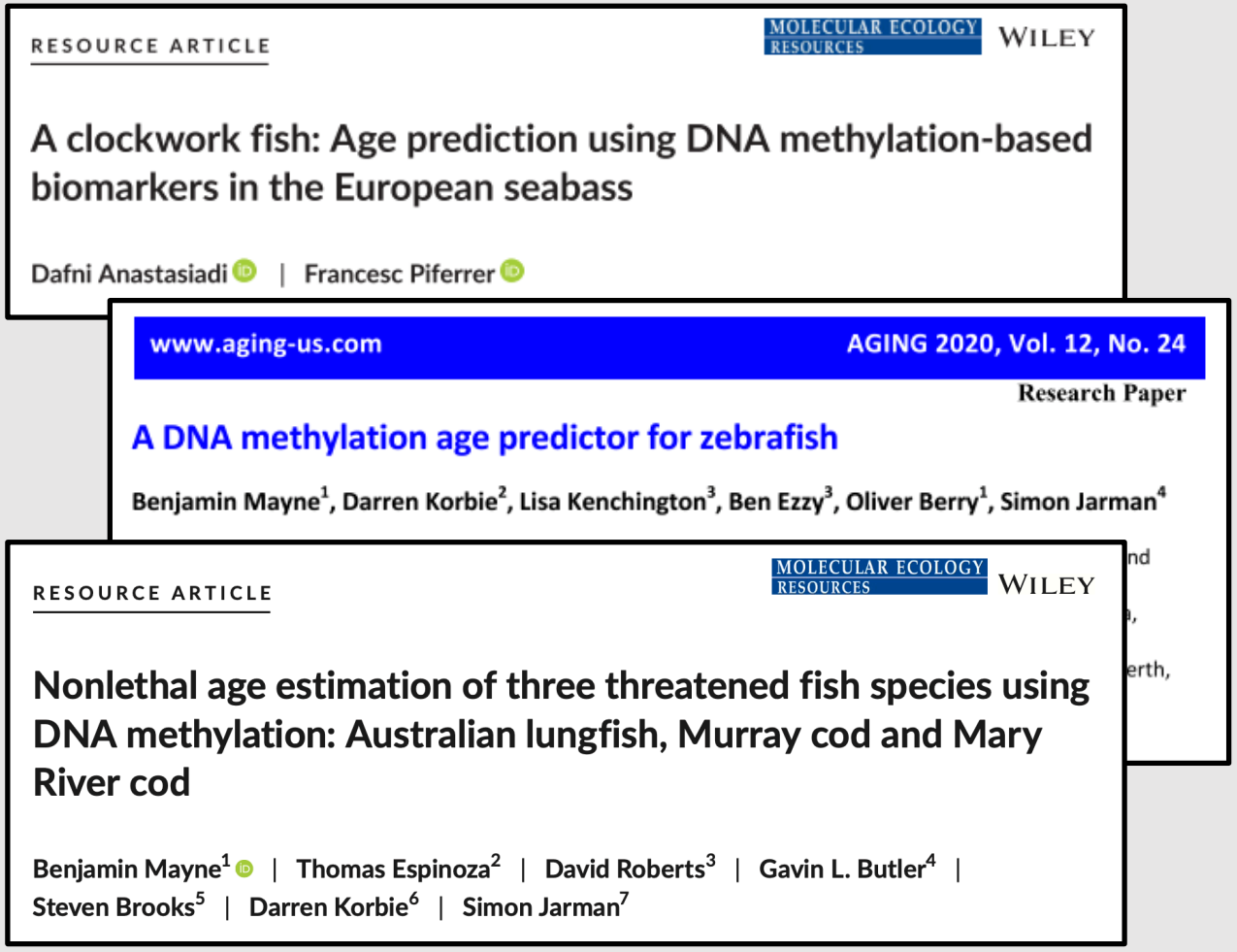
- Reared in laboratory

2020: Zebrafish

- Reared in laboratory

2021: Australian lungfish and cod

- Used zebrafish CpG sites



RESOURCE ARTICLE

MOLECULAR ECOLOGY RESOURCES WILEY

A clockwork fish: Age prediction using DNA methylation-based biomarkers in the European seabass

Dafni Anastasiadi | Francesc Piferrer

www.aging-us.com AGING 2020, Vol. 12, No. 24

Research Paper

A DNA methylation age predictor for zebrafish

Benjamin Mayne[1], Darren Korbie[2], Lisa Kenchington[3], Ben Ezzy[3], Oliver Berry[1], Simon Jarman[4]

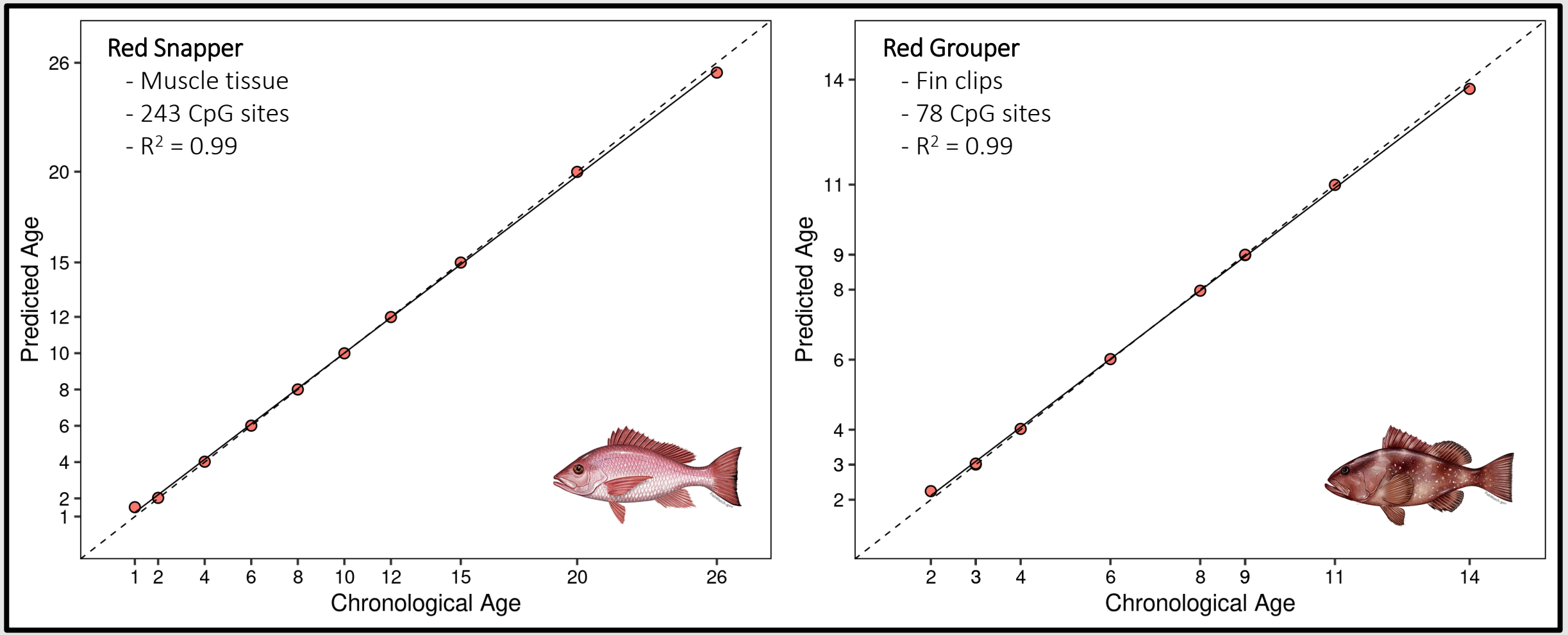
RESOURCE ARTICLE

MOLECULAR ECOLOGY RESOURCES WILEY

Nonlethal age estimation of three threatened fish species using DNA methylation: Australian lungfish, Murray cod and Mary River cod

Benjamin Mayne[1] | Thomas Espinoza[2] | David Roberts[3] | Gavin L. Butler[4] | Steven Brooks[5] | Darren Korbie[6] | Simon Jarman[7]

Novel epigenetic age estimation in wild-caught Gulf of Mexico reef fishes. 2022. D. Nick Weber, Andrew T. Fields, William F. Patterson III, Beverly K. Barnett, Christopher M. Hollenbeck, David S. Portnoy. *Can. J. Fish. Aquat. Sci.*, **79**:1–5.



