Finding the optimum sample volume for eDNA detection of *Faxonius rusticus*

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Introduction

Environmental DNA fragments are deposited into the environment (soil, water column, ground etc.) via cellular material shed by organisms such as mucus, feces and skin. Environmental DNA can be used as a detection tool for species including invasive and difficult to detect organisms (Dougherty et al., 2016).

Because of the increasingly widespread use of this mini barcoding method, it is paramount that there is a level of standardization in how eDNA is being used in detection of species. However, due to the precise nature of capturing environmental DNA and using it as a detection tool, the protocol must be altered on a case-by-case basis because of the fundamental differences that lie between each species and ecosystem.

As a result of this, our aim in this study was to first gain a sound understanding of environmental DNA and to develop a protocol for the optimal method to detect the presence of the Rusty Crayfish (*Faxonius rusticus*), an invasive species found in the streams and ponds of Ithaca.

Figure A (left) shows the *Faxonius rusticus*. Figure B (right) shows the Tridacna crocea embedded in a coral.

Research question

The aim of our study was to find the optimum volume at which we could detect the presence of the Rusty Crayfish (*Faxonius rusticus*), an invasive species of crayfish which has been previously sighted in the streams and lakes in Ithaca NY. We hope to use this information to inform for a sampling strategy of collecting the environmental DNA of a cryptic marine species living in a tropical environment, the *Tridacna Crocea* (Crocus clams).

Methodology

- Our research was carried out in two visits, over a two-week period.
- Our sampling sites were located on Fall Creek, Ithaca, NY.
- In the first week we collected three replicates of sample volumes of 500mL, 1L, 1.5L and 2L including field blanks for each volume.
- We adjusted the volumes picked for the second week and instead collected two replicates of samples of 300mL, 1L and 1.5L.
- DNA was extracted from the sample using QIAAmp mini kit. QPCR analysis was carried out on the extracted DNA.

Figure C shows the set-up of the filtering station.

Results

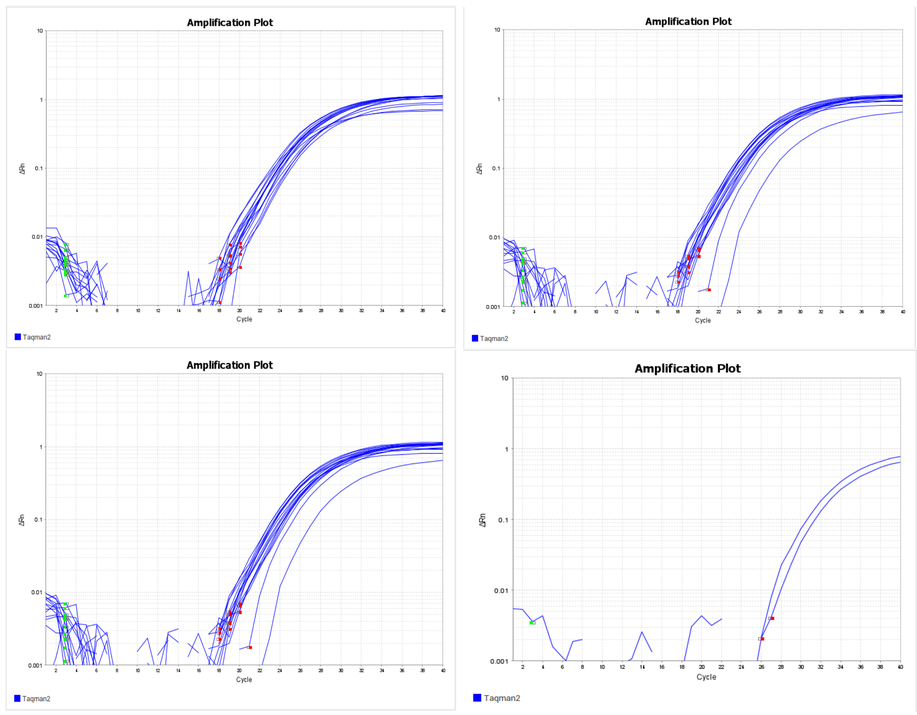

Figure E (top left) shows amplification of the sample between June 24th-26th. Figure F (top right) shows amplification of samples between July 4th-6th. Figure G (bottom right) shows the amplification curves of the field blanks between July 4th-6th. Figure H (bottom right) shows the amplification curve of two different volumes, 300mL and 1.5mL.

Discussion

- Positive amplification curves support the fact that the *Faxonius rusticus* are present in Fall Creek, Ithaca, NY.
- Following visual observation, there was no significant difference between the rate of amplification of the different volume being used.
- The cycle thresholds of the samples were between 22 and 24, indicating high copy numbers of eDNA, which may be due to contamination from the FaRu standards
- We detected amplification in our field blanks and in our No Template controls This tells us that there was DNA contamination in our method, and because of this, we cannot be certain that our positive sample plots are also not the result of contamination as well
- Upon further validation, we found that the master mix, primers and probes were contaminated with standards which may be a cause of the contamination of our field blanks.
- Despite the lack of confidence in our results, thorough literature review has allowed us to endorse the use of smaller volumes in eDNA sampling for the Rusty Crayfish (Muha et al. 2019).

Future Research

- Further experimentation must be carried out to create a protocol for the optimum sampling method to attain the environmental DNA of the Giant clam.
- However, since the Crocus clam lives in a Tropical marine environment, there are other factors which we must consider in our approach including transport and storage of the samples, eDNA degradation as well as the volume of samples.
- Our desired approach includes combining multiple species detection methods in addition to eDNA.

References

Dougherty MM, Larson ER, Renshaw MA, Gantz CA, Egan SP, Erickson DM, Lodge DM. 2016. Environmental DNA (eDNA) detects the invasive rusty crayfish. Orconectes rusticus at low abundances. Journal of Applied Ecology. 53(3):722–732.

Muha, T. P., Robinson, C. V., De Leaniz, C. G., & Consuegra, S. (2019). An optimised eDNA protocol for detecting fish in lentic and lotic freshwaters using a small water volume. PLoS ONE, 14(7), e0219218

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