Applying eDNA for Species Detection: Tips for Successful Integration

EnviroTech 2021

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SLR Consulting (Canada) Ltd.

global **environmental** and **advisory** solutions

Overview

- Environmental Biomonitoring & Conventional Techniques
- What is eDNA?
- How is eDNA testing conducted?
- eDNA Benefits
- Tips for Implementing eDNA Surveys
- Questions/Comments

Environmental Biomonitoring Programs

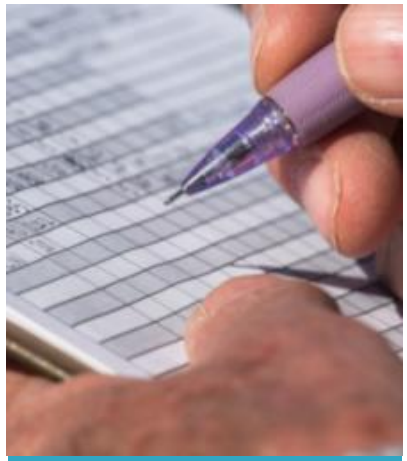
Environmental Biomonitoring Programs

Conventional Techniques – Potential Drawbacks

Resources

Cost

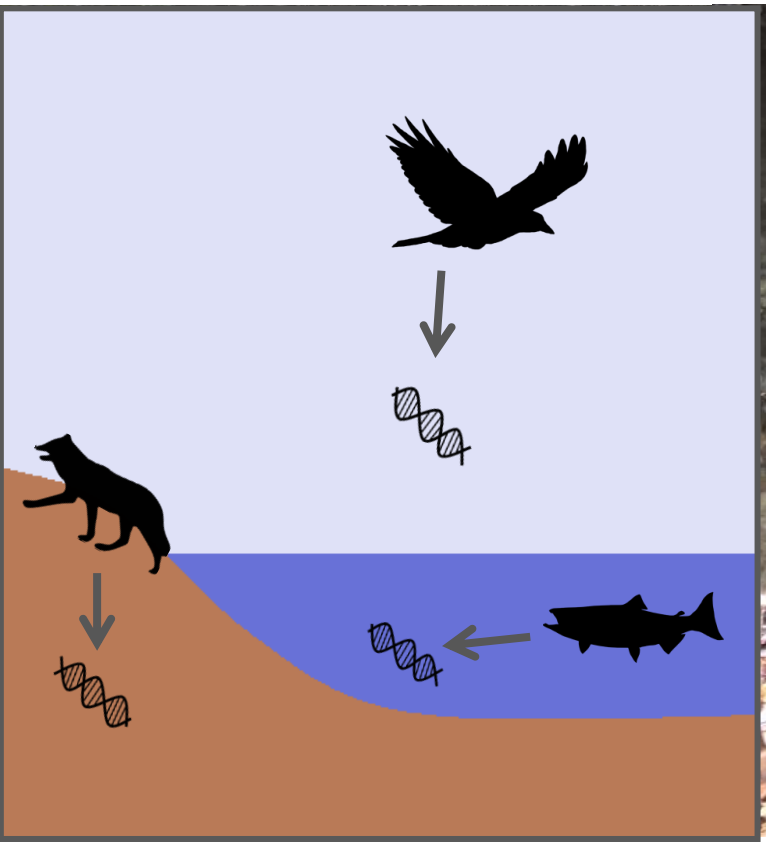
Time



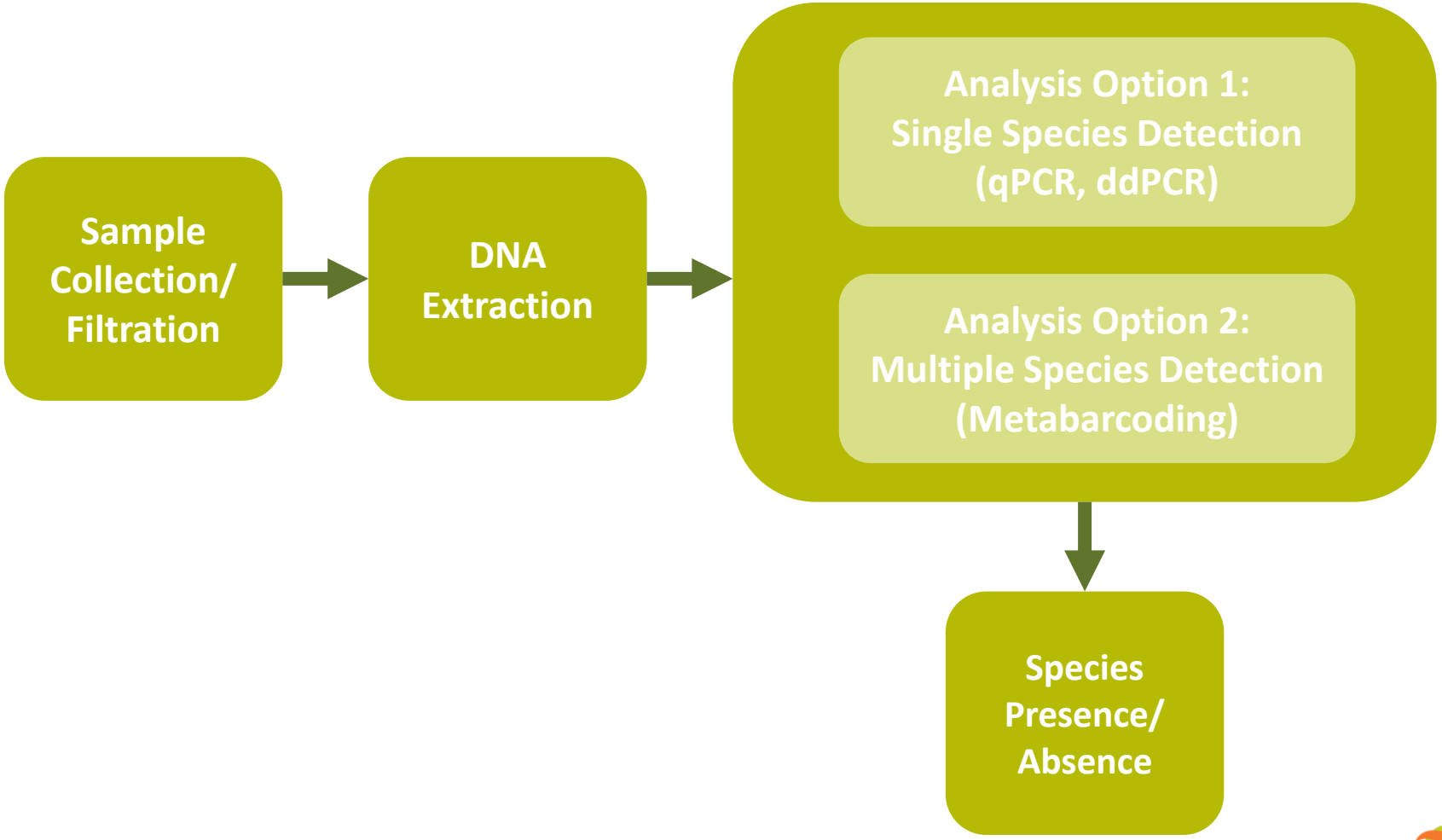
Accuracy

Environmental Biomonitoring Programs

What is “eDNA?”

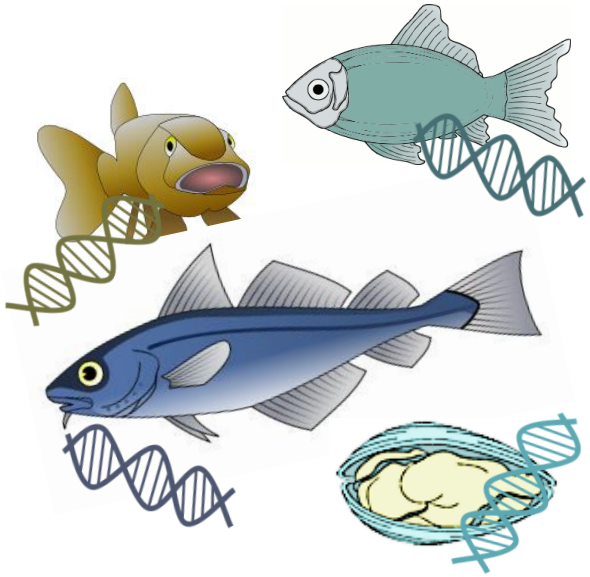


How is eDNA Testing Conducted?

