eDNA Assay Development for the Detection of the Native Crayfish Species *Faxonius virilis* (Northern Crayfish)

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PROJECT OBJECTIVE

The goal of this project is to develop an assay for the detection of *Faxonius virilis* (Northern Crayfish) environmental DNA collected from water samples and quantified using qPCR.

METHODS

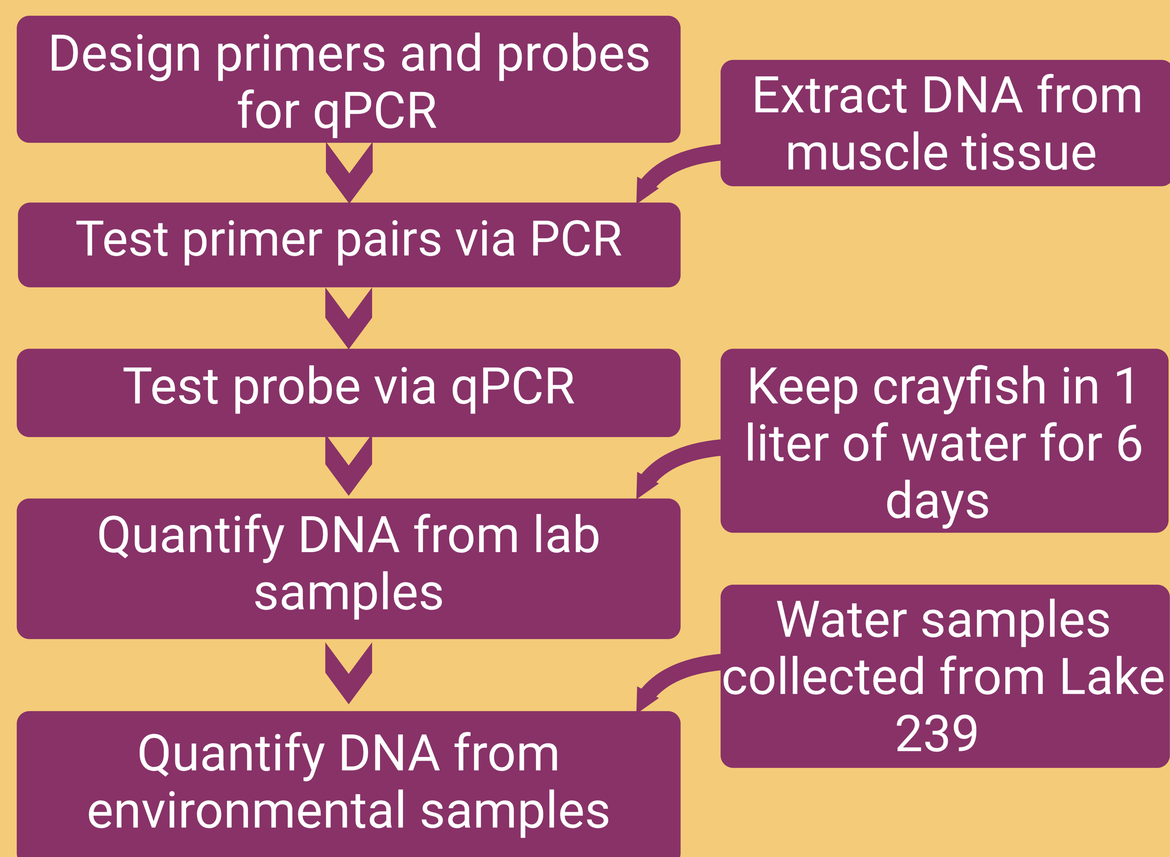

Figure 1: Flow chart showing the process of designing and validating a qPCR assay for detection of the Northern Crayfish.

- Assay development for this project followed the steps laid out in Figure 1
- Lab sample DNA was quantified to validate that the assay is capable of detecting target DNA under controlled conditions
  - Additionally, a gravel and no gravel treatment were compared with a paired t-test to assess for differences in DNA detected (Figure 2)

Figure 2: Northern Crayfish housed in a small volume of water to produce lab samples for assay validation and testing, no gravel (left) and gravel (right) treatments shown.

