

Development of a faster and cheaper method to detect *Ichthyomyzon* species lamprey using eDNA



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Background

- Invasive sea lamprey (*Petromyzon marinus*) damage commercial & recreational fish populations in the Great Lakes
- Sea lamprey control has been used in the Great Lakes since 1955
- Lamprey control is the greatest threat to the four native species of lamprey (Fig. 1)^{1,2,3,4}
- Three native species belong to the *Ichthyomyzon* genera & are also native to Manitoba; silver (*Ichthyomyzon unicuspis*), chestnut (*Ichthyomyzon castaneus*) & northern brook (*Ichthyomyzon fossor*) lamprey⁵



Figure 1. Invasive sea lamprey (*Petromyzon marinus*, right) with the four native species of lamprey (left) in the Great Lakes.

Hypothesis

Ichthyomyzon spp. lamprey have a section of their genome that is distinct from other genera of lamprey.

Methods

- Choose target sequence by comparing sequences of native species
- Design primer & probe using Primer-BLAST & Primique software programs
- Assess specificity & properties of primer & probe
- Determine efficiency, reproducibility & validity of primer & probe
- Use samples of tissue-derived lamprey DNA & water from tanks containing live lamprey
- Collect & analyze water samples from known habitats for native lamprey

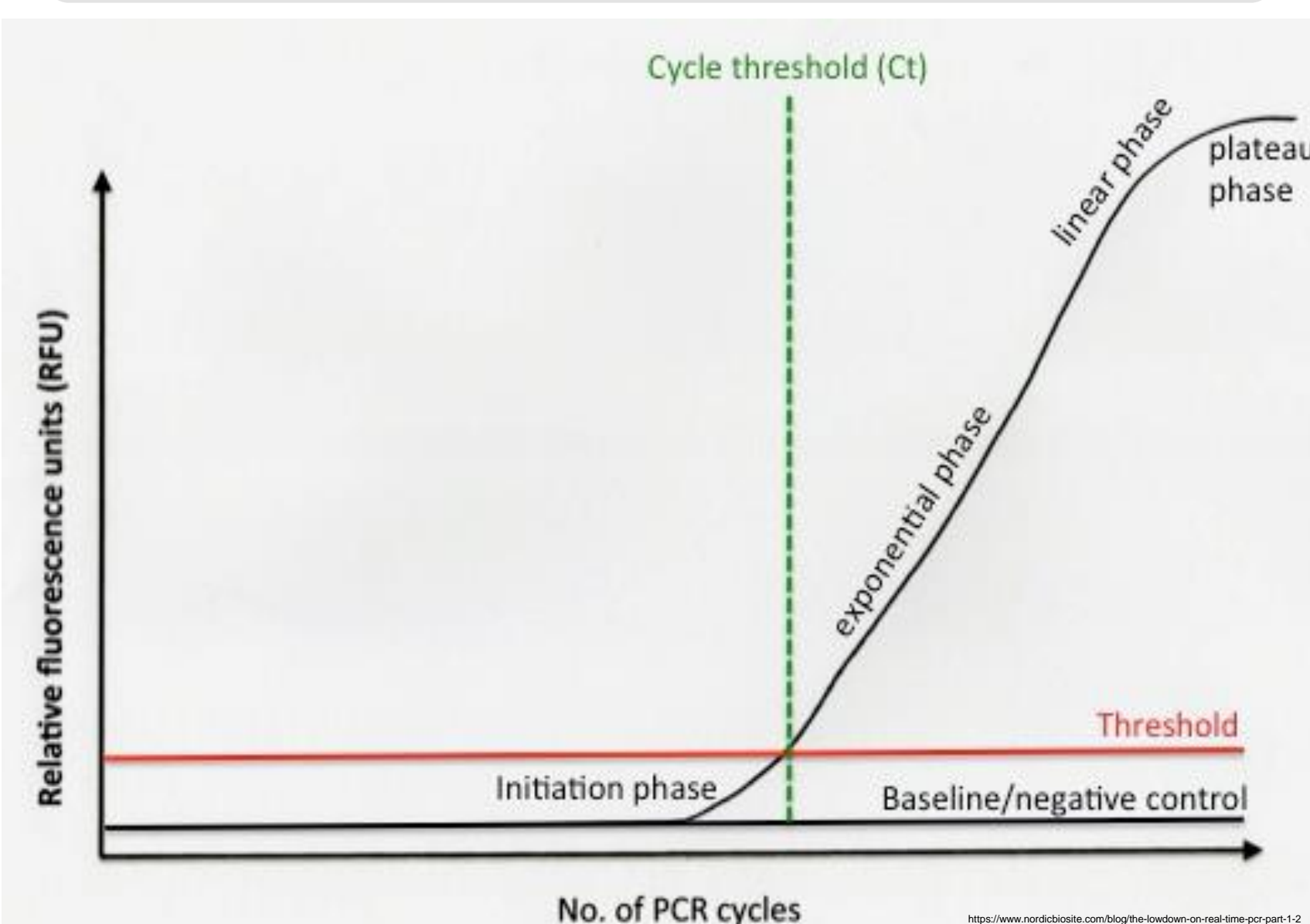


Figure 2. An amplification curve, generated by the qPCR machine in real time when detection of target has occurred.

