

The background is a dark blue gradient with a starry texture. On the left side, there are several overlapping circular elements. A prominent one is a large arc with a scale from 140 to 260 in increments of 10. Other circles include dashed lines, solid lines, and arrows, suggesting a technical or scientific theme.

DESIGNING PILOT STUDIES FOR EDNA APPLICATIONS

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CRITICAL CONSIDERATIONS – RECOMMENDATIONS

(GOLDBERG ET AL. 2016)

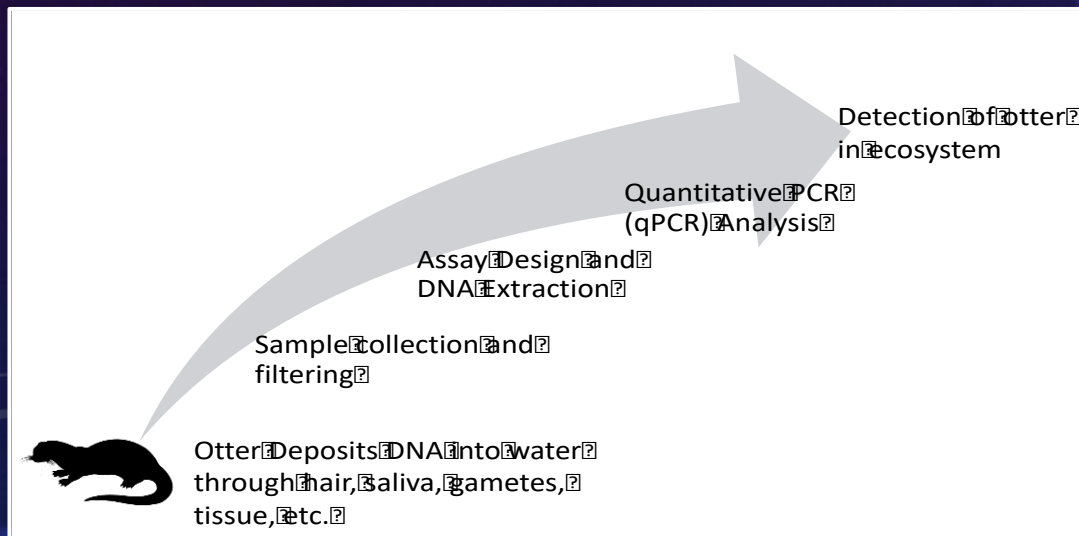
PILOT STUDY

- 🛡️ Implement field sampling protocol and evaluate detection rates
- 🛡️ Test extraction and analysis protocols
- 🛡️ Validate eDNA assays *in silico*, *in vitro*, and *in situ*



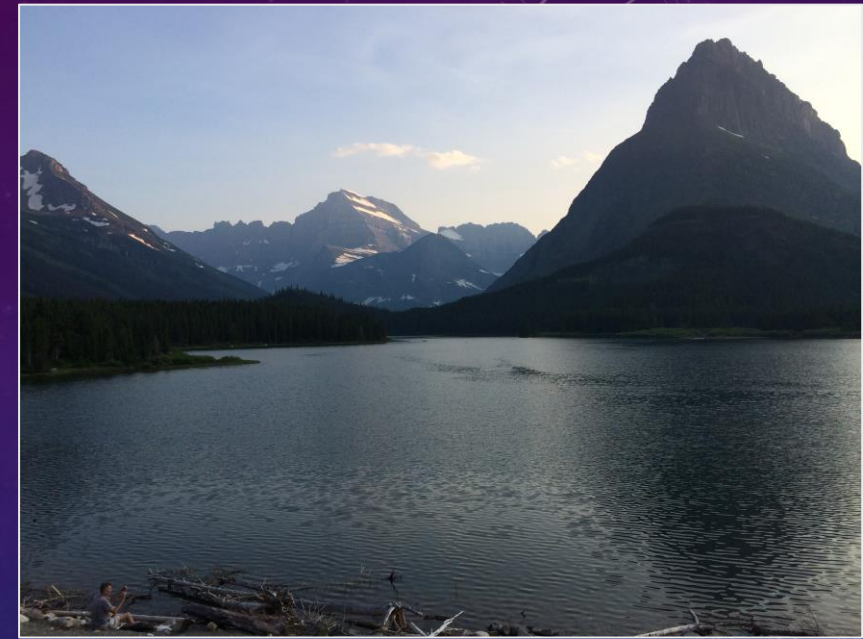
WHAT IS A PILOT STUDY?

- A study where you apply the exact same protocol that you will use in the larger application to a select number of sites.
- This is **both field and lab protocols.**
- Can also be used to test protocol variations (filter type, etc.)



WHY DO WE NEED PILOT STUDIES?

- eDNA is not one method that can be universally developed and applied



WHY DO WE NEED PILOT STUDIES?

Detect issues with sample collection

- ☛ Contamination between samples
- ☛ Sample degradation
- ☛ Filter clogging



WHY DO WE NEED PILOT STUDIES?