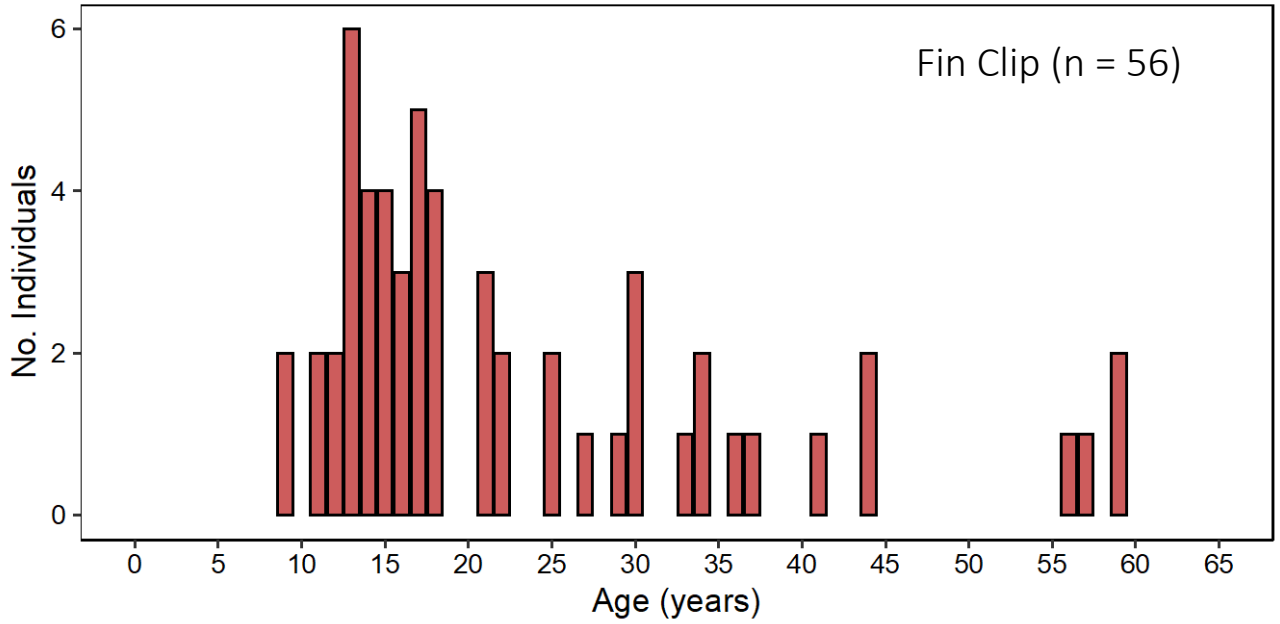
1: Can this epigenetic ageing technique be applied to a deepwater fish?

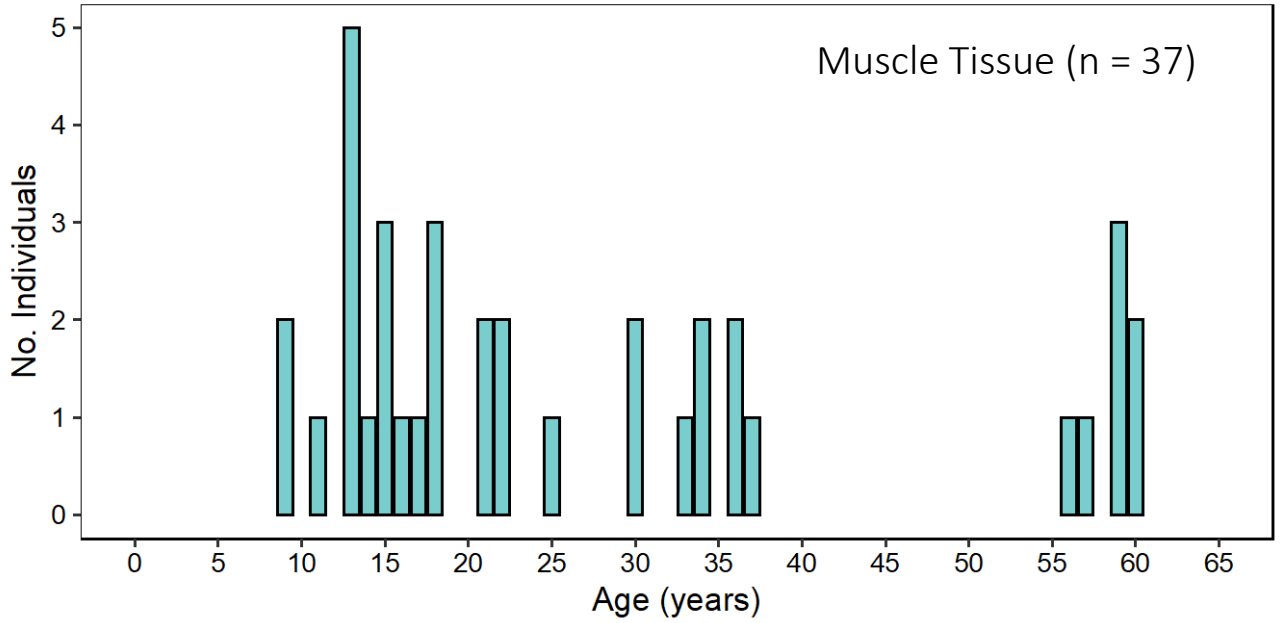
2: Are age-correlated CpG sites shared across tissue types (fin clips and muscle tissue)?

Blackbelly Rosefish

Helicolenus dactylopterus

- Deepwater scorpionfish (150 to 600 m)
- Long-lived (>90 years)
- Widely distributed in Atlantic Ocean
    - Potentially in Indian Ocean
    - Likely several subspecies in Atlantic
- Difficult to age





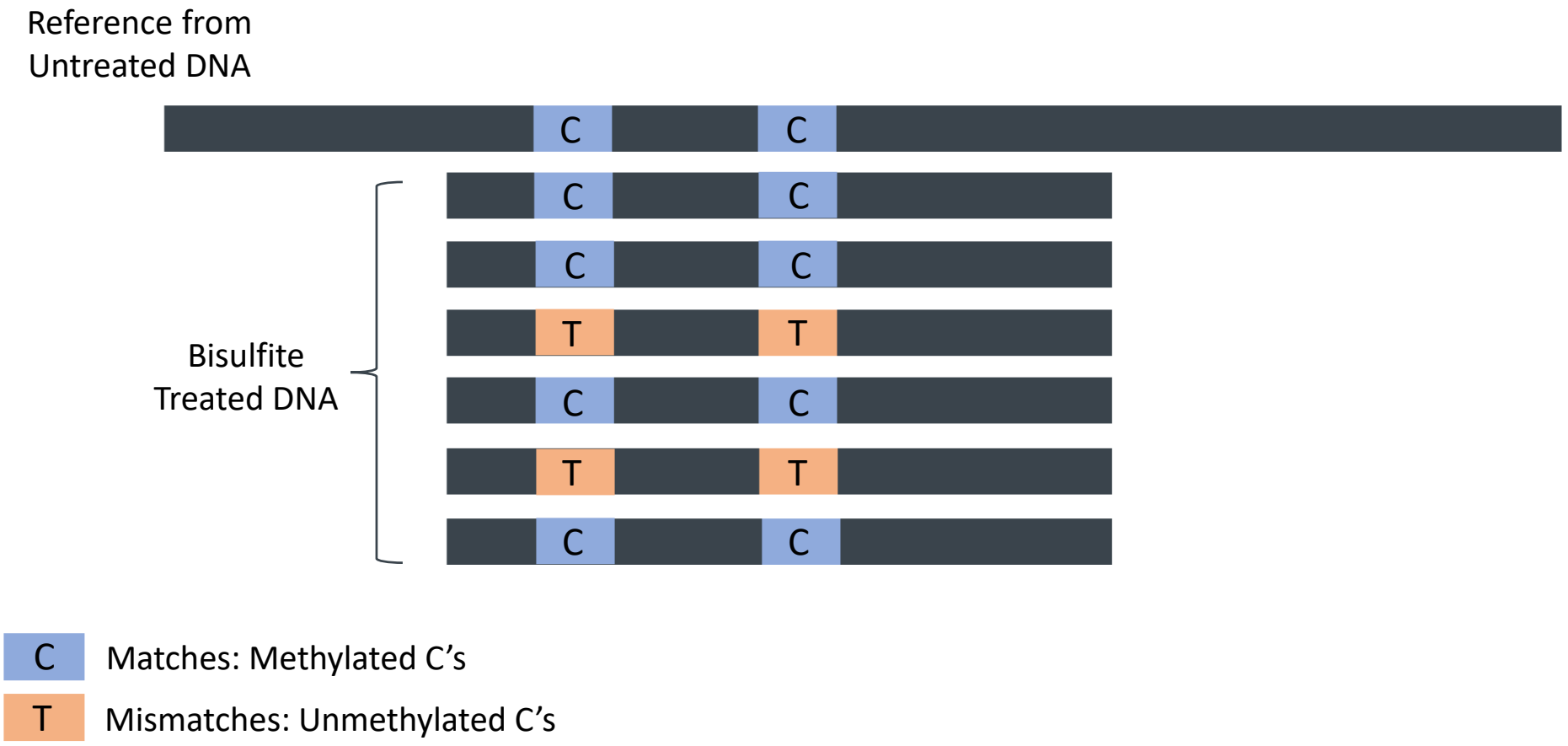
61 individuals

- 56 fin clip samples
- 37 muscle tissue samples

All ages bomb ^{14}C-validated (range: 9 to 60 years)