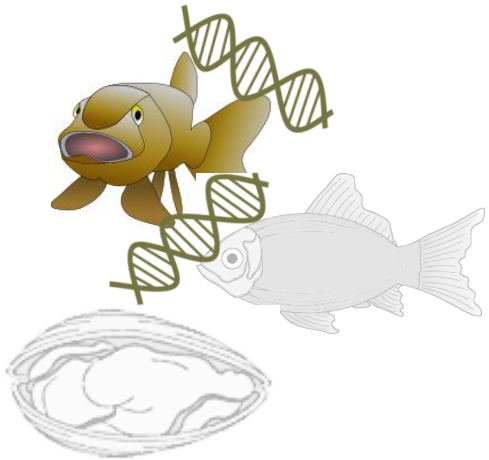


eDNA for Biomonitoring

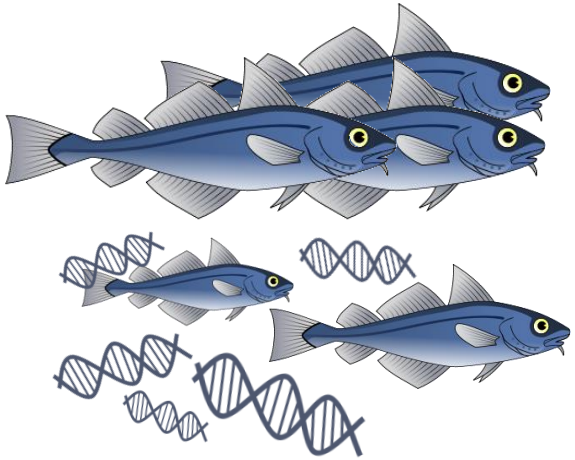
- eDNA can characterize diversity on several scales:



Community



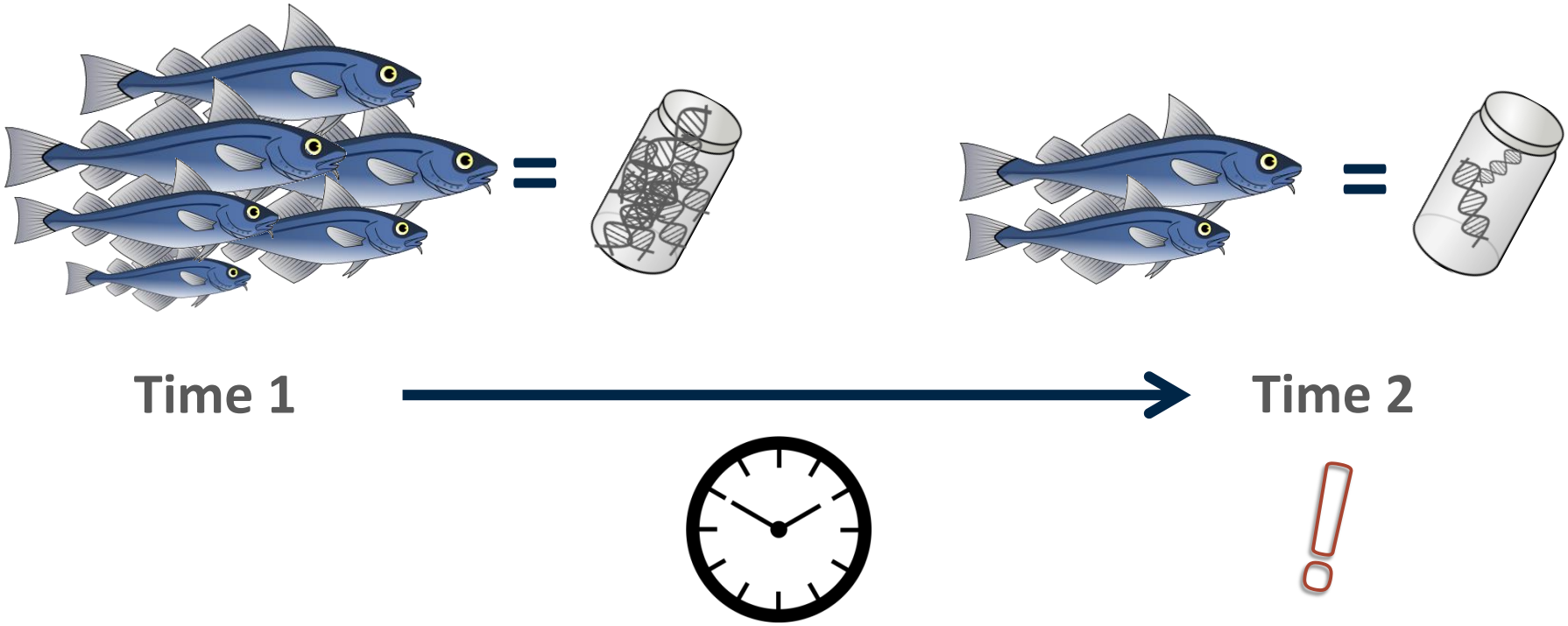
Species



Population

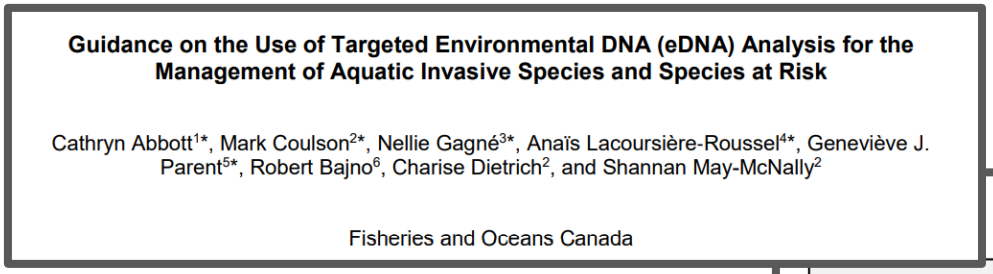
eDNA for Biomonitoring

- eDNA can also reflect seasonal or temporal changes in relative abundance:



Other Benefits

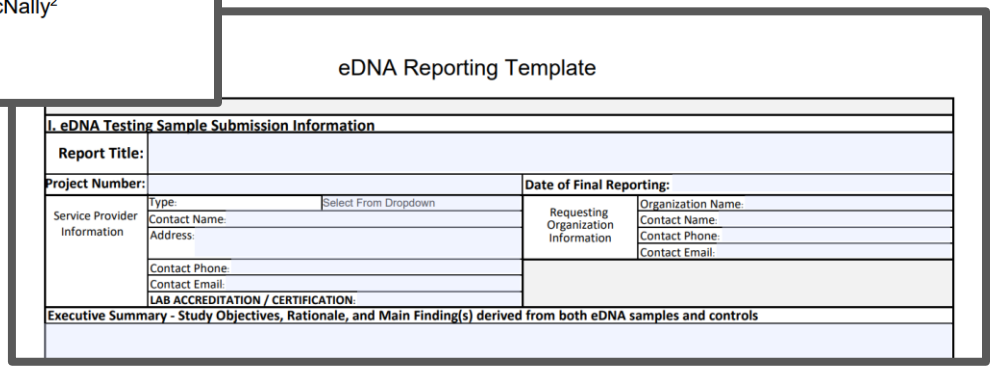
- Can eliminate the need for collection permits
- Suitable for rare, invasive, or elusive species detection
  - Early warning system
- Increasing application in industry and government sectors:
  - **Department of Fisheries and Oceans (DFO):** eDNA Guidance Doc + Reporting Template



Guidance on the Use of Targeted Environmental DNA (eDNA) Analysis for the Management of Aquatic Invasive Species and Species at Risk

Cathryn Abbott[1]*, Mark Coulson[2]*, Nellie Gagné[3]*, Anaïs Lacoursière-Roussel[4]*, Geneviève J. Parent[5]*, Robert Bajno[6], Charise Dietrich[2], and Shannan May-McNally[2]