Polymerization

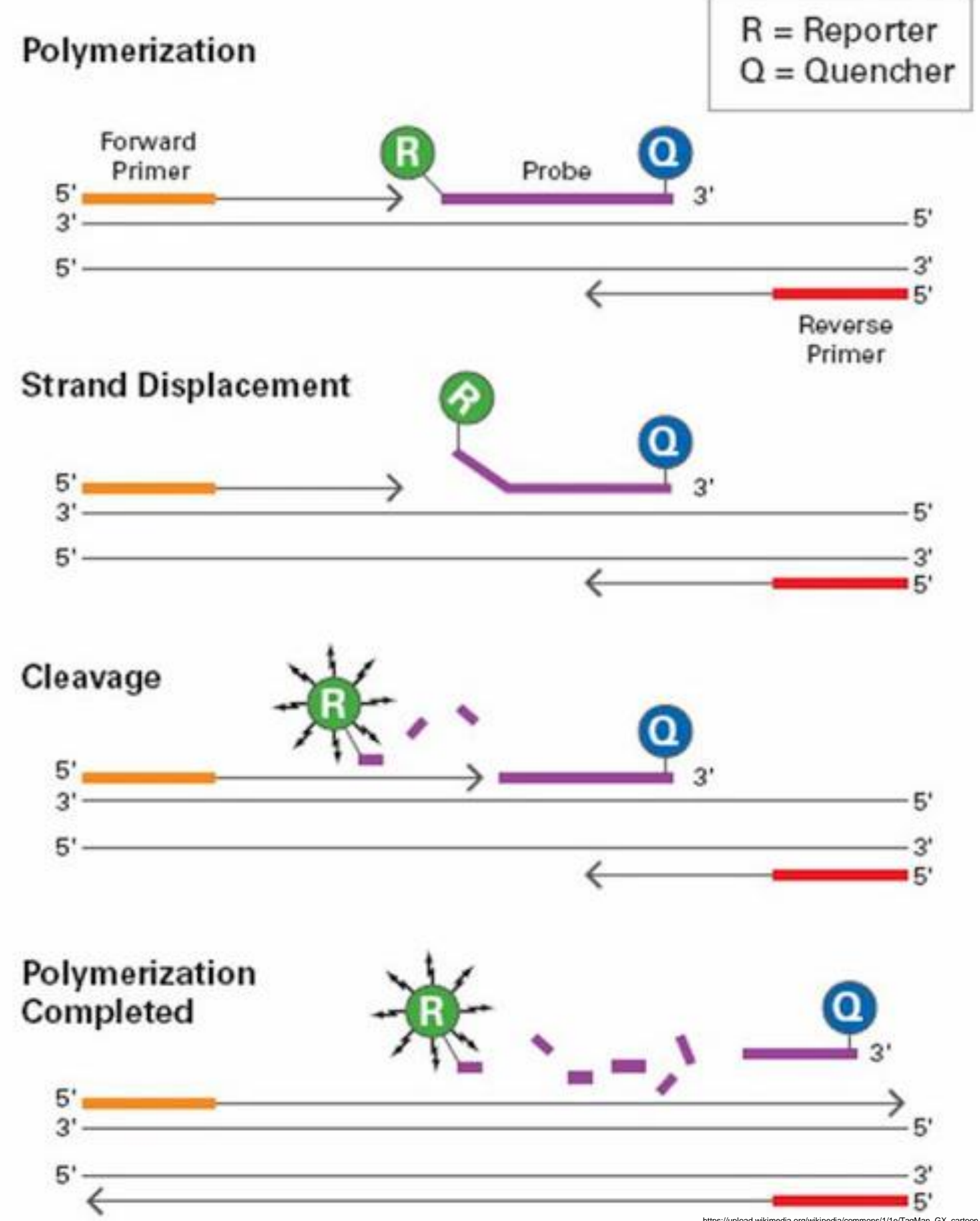


Figure 3. Process of PCR using Taq™ polymerase, sequence-specific primers & a fluorescent probe.

Results

- The shared sequence in the genome of *Ichthyomyzon* spp. lamprey can be used to create genus-specific primers & probes for qPCR assays

Statistical Analyses:

- ANOVA to compare cycle threshold values among treatments (Fig. 2)
- Fisher's Exact Test to compare percentage of samples & technical replicates with successful amplification

Conclusion

- Fast & cost-effective confirmation of presence/absence of native lamprey in sites of interest for sea lamprey control is essential to the conservation of the four native lamprey species
- Development of a genus-specific qPCR assay for *Ichthyomyzon* spp. lamprey would allow for detection of three native species of lamprey in the Great Lakes & Manitoba at once
- Future research could focus on the development of lamprey control that is specific to sea lamprey

References

- ¹COSEWIC (2007) COSEWIC assessment and update status report on the northern brook lamprey *Ichthyomyzon fossor* (Great Lakes-Upper St. Lawrence populations and Saskatchewan-Nelson population) in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa
