Identifying historically collected larval coregonines to species using a redesigned genetic assay

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Background

- Cisco (*Coregonus artedi*) and Lake Whitefish (*Coregonus clupeaformis*) belong to the subfamily Coregoninae.[1]
- Both species were once abundant in the Great Lakes in the early-mid 20th century but have since declined due to overfishing and competition with the non-native Alewfie (*Alosa pseudoharengus*).[1]
- Coregonines are essential to the Great Lakes food webs in providing Thiamine-rich diets for other native fish species.[2]
  - Specifically, Lake Trout (*Alvelinus namaycush) and* Atlantic Salmon *(Salmo Salar)*
- Cisco are hard to distinguish from Lake Whitefish visually at larval stage and difficult to study due to phenotypic plasticity.[1]
- Larval Coregonine genomic data vastly improves the ability to assess early life dynamics and recruitment processes in the efforts to restore and manage these species.[3]
- Successfully identifying Coregonine species at early life stages is necessary for understanding habitat use, growth, and mortality rates in ongoing restoration efforts.[3]

Objectives

- Develop a new, shorter Polymerase Chain Reaction (PCR)-based assay to infer species for unknown Coregonine larvae with degraded DNA.

General Methods

- Larvae were collected from Chaumont Bay, NY, and preserved over the past decade.
- Cisco are typically collected at a higher density offshore compared to Lake Whitefish.[4]
- Larvae were cut in half and placed in 96-well trays.
- Replicated the PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) assay created by George et al. (2018),[1] modified to improve amplification efficiency.
- PCRs amplified a 202 base pair (bp) locus using the same molecular conditions while amplifying at smaller amplicon size, which were then digested using EcoO109I to infer the presence of species-specific polymorphism with the cytochrome oxidase I gene.
- Digested amplicons were size-separated via 2% agarose gels, stained with ethidium bromide and visualized under ultraviolet light.

Species are difficult to identify visually at larval stage

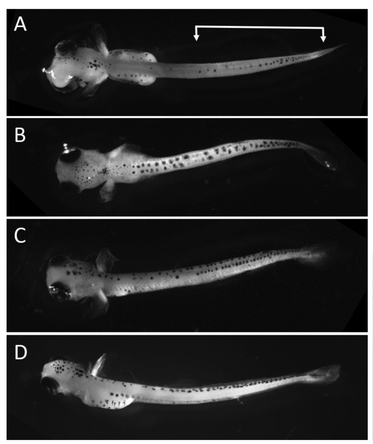

Figure 1. Identified Cisco (A-C) and identified Lake Whitefish (D)[1]

Figure 2. Adult Cisco reared at Tunison Lab of Aquatic Science, Cortland, NY

Figure 3. Adult Lake Whitefish[5]

Assay design has significant role in genotyping success

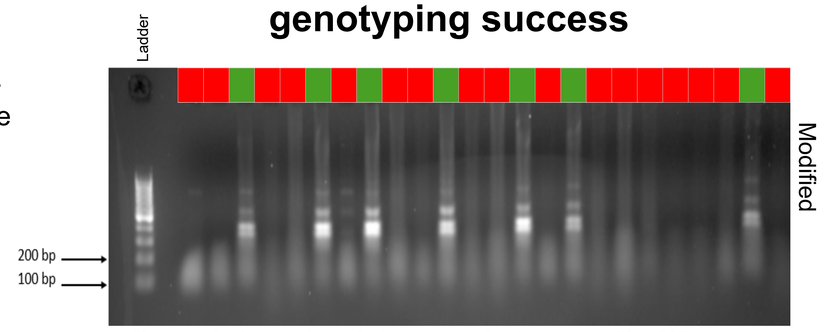

Figure 4. Gel image of row 3 of Larval tray 5 using the modified 707 bp assay, labeled with identified Coregonines. Ciscoes are identified in (Dark Green), Failures are seen as (Red).

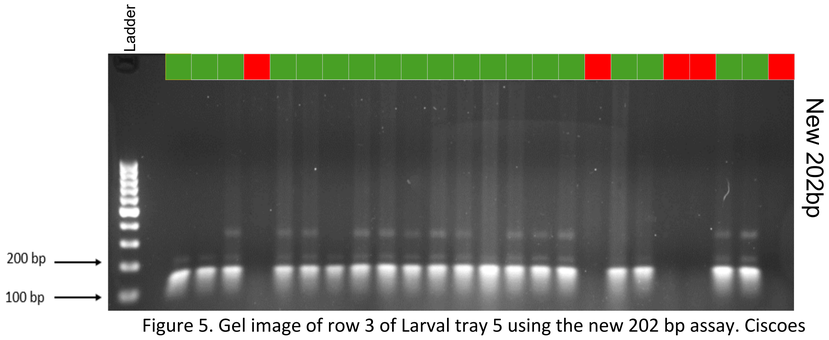

Figure 5. Gel image of row 3 of Larval tray 5 using the new 202 bp assay. Ciscoes are identified in (Dark Green), Failures are seen as (Red).

New test improved species identification success

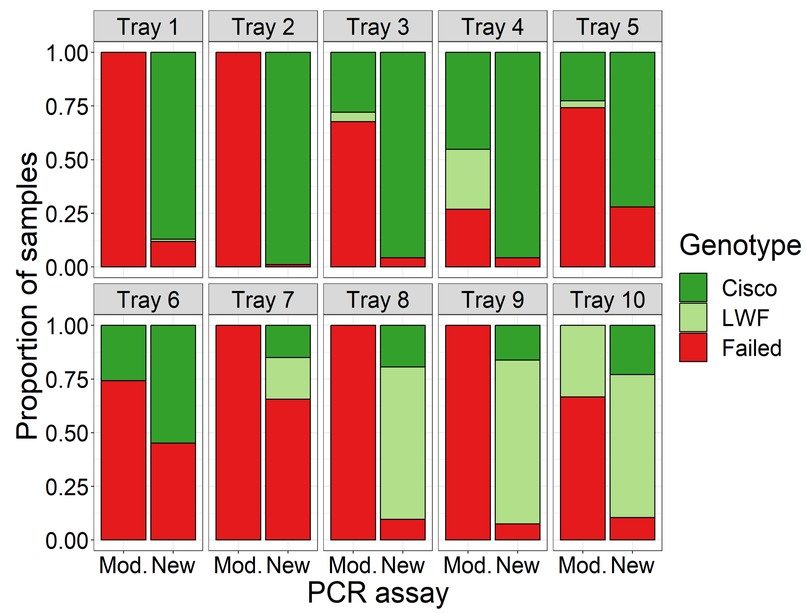

Figure 6. Proportion of genotype calls from each tray and the respective assay design used (Modified 707 bp) and (New 202 bp)

- The modified George et al. (2018)[1] assay successfully identified 19.0% ± 22.8% of larval coregonines to species.
- The new assay successfully identified larval coregonines to species 81.2 ± 20.2%.
  - Resulting in a 62.2% ± 21.5% increase relative to the modified assay in species identification (V = 0, p = .002).

Discussion and Future Work

- The new assay provided more confidence and ability to make accurate genotyping calls. Tray 4 saw a loss of 26 LWF in the new assay, as the modified had difficult to interpret genotypes.
- Currently working to validate the new assay working with contemporary samples and using the modified George et al. (2018)[1] to compare effectiveness.
- We may convert assay to more modern genotyping approaches:
  - Quantitative Polymerase Chain Reaction (qPCR TaqMan) Assay
  - Genotyping-in Thousands by sequencing (GT-Seq).

Learn more at sardlab.com/

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References

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