Genomic Approach

bsRADseq: bisulfite-converted restriction site-associated DNA sequencing

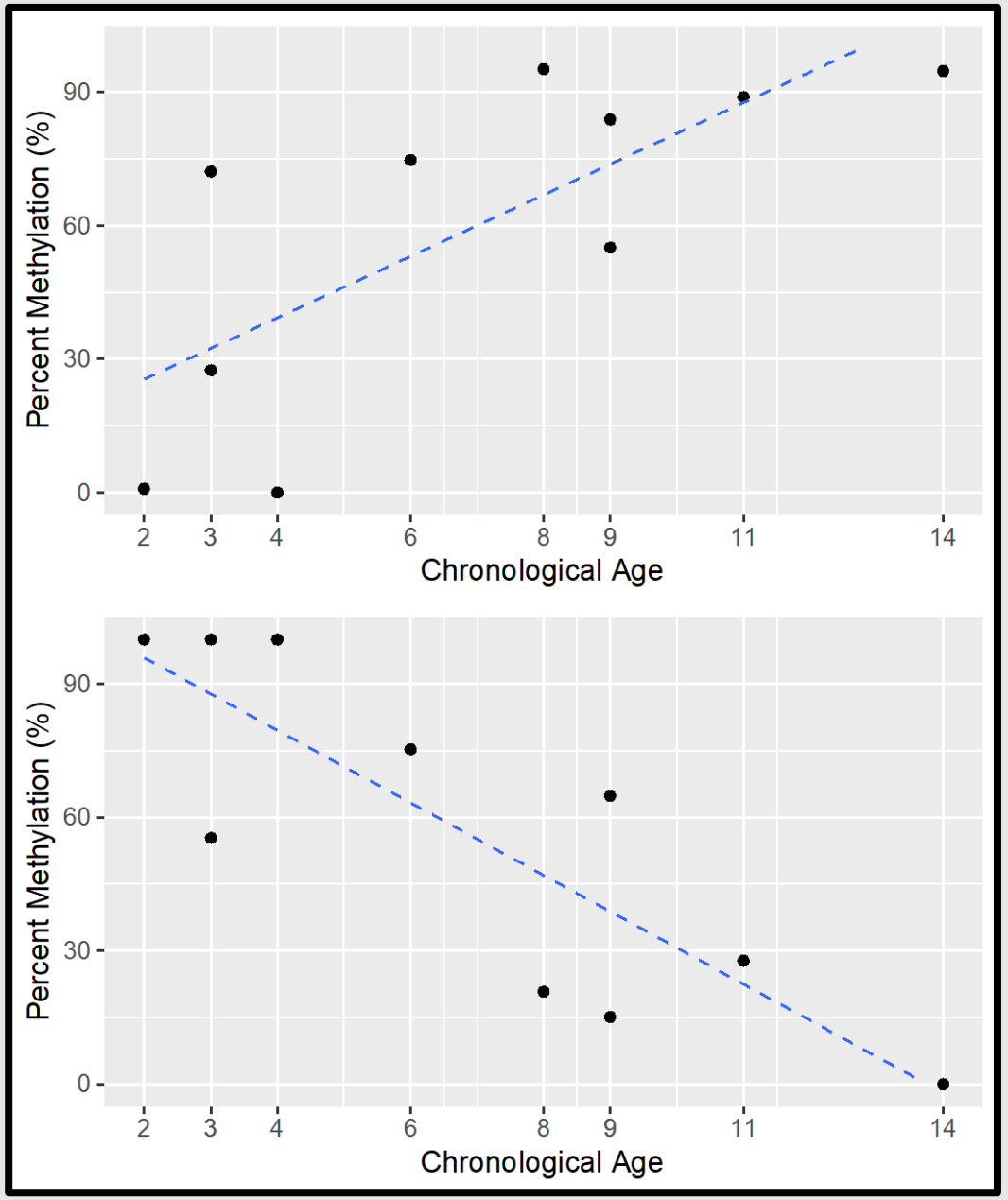


Data Analysis

1) Identify all CpG sites that exhibit age-correlated methylation

Bayesian GLM

- Age as fixed factor
- Sample as random factor
- $\frac{\#\ \text{methylated reads}}{\#\ \text{total reads}}$ as response
- 95% HPDI's

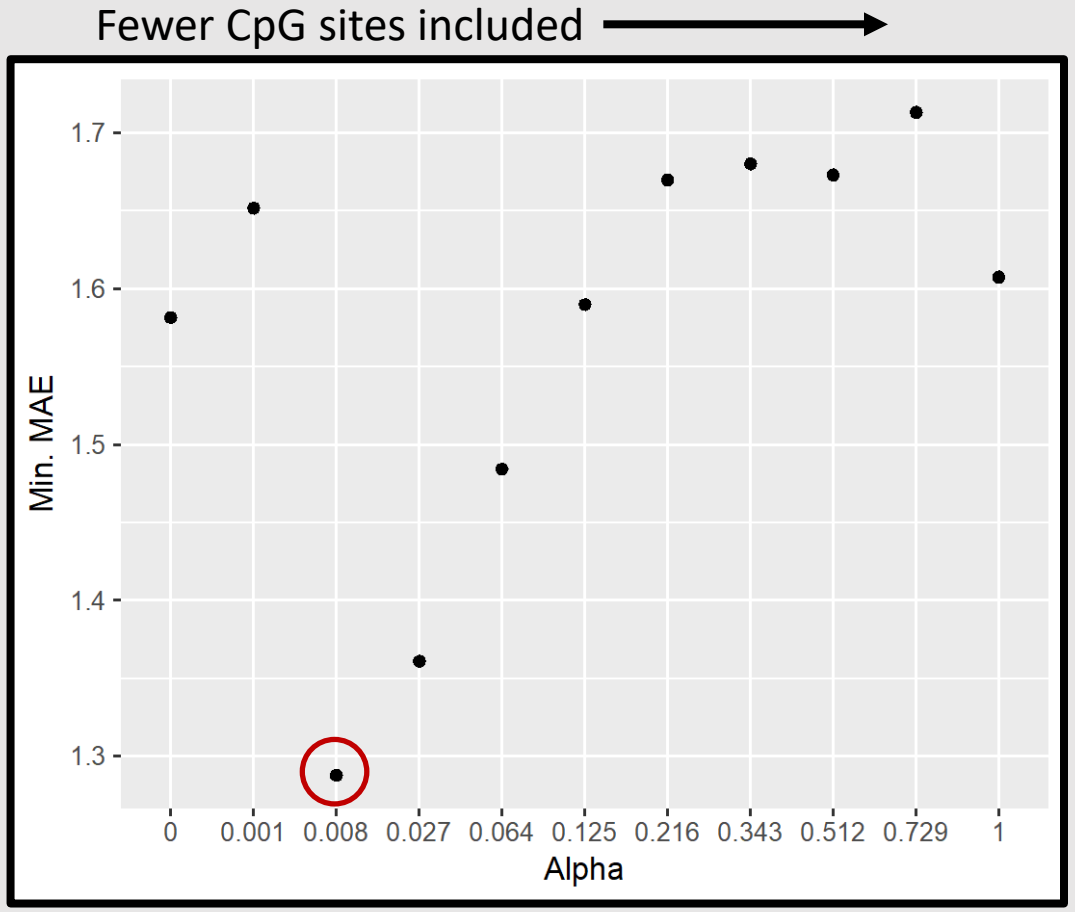


2) Identify the subset of CpG sites that best predict age

Penalized Regression

using “elastic net” version of `glmnet` in R

- Penalty (α) ranges from 0 to 1
- α can be optimized
- Tuning parameter (λ) influences penalty strength
- λ is internally cross-validated