Fisheries and Oceans Canada



eDNA Reporting Template

I. eDNA Testing Sample Submission Information				
Report Title:				
Project Number:			**Date of Final Reporting:**	
Service Provider Information	Type:	Select From Dropdown	Requesting Organization Information	Organization Name:
	Contact Name:			Contact Name:
	Address:			Contact Phone:
	Contact Phone:			Contact Email:
	Contact Email:			
	LAB ACCREDITATION / CERTIFICATION:			
Executive Summary - Study Objectives, Rationale, and Main Finding(s) derived from both eDNA samples and controls				

Fisheries and Oceans Canada

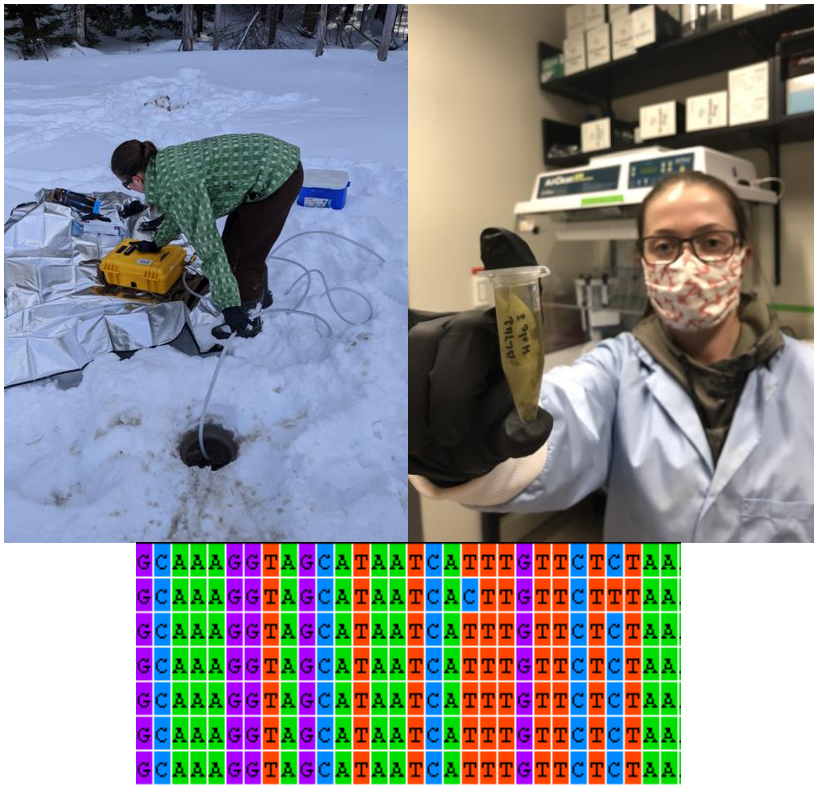
eDNA Survey Design

Multiple Stages:

- Field
- Laboratory
- Data Analysis

Survey Aspects:

- Pilot Studies
- Field Collections/Sampling Design
- Assay Selection
- Results Interpretation
- Strengths and Limitations



eDNA Survey Design – Pilot Studies

- Allow clients to optimize eDNA methodologies before scaling up
- **Other Benefits**:
  - Opportunity to test the chosen analysis method on locally sourced specimen(s) or any subsequent sequence data, if available, to verify positive amplification/assay binding from a geographically relevant source
  - Gain further insight on the number of samples to collect based on site characteristics
  - Impact of seasonality on field collection, target species activity, eDNA persistence
  - Can alter DNA extraction and amplification techniques based on the pilot if environmental inhibition is encountered
  - Compare/contrast eDNA species detection to traditionally collected data
    - (e.g. Bat acoustic surveys, electrofishing, frog call surveys)

eDNA Survey Design – Field Collections

Questions to ask:

- **What will you be collecting?**
  - Water, Feces, Soil?

eDNA Survey Design – Field Collections

Questions to ask:

- **How will you collect it?**
  - Pump/Filter, Bottle, Collection Tubes?

eDNA Survey Design – Field Collections

Questions to ask:

- **How will you preserve your samples in transport and before processing?**
  - EtOH, silica gel, frozen?

eDNA Survey Design – Field Collections

Questions to ask:

- **How will you prevent contamination when handling samples?**
  - Decontaminate all field gear with 10-50% bleach solution, Eliminase, etc. between surveys
  - PPE & consumables: nitrile gloves, forceps, plastic bags, dedicated coolers/containers
  - eDNA “Clean Rooms” (for post-collection processing)

eDNA Survey Design – Field Collections

Questions to ask:

- **What field controls will you have?**
  - E.g., Deionized water samples filtered onsite per day

eDNA Survey Design – Field Collections

Questions to ask:

- **What is the waterbody type?**
  - Wetland? Flowing river? Stagnant pond? Lake?

eDNA Survey Design – Field Collections

- **Who are your target species?**
  - What are their life histories? What time of year are they most active (i.e. shedding more eDNA)?

eDNA Survey Design – Field Collections

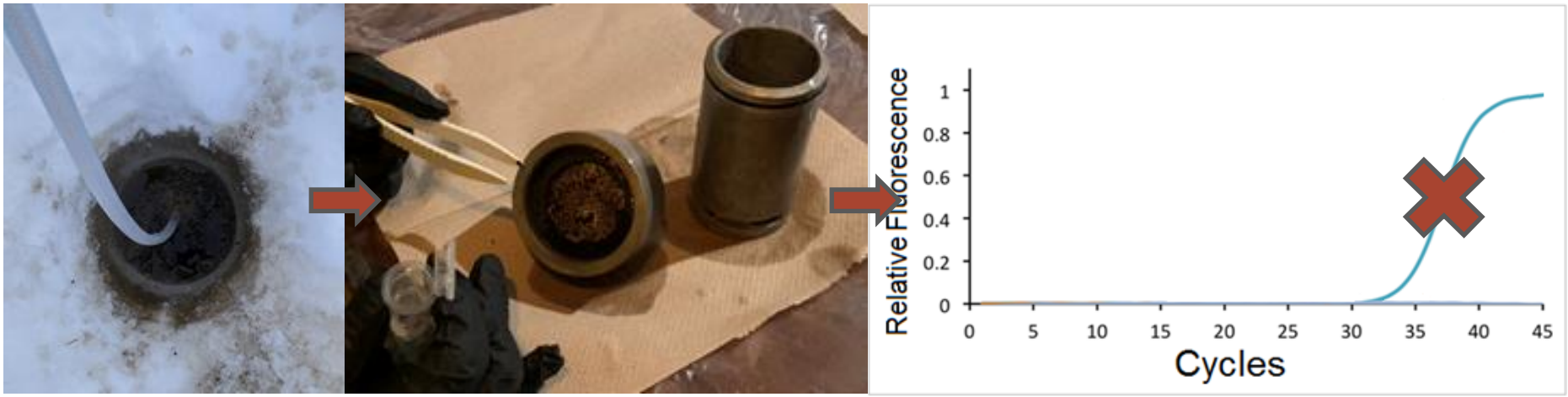
- **How many samples should you collect? What volume of water?**
  - What volume: will be limited by sediment loads and filter pore size
  - Smaller pore sizes (1.0 – < 5 µm) will clog faster than larger pore sizes (5 – 10 µM), but may capture smaller genetic materials
  - How many: maximize spatial coverage if samples are limited, while noting any onsite activities or observations that may redirect sampling efforts

eDNA Survey Design – Field Collections

- **What data should you record?**
  - Criticals: sample ID, location, date, site & sample photos, volume recovered, filter specs
  - Extras: water quality metadata (pH, turbidity, DO, etc.)
  - Standardization: DFO reporting sheet

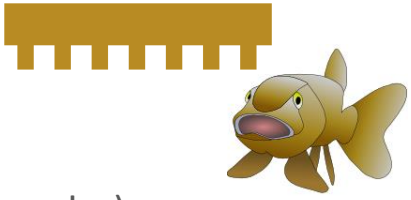
eDNA Survey Design – What is Inhibition?

