

Introduction

- **Environmental DNA (eDNA):** the presence of small fragments of an organism's genetic material left behind in their environment. (Diaz-Ferguson & Moyer, 2014)
 - These fragments of eDNA can be released into the water column through shed skin cells, waste, and mucus.
 - eDNA is especially useful when looking for animals whose populations are of great concern, such as rare, endangered, elusive, or invasive species. (Bessell et al., 2023)
- **eDNA persistence,** the length of time eDNA fragments remain in the water column, can vary in different aquatic systems depending on environmental factors such as stream flow, pH, salinity, and temperature. (Barnes et al., 2014; Shogren et al., 2018; Curtis et al., 2021)
- **Rusty crayfish (*Faxonius rusticus*):** an invasive species in the Cayuga Lake watershed that has the potential to disrupt delicate balances within this freshwater system. (Larson et al., 2017)
 - Known to cause a decline in aquatic plants, crayfish that are native to the system, and macroinvertebrates
- **Crocus clam (*Tridacna crocea*):** a member of the giant clam family that faces anthropogenic pressures such as habitat destruction and over-harvesting. (Neo et al., 2017; Li et al., 2024)
- **Commons Biodiversity Project:** In collaboration with a team of scientists at the Universiti Malaysia Terengganu, the Commons Biodiversity Project aims to use eDNA techniques to help conserve Crocus clams found in the South China Sea off of Terengganu. This project aims to foster and grow a passion for marine wildlife conservation in the local population of Terengganu, and highlights the importance of collaboration between scientists for opportunities rich in learning and sharing different techniques, asking questions together, and furthering a foundation for the project based on community involvement.
- **Research Question:** How do detections of the invasive rusty crayfish (*Faxonius rusticus*) compare between Fall Creek and two ponds in the Cornell Arboretum?