Preliminary Results

No. raw CpG sites: 2,959,164

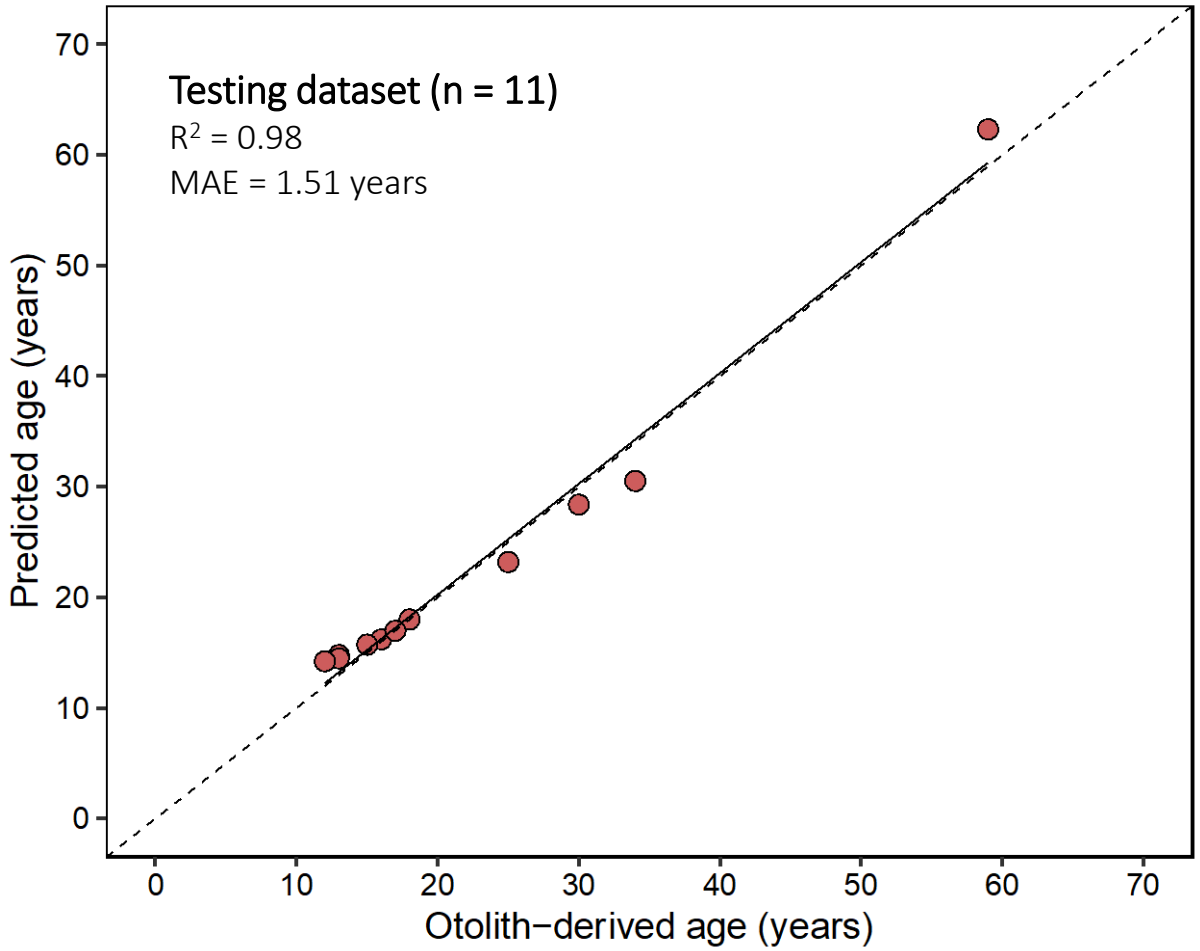
Model	n	Age Range (Years)	No. CpG Sites Post-Filtering	No. CpG Sites Age-Correlated	No. CpG Sites in Final Model
Fin Clip	56	9–59	156,529	10,139	315
Muscle	37	9–60	115,569	5,886	623
Combined Tissues	61	9–60	129,916	15,071	524