- ²COSEWIC (2010) COSEWIC assessment & status report on the Chestnut Lamprey *Ichthyomyzon castaneus* (Great Lakes-Upper St. Lawrence populations and Saskatchewan-Nelson River populations) in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa
- ³COSEWIC (2011) COSEWIC assessment and status report on the Silver Lamprey, Great Lakes-Upper St. Lawrence populations and Saskatchewan-Nelson Rivers populations *Ichthyomyzon unicuspis* in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa
- ⁴Maitland, P.S., Renaud, C.B., Quintella, B.R., Close, D.A., Docker, M.F. (2015) Conservation of Native Lamprey. In: Docker M. (eds) Lampreys: Biology, Conservation and Control. Fish & Fisheries Series 37, Springer, Dordrecht, pp 375-428
- ⁵Renaud, C.B., Docker, M.F., Mandrak, N.E. (2009) Taxonomy, Distribution, and Conservation of Lampreys in Canada. In: Brown LR (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society, Symposium 72, Bethesda pp 293-310

Objective

To develop a method to detect native lamprey species in areas of interest for sea lamprey control in the Great Lakes that is more rapid & cost-effective using eDNA & a genus-specific quantitative PCR (qPCR) assay (Fig. 3).

Acknowledgements

Thank you to Dr. Jane Waterman, Dr. Margaret Docker, Arfa Khan & the class of BIOL 3100 for their guidance & advice in the development of this project.