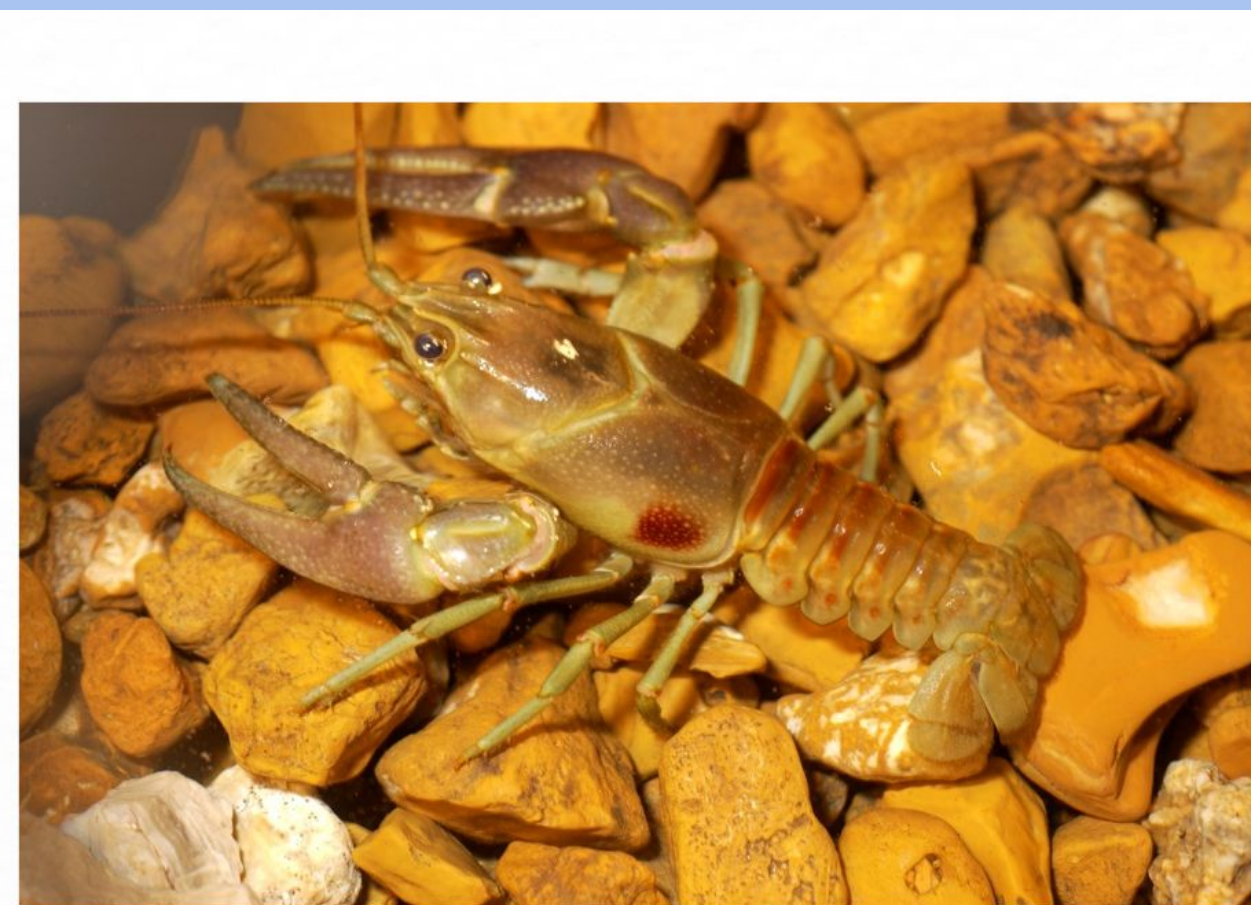


Figure 1. Rusty crayfish (*Faxonius rusticus*)



Figure 2. Crocus clam (*Tridacna crocea*)

Methodology

- Sampling sites (see **Figure 1**): Fall Creek (42.4540029, -76.4569845) and two ponds in the Cornell Arboretum - Houston Pond (42.4508221, -76.4555532) and Houston Pond Adjacent (42.452086, -76.4545786)
 - Samples collected the weeks of June 10, 2024 and July 8, 2024
 - 2 or 3 replicates of samples collected at each site
- Active filtering of 1 L samples and 300 mL nanopure water field blank through filter paper. Filtration was done using a hand pump apparatus (see **Figure 2**)
- DNA extraction from each sample and control using QiaAmp DNA Mini Kit
- Real-time quantitative PCR (qPCR) analysis to quantify any rusty crayfish eDNA collected