Bayesian Model

Elastic Net Regression

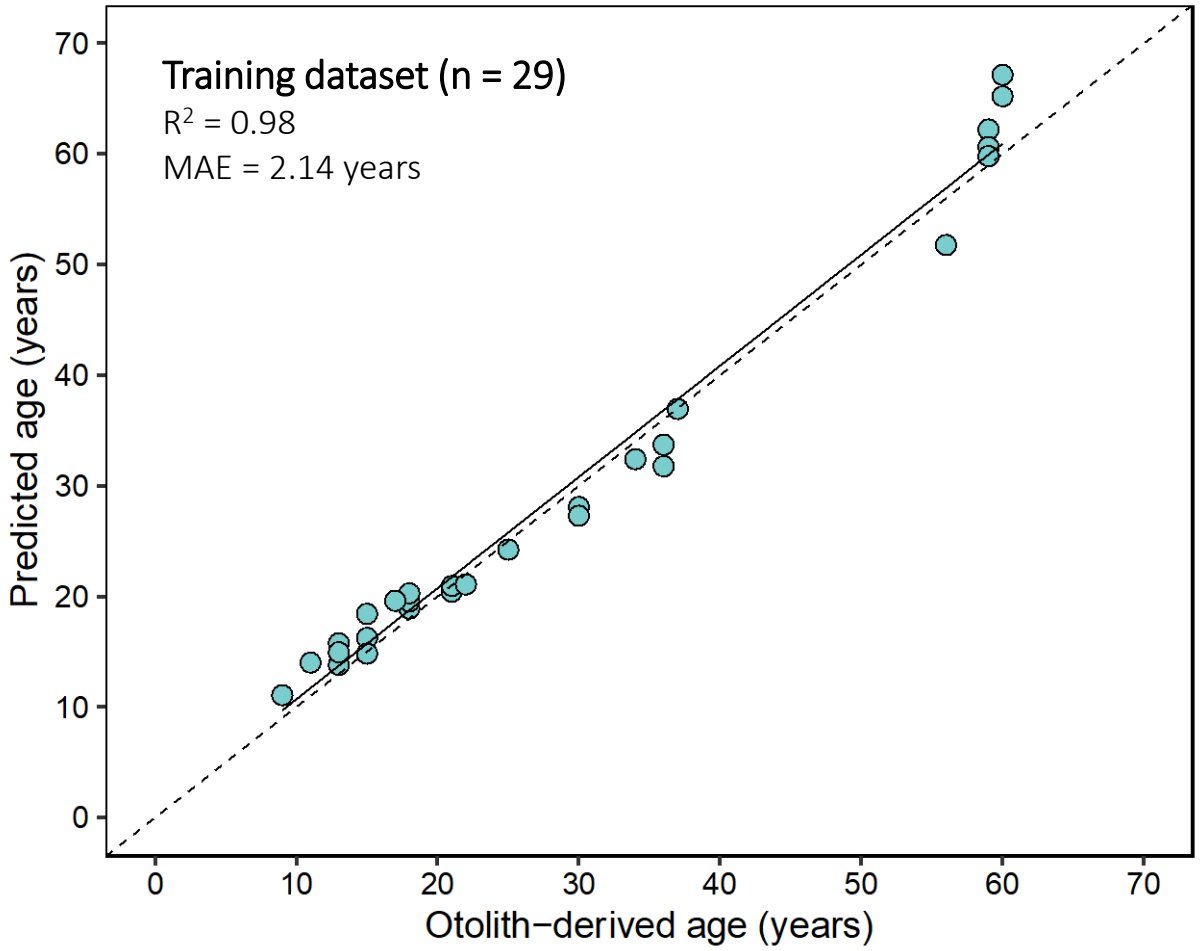
Fin Clip Clock

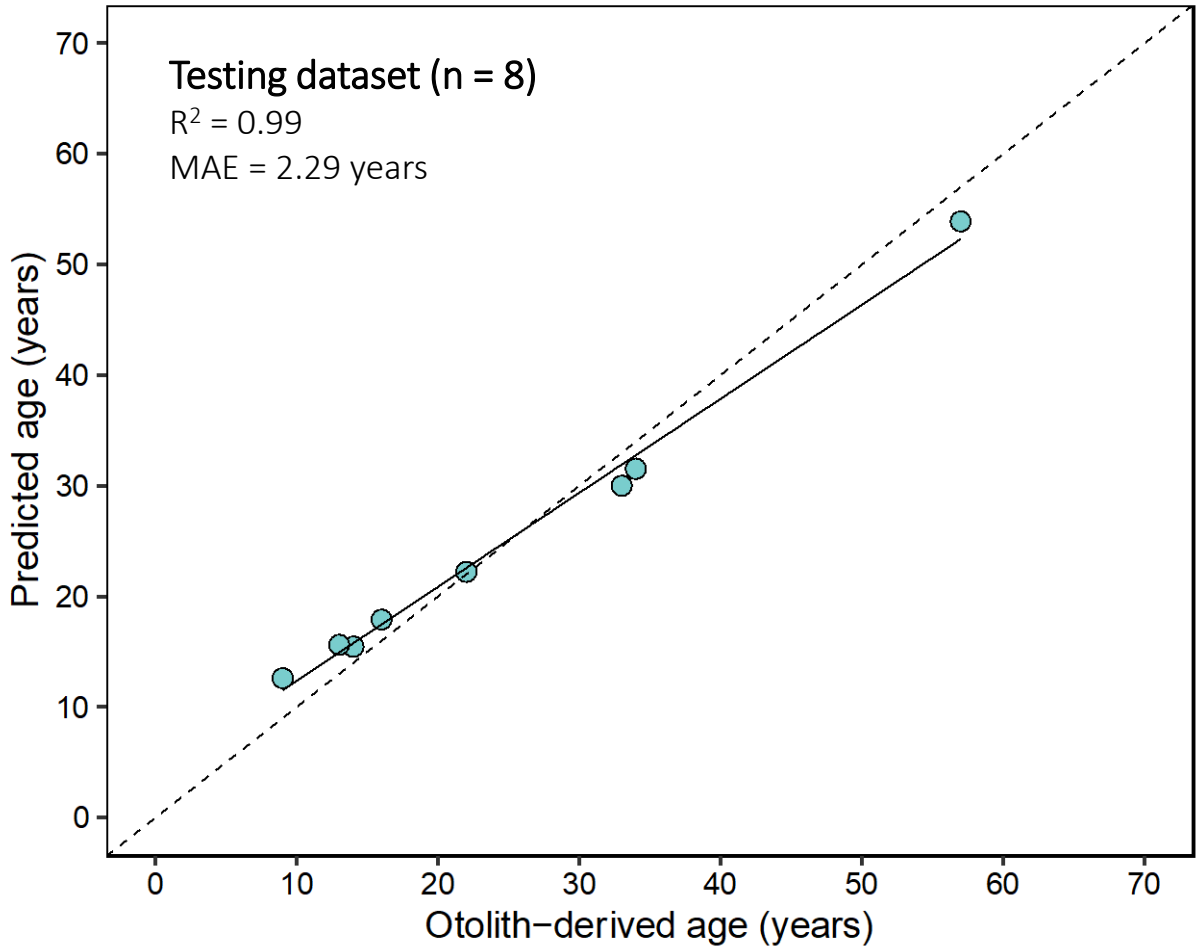
- 56 individuals
- 315 CpG sites



Muscle Clock

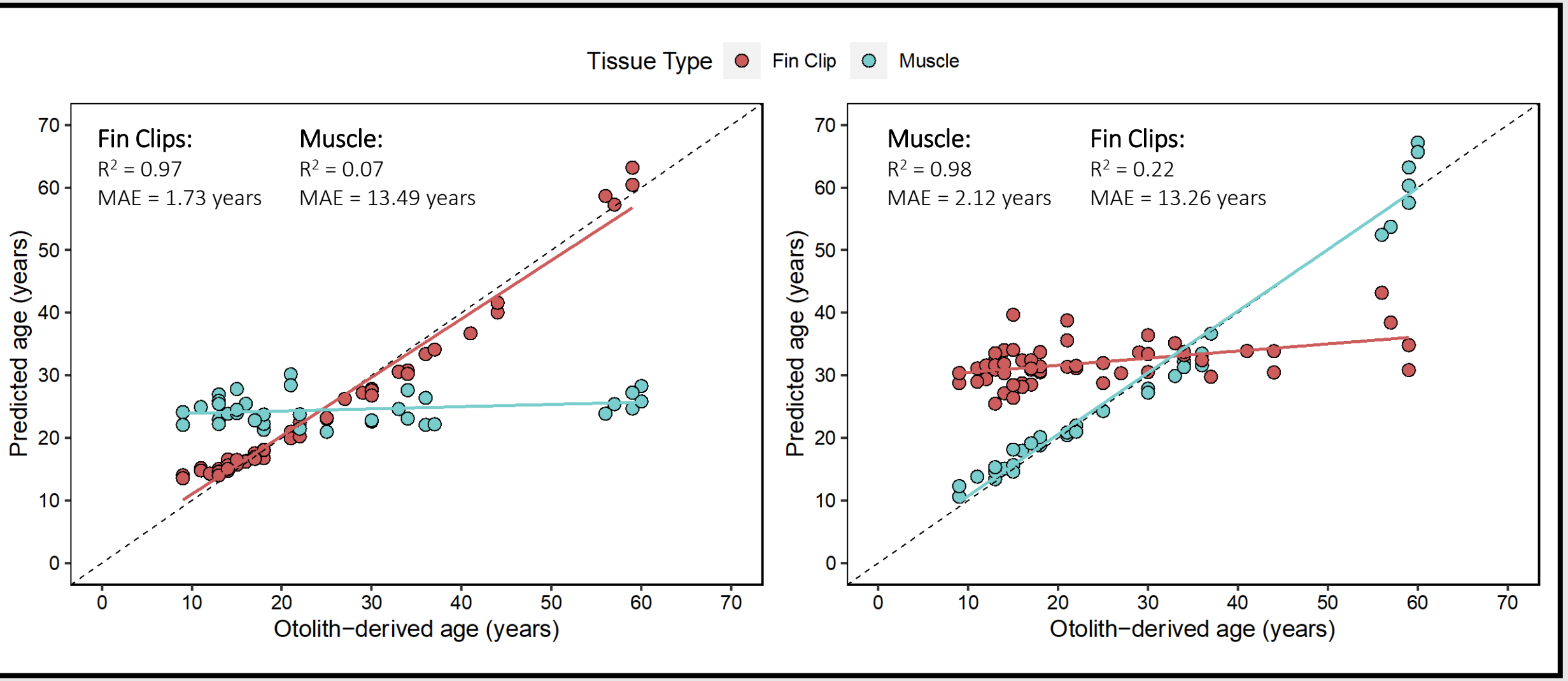
- 37 individuals
- 623 CpG sites





Predicting Muscle from Fin Clip Clock

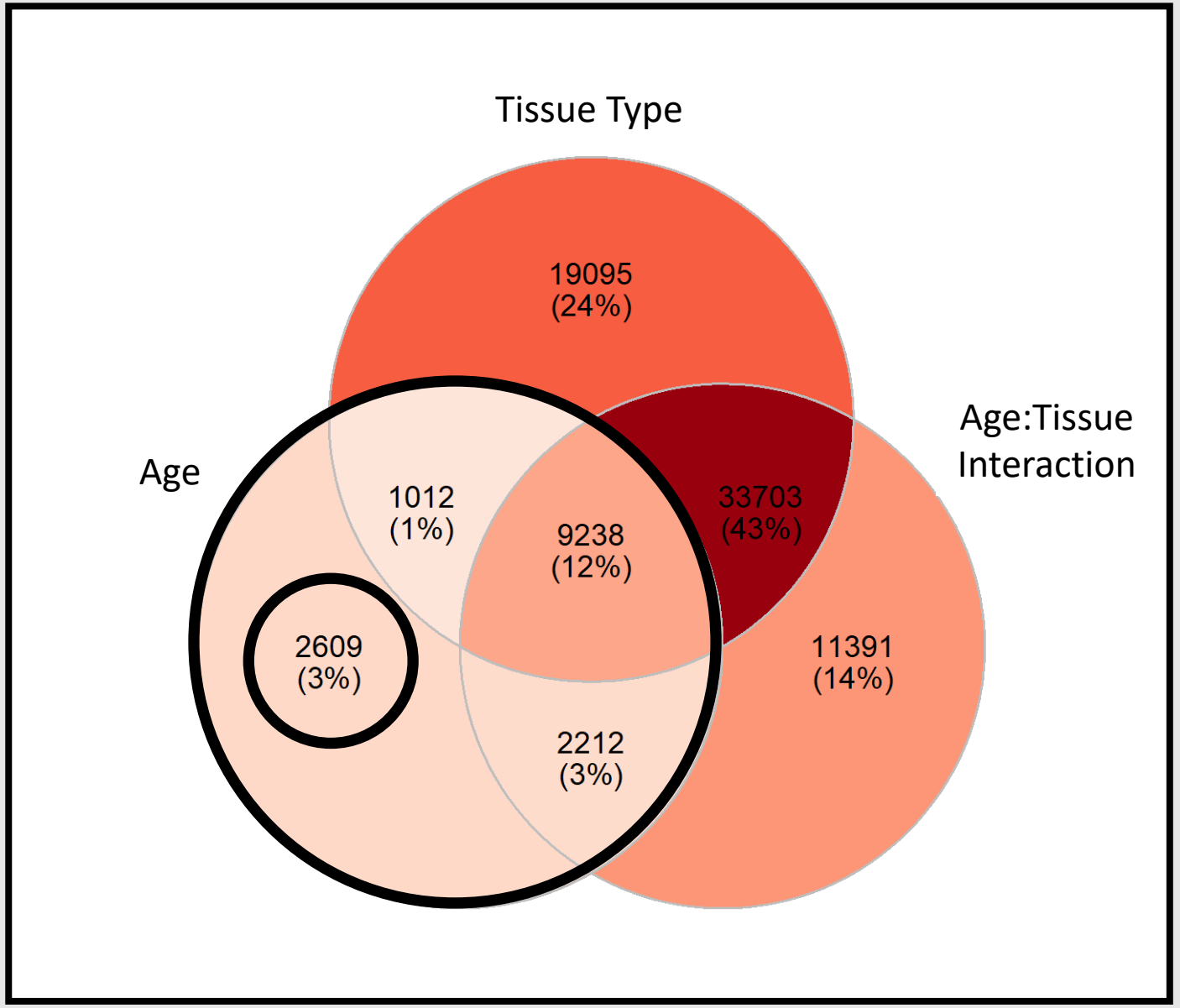
Predicting Fin Clip from Muscle Clock



Combined Tissue Clock

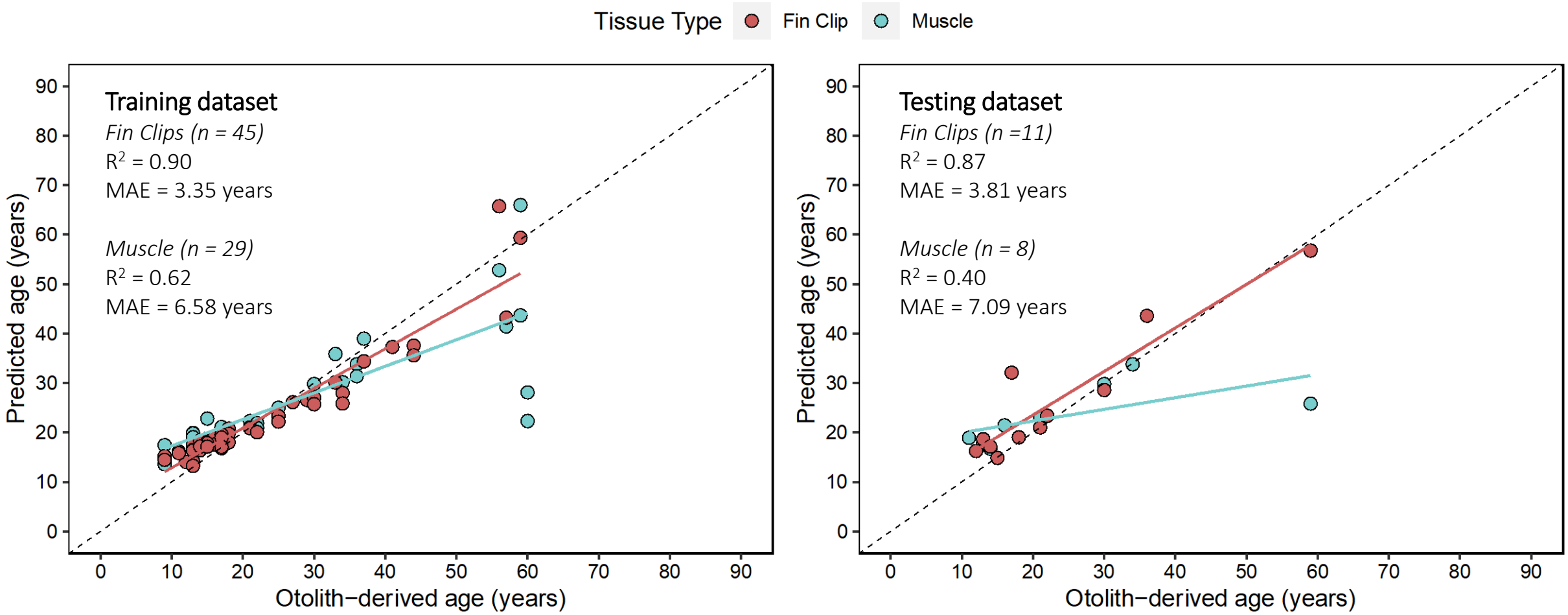
Bayesian GLM

- Age as fixed factor
- *Tissue type as fixed factor*
- Sample as random factor
- $\frac{\#\ methylated\ reads}{\#\ total\ reads}$ as response
- 95% HPDI's



Combined Tissue Clock

- 524 CpG sites



Combined Tissue Clock

- Leave-one-out
- 524 CpG sites