Detect issues with sampling design

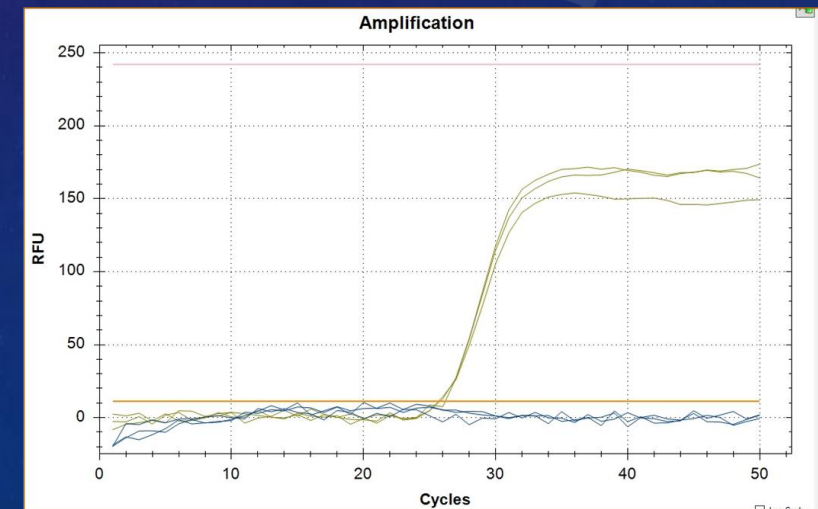
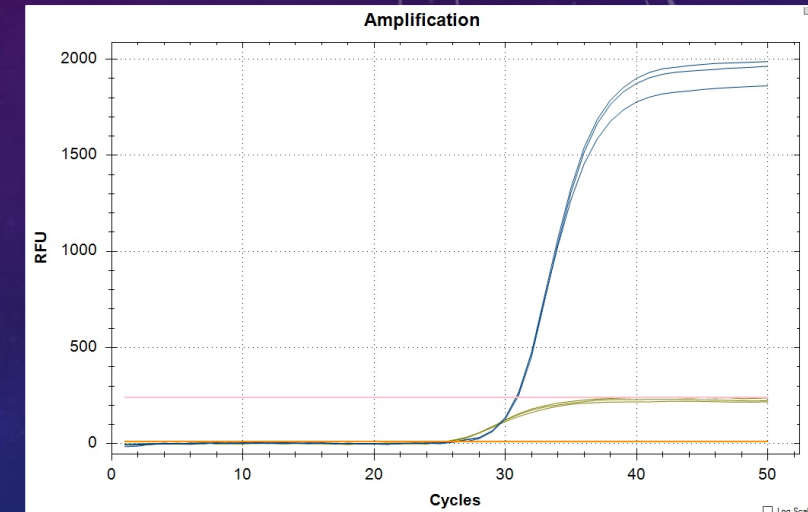
- Lack of detection indicating need for more samples (or more volume, etc.)
- Consistent detections indicating optimal number of samples



WHY DO WE NEED PILOT STUDIES?

Detect issues with assay validation

- 🐛 **False positives** (cross-amplification with other species)
- 🐛 **False negatives** (lack of amplification when the species is present)



GENERAL PILOT STUDY DESIGN

- A few sites where the species is fairly common
- A few sites where the species is likely absent
- A few sites where the species is at low density

More if you have a gradient of:

- Inhibitors (tannins, etc.)
- Degradative conditions (high temperatures, acidity)
- Other variation (salinity)



EXTERNAL VALIDATION

It can be helpful to pair a pilot eDNA study with field sampling **but...**

...we are often trying out eDNA sampling because field sampling is inaccurate or really challenging



PILOT STUDIES IN REALITY

- Typically these elements are incorporated into a study in a new environment rather than done first
- Sometimes this saves money, other times an error is made that causes a failure to meet project goals

Strengths, limitations, and practical considerations of using eDNA techniques to detect wildlife and their pathogens. A webinar hosted by the **Canadian Herpetology Society on Zoom** on 18 Nov 2020

Increasingly, environmental DNA (eDNA) approaches are being incorporated into projects that seek to detect and monitor wildlife and their pathogens. The long list of highly desirable attributes of eDNA approaches includes the relative ease and non-invasive nature of collecting samples. However, eDNA approaches are not a panacea. They are vulnerable to poor study design, improper sample collection and storage, and a slate of potential issues in the lab come time to test the samples using PCR-based assays. **It has been our collective experience that many studies that start out with the best of intentions end in meaningless and unpublishable eDNA results, frequently as a result of avoidable pitfalls.** Please join us for a brief overview of what eDNA approaches entail followed by a panel discussion that will touch on practical considerations and recent developments from both field and lab perspectives.

“It has been our collective experience that many studies that start out with the best of intentions end in meaningless and unpublishable eDNA results, frequently as a result of avoidable pitfalls.”

OVERALL CONCLUSIONS

- Pilot sampling is very useful to identify and solve problems with lower investment of resources
- Many eDNA studies so far are actually pilot studies – more about demonstrating possibilities than wide-ranging monitoring
- If done well, pilot studies can inform the best way to sample (including whether sample size is adequate)