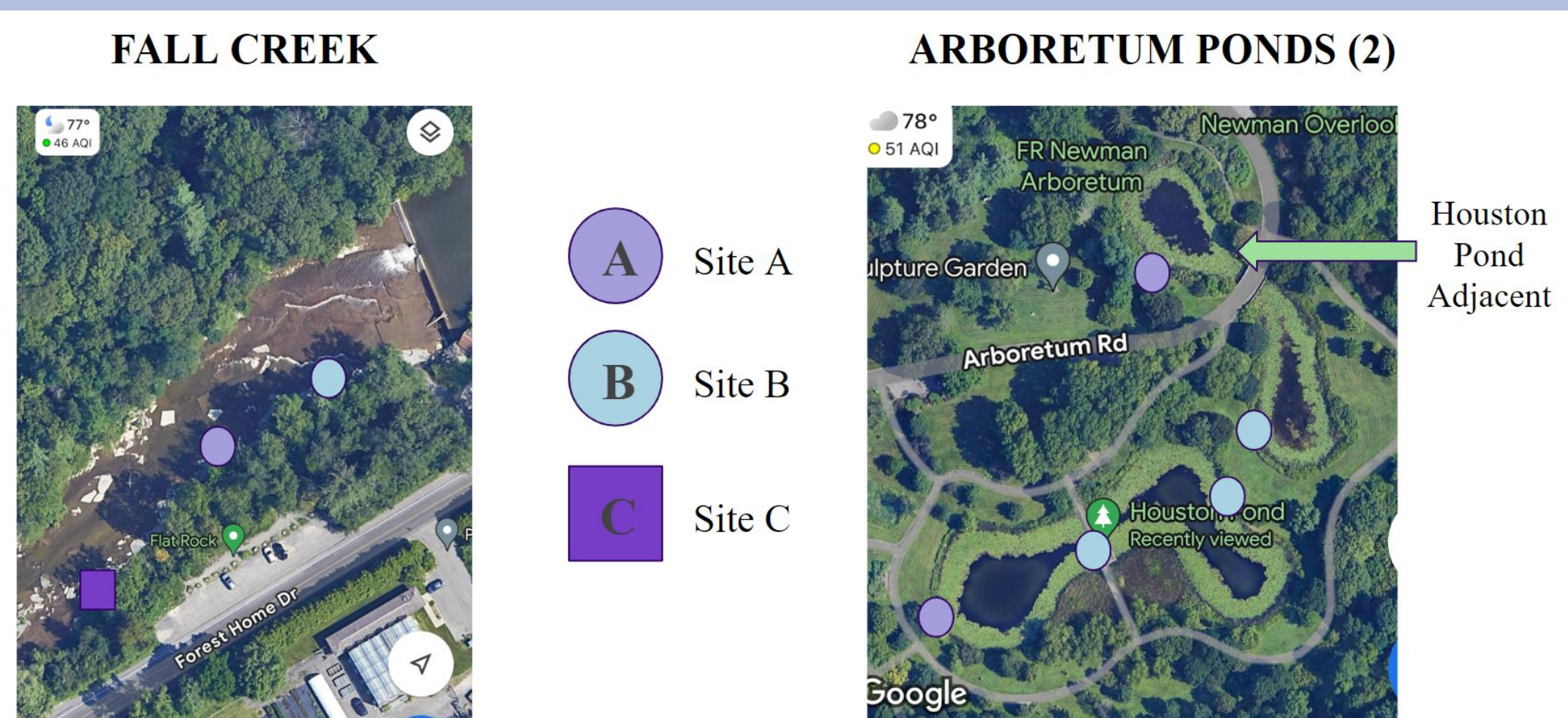


Figure 3. Stream vs. Pond sampling sites Fall Creek (left), Houston Pond (bottom right), and Houston Pond Adjacent (top right).



Figure 4. Active filtering apparatus assembled at Fall Creek.



Figure 5. Fall Creek (left) and Houston Pond Adjacent (right)