Fin Clip:

R^2 = 0.92

MAE = 3.05 years

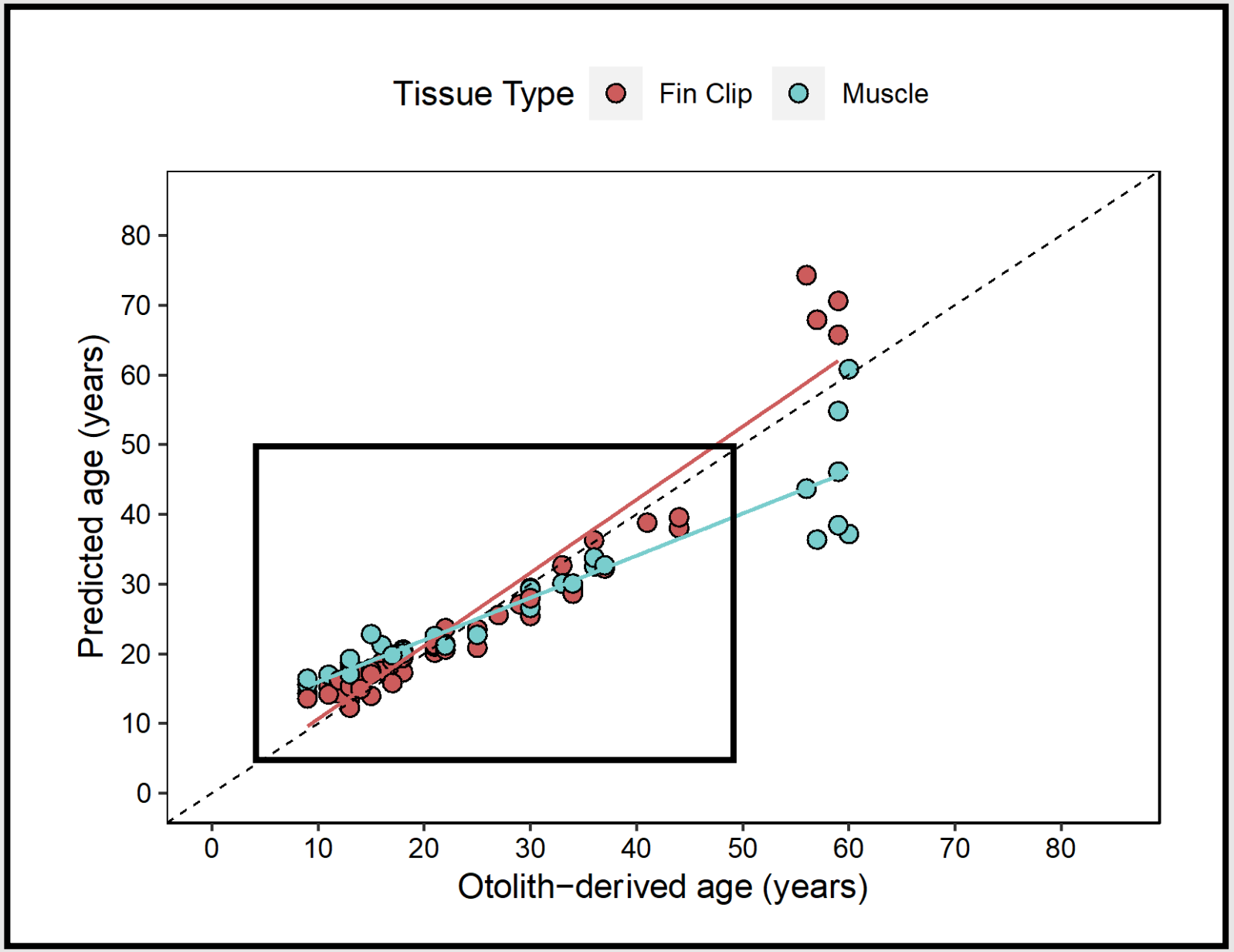
MAE for ages <50 = 2.37 years

Muscle:

R^2 = 0.87

MAE = 5.55 years

MAE for ages <50 = 3.70 years

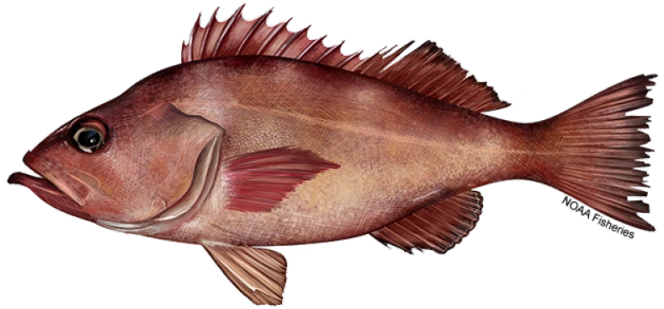


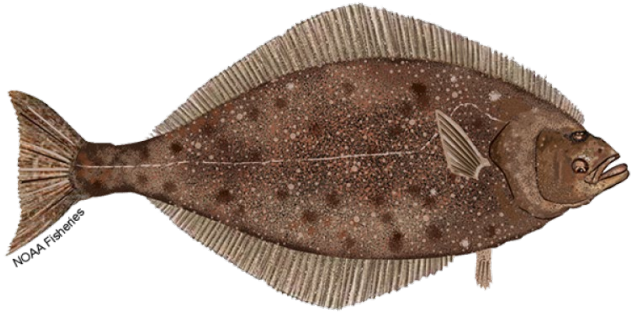
Conclusions

- Accurate epigenetic clocks can be developed for deepwater fishes
- Age-correlated CpG sites were identified across tissue types, suggesting the potential for a multi-tissue clock

Potential Benefits

- Non-invasive
- More time- and cost-efficient generation of age estimates
  - $10 per sample
  - Age thousands of individuals per month
- Accurate/precise for difficult to age species
  - MAE = 1.51 years in fin clip clock