- **Sources:** organic compounds, enzymes, chemicals in the environment
  - Common in turbid waters, soil, feces
- Can adversely affect PCR (eDNA amplification) and ultimately species detection
- Can be addressed at both DNA extraction and/or PCR laboratory steps



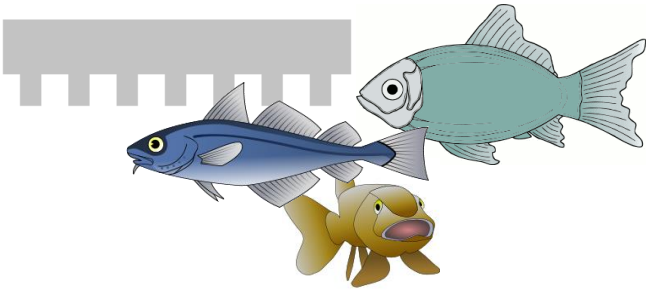
eDNA Survey Design – Assay Selection

- **Assays** bind to the eDNA of the target species in a sample
  - What kind of assay(s) you will use depend on your goal:

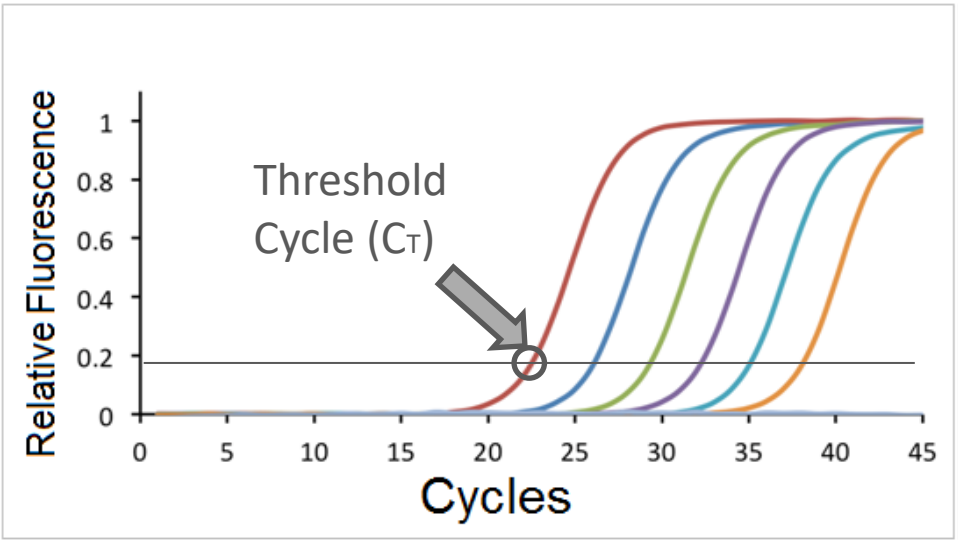


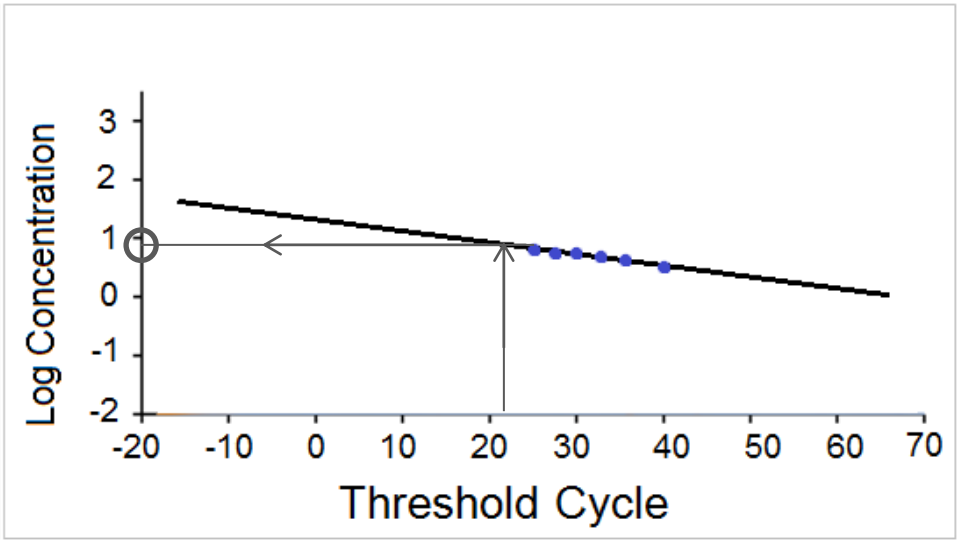
- For single-species detection studies, these will be **qPCR assays**
  - sensitive, specific
  - Suitable for detection of a single, rare species (e.g. a SAR or early invader)