PROGRESS AND RESULTS

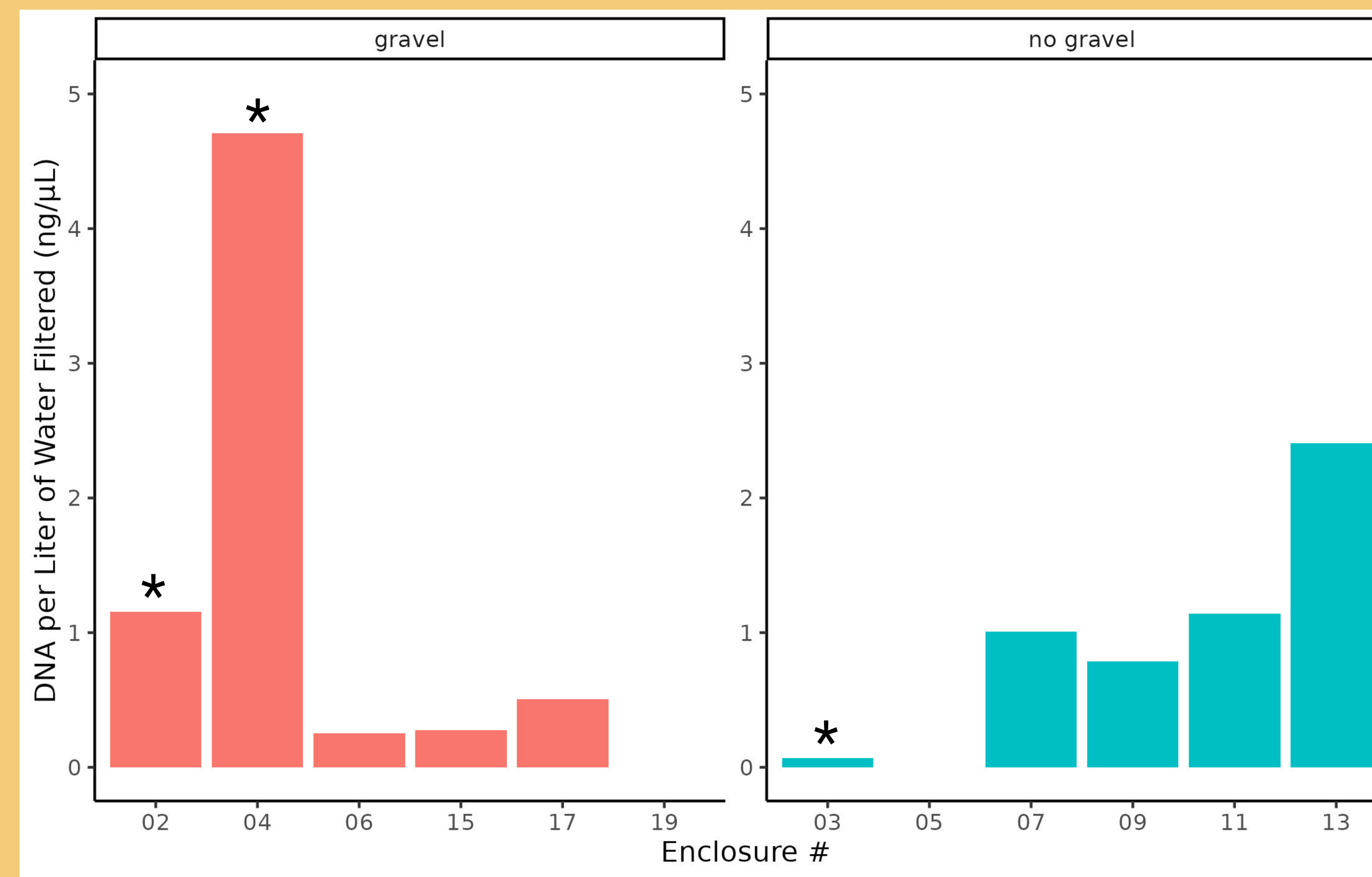

Figure 3: Plot showing the concentration of DNA detected per enclosure (n=12). All control enclosures resulted in a detected DNA concentration of 0 ng\µL (n=6, not shown), enclosures marked with * indicate enclosures in which the crayfish molted during the experiment.

- The assay was successful at detecting DNA in 10/12 enclosures (Figure 3)
  - No DNA was detected in 2 experimental enclosures (05, 19)
  - All control enclosures resulted in no DNA being detected
- Several crayfish molted during the experiment, this was determined by t-test to be insignificant
  - T-test values: $t(2.06)=-1.099$, p-value=0.3834
- Initial analysis suggests the difference in DNA concentration between treatments is not significant
  - T-test values: $t(12.33)=0.287$, p-value=0.7792

CHALLENGES AND NEXT STEPS

- Two sets of primers were ordered and tested *in vitro*.
  - Both primer sets were unsuccessful, did not amplify target DNA during qPCR testing
- Additional primer pairs being designed *in silico* were being marked as having the potential to form hairpin structures and primer dimers
  - The potential for hairpin structures and primer dimers was acknowledged but minimized as much as possible by applying recommendations from the literature.
- The gravel and no gravel treatments are to be evaluated further by pairing crayfish individuals by weight and re-analysing
- Additional tasks remaining are to quantify the DNA content of field samples and evaluate the statistical significance of the detection ratios

ACKNOWLEDGEMENTS

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