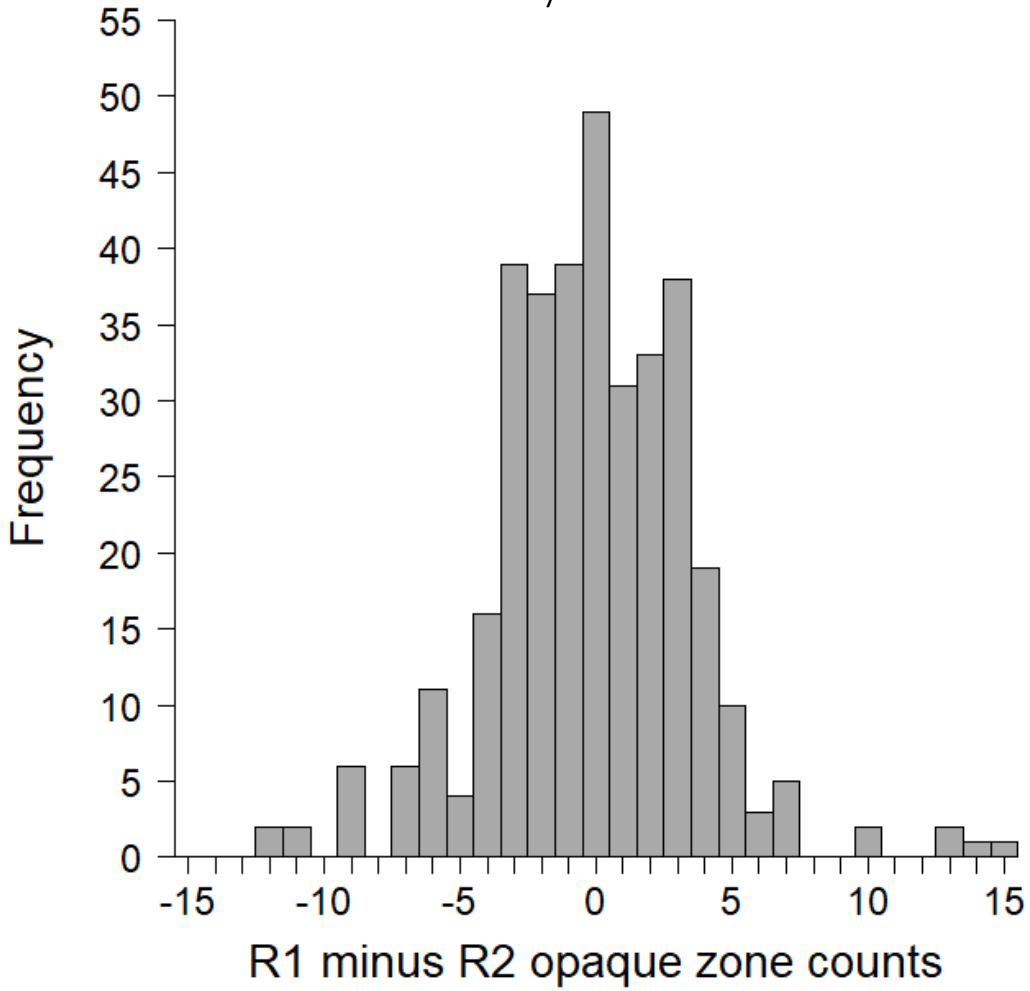




Chamberlin et al. 2023, in review

Otolith-derived

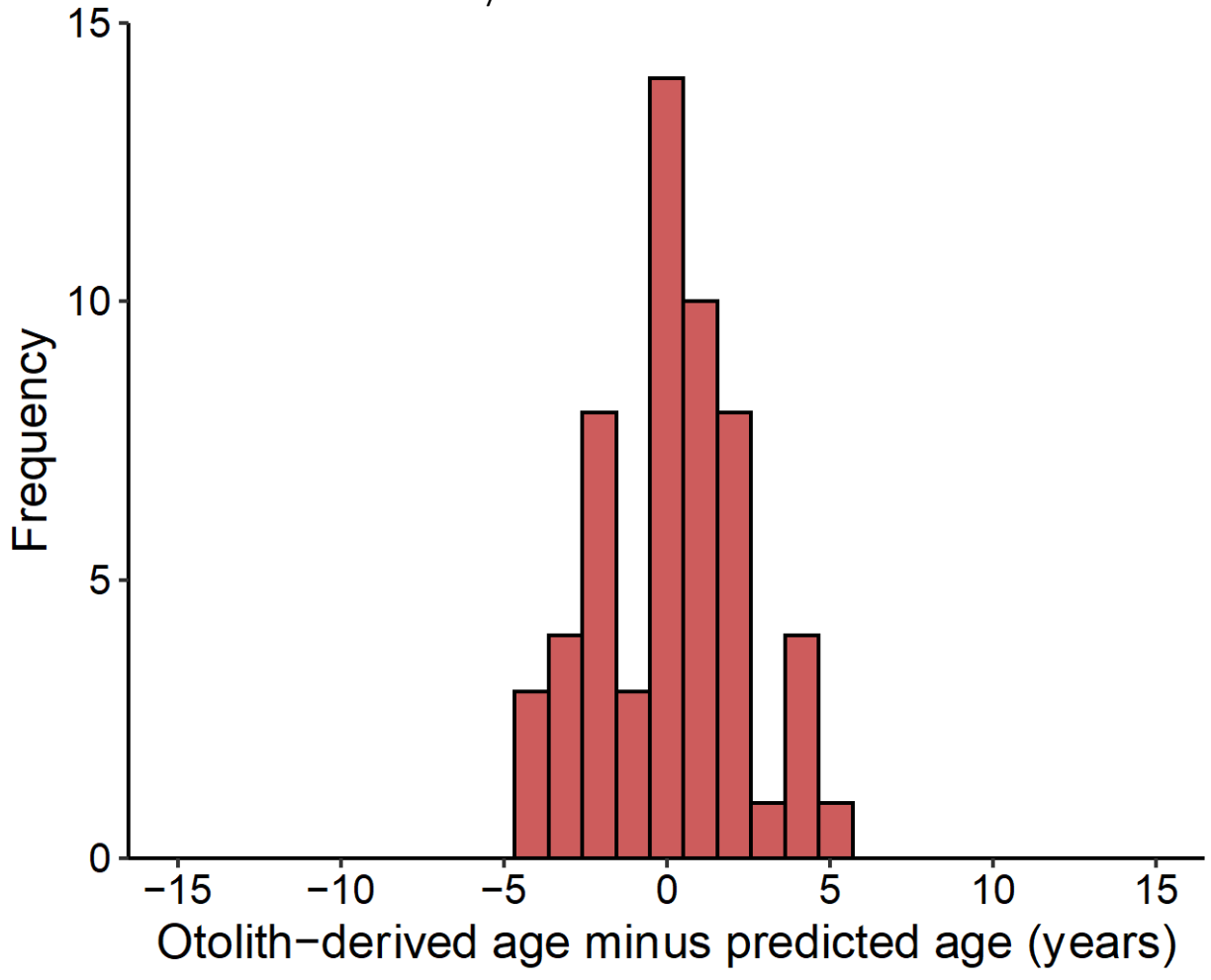
n = 356 blackbelly rosefish



Present study

Fin clip epigenetic clock

n = 56 blackbelly rosefish



Acknowledgements

Sample Acquisition:
Captain John Greene
Captain Donnie Waters
Joe Tarnecki
Joe Moss
Bob Dahl
Kristen Dahl
Steve Garner
Justin Lewis
Holden Harris

Sample Processing:
Miaya Glabach

Funding:
National Marine Fisheries Service
National Sea Grant College Program
Texas Sea Grant

Contact:
D. Nick Weber
Ph.D. Candidate
Texas A&M University–Corpus Christi
dweber@islander.tamucc.edu

Application to Fisheries Management

Goal: more time- and cost-efficient ageing approach

- bsRADseq is necessary to develop epigenetic clock (i.e. determine CpG sites of interest)

- Once identified, simply target those CpG sites in subsequent individuals using multiplex PCR

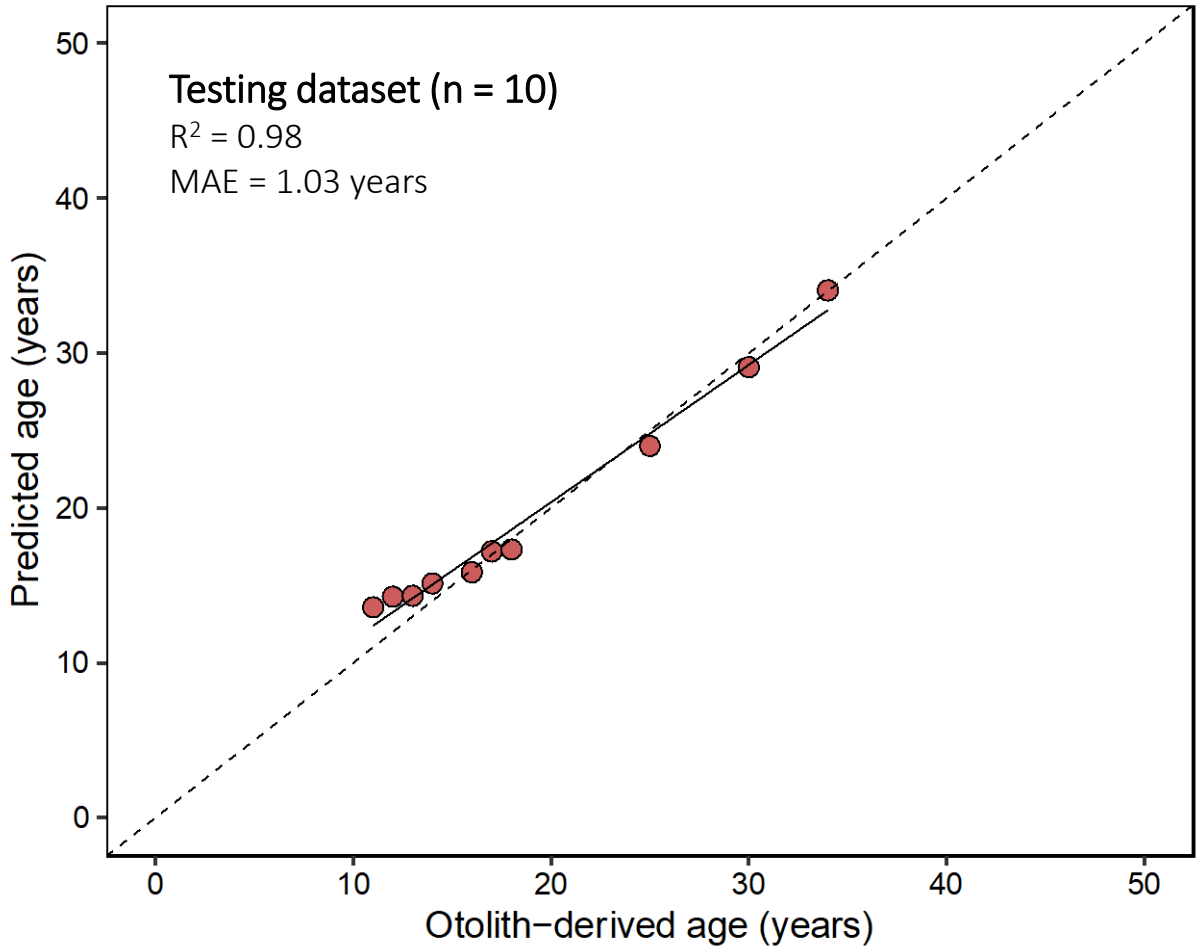
- Design primers (25 bp in length) to target age-correlated CpG sites