Results

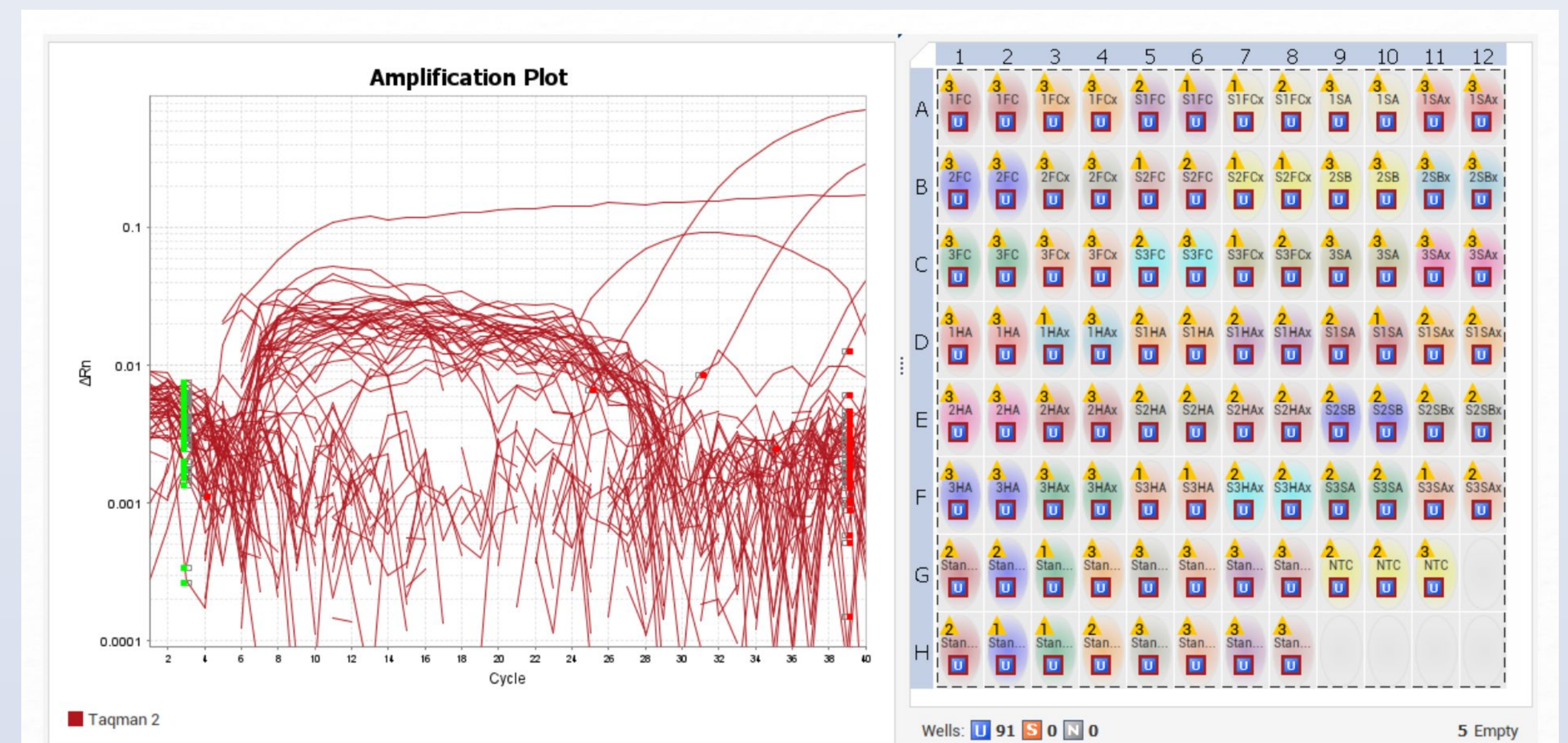


Figure 6. The post-qPCR run amplification plot produced in QuantStudio 3 Analysis & Design Software for the Stream vs. Pond assay performed on June 24, 2024.