- For multi-species detection studies, these will be **metabarcoding assays**
  - Less specific: designed to bind to eDNA of multiple, related species
  - Suitable for the detection of species communities (e.g. fishes, amphibians, mammals)



eDNA Survey Design – Assay Selection

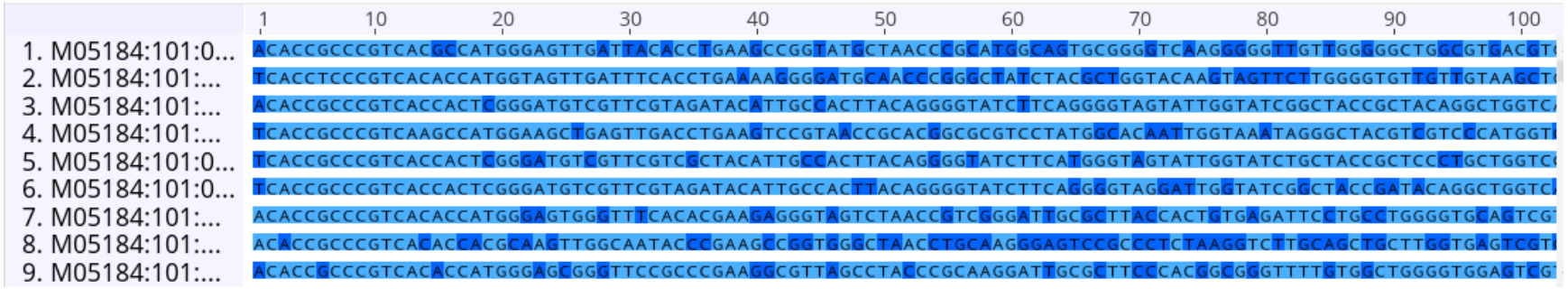




qPCR Data:

- Presence/absence and quantities of eDNA
  - Lower cycle number = more eDNA in the original sample
- Each assay has a **Limit of Detection** and a **Limit of Quantification** that specify the boundaries of target species detection by that assay (to prevent false positives)

eDNA Survey Design – Assay Selection



Metabarcode Data:

Thousands of sequences generated **per sample** → bioinformatics processing → species identifications (typically a 98% sequence match required)

- Bioinformatics pipelines: software algorithms that process raw sequence data
  - Pipelines are tailored to specific species groups and/or target genes
- **Cannot be used for relative abundance estimates***

Results – Interpretations and Limitations

Positive Detections (Presence):

- Suggest the DNA of the target species is present at the sampling location at the time of collection
- Cannot distinguish living or dead organisms, age, sex*, or stationary vs. migratory (due to eDNA transport in water)
- Repeated spatial and temporal sampling efforts increase confidence in results
  - required for relative abundance estimations from eDNA
- Can inform where to direct traditional sampling methods
- **False positives:** avoided with field and laboratory controls

Negative Detections (Absence):

- Doesn't outright confirm species absence: must also consider environmental inhibitors, sampling effort; organism possibly too rare or below limits of detection with eDNA

Conclusions

- eDNA can **complement** and **inform** traditional sampling methods as a cost-effective, **first-pass look** to direct further monitoring effort
- Applications of eDNA should be aware of its **strengths** and **limitations**
- Consult with experts (**Molecular Ecologists** and **Field Biologists**) before initiating biomonitoring based on interpreted eDNA results

SLR Consulting – Molecular Services

End-to-end eDNA surveys

- Customized survey design
- Informed field collections
- Laboratory analysis (single or multiple species) at the University of Guelph
- qPCR assay design available
- Guano/faecal, tissue, hair, water samples
- National team of Ecologists (Field & Molecular) for integrated results

SLR's eDNA Projects

- **E-fishing vs. eDNA** for Brook Trout stream detection
- **eDNA Gear Comparison** using **Citizen Science** (University of Guelph students)
- **Research Study:** qPCR vs. ddPCR vs. metabarcoding (ongoing)
- **Other Applications:** DNA tracers for groundwater/flow monitoring

THANK YOU!

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