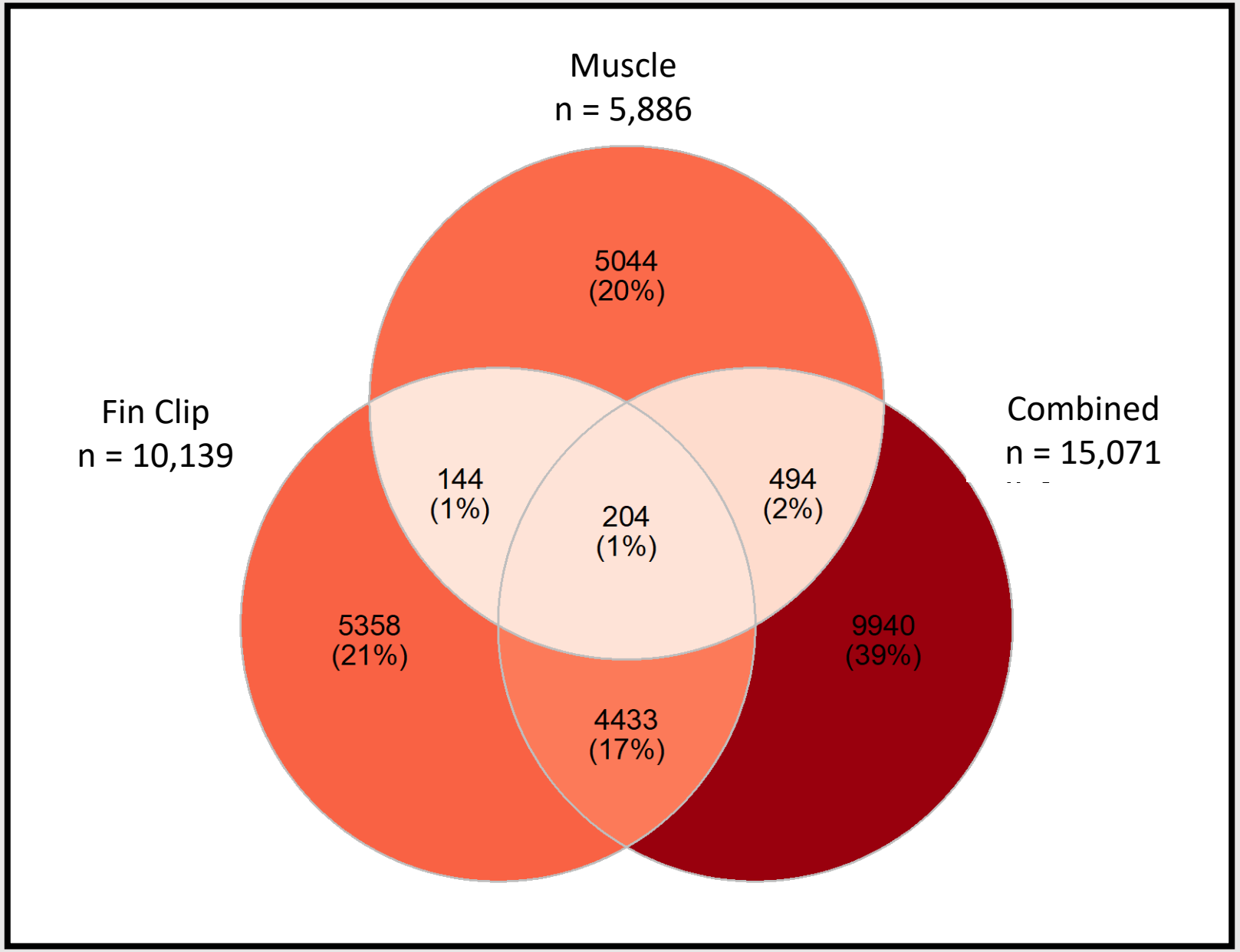
→ Target up to 500 CpG sites in thousands of individuals at one time

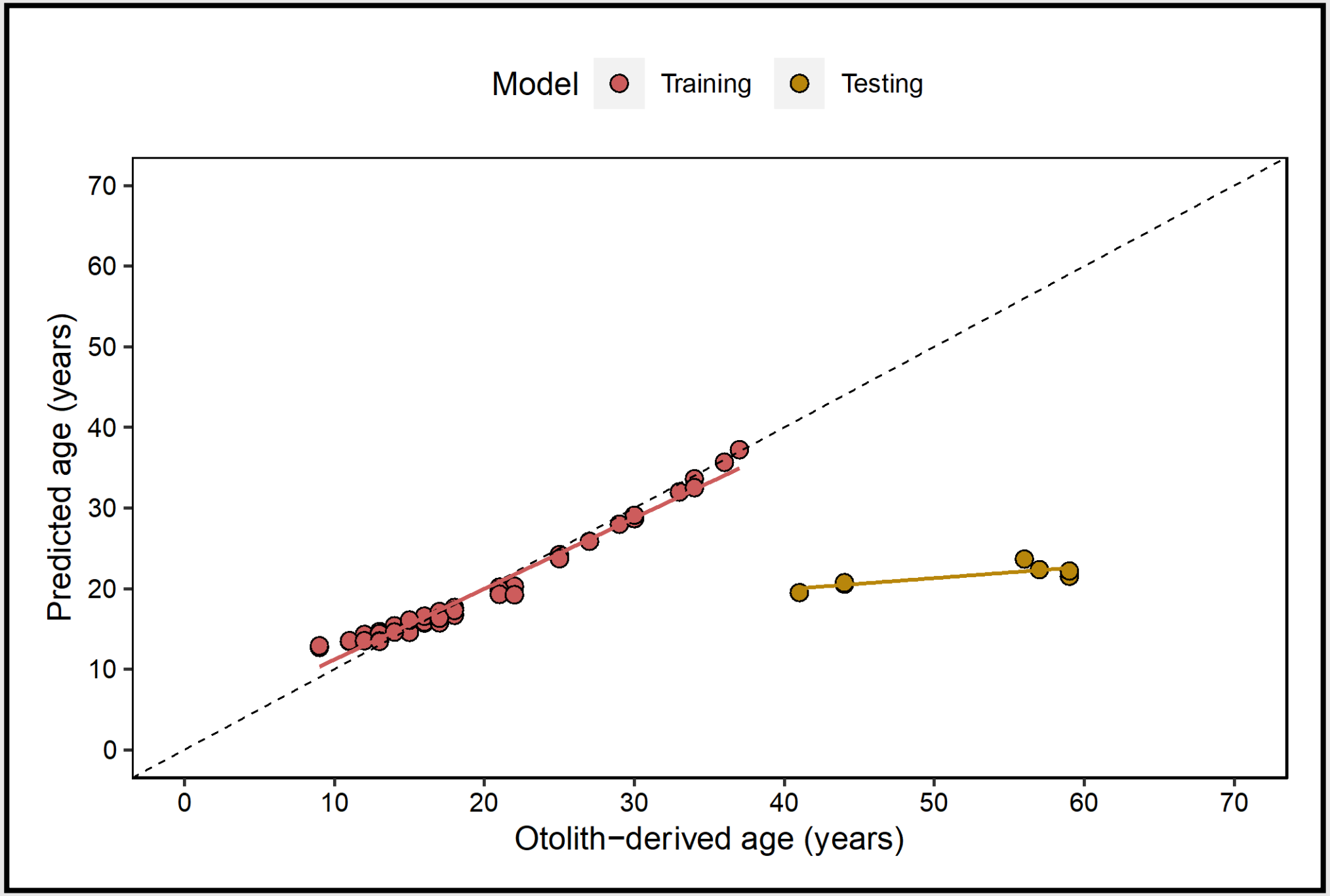
→ Age tens of thousands of individuals per month ($10 per individual)

Fin Clip Clock for Individuals <40 Years Old

- 49 individuals
- 222 CpG sites




Muscle
n = 5,886
Fin Clip
n = 10,139
Combined
n = 15,071
5044
(20%)
144
(1%)
204
(1%)
494
(2%)
5358
(21%)
9940
(39%)
4433
(17%)


Model
Training
Testing
Predicted age (years)
Otolith−derived age (years)
0
10
20
30
40
50
60
70