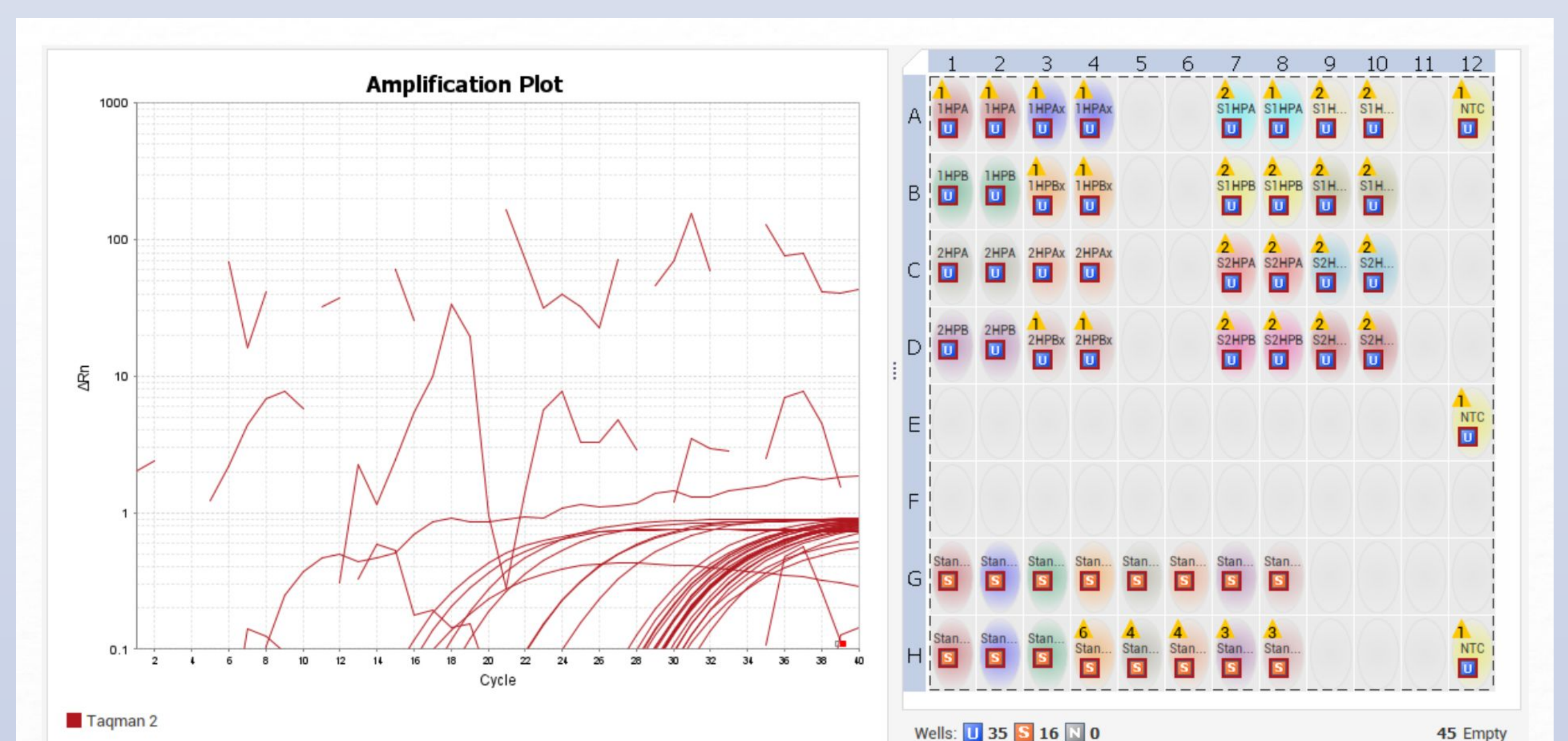


Figure 7. The amplification plot for the Houston Pond assay performed on July 12, 2024.

- June 24, 2024 Assay (**Figure 6**): No amplification observed in any Fall Creek Site C samples or controls, and the same was observed for the Houston Pond Site A and Houston Pond Adjacent samples and controls. However, for the standards included in the assay, only Standard 3 had amplification, while the other standards did not.
- July 12, 2024 Assay (**Figure 7**): We observed amplification in samples, field blanks, and NTCs. However, the spiked samples did not appear at all on the amplification plot.

Conclusion

- Despite thorough decontamination procedures, using the same set of micropipettes for samples, qPCR reagents, and standards resulted in the contamination of the micropipettes, which affected the assays.
- Using too high concentration in the standards not only had the potential to contribute to contamination, but also affected to amplification we saw in serial dilutions and spiked samples.
- Our technique was successful in confirming the presence of the rusty crayfish in Fall Creek, where we have seen it before.
- Due to amplification in our no-template controls and field blanks, we are not able to confidently conclude the eDNA assay's detection of this species in the Cornell Arboretum ponds.
- We will apply these takeaways regarding proper decontamination, plating techniques, and sources of contamination next summer, as we work for the Commons Biodiversity Project to conserve the Crocus clam (*Tridacna crocea*) in Terengganu, Malaysia.

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