Developing lab standard procedures and guidelines to improve uptake of eDNA methods by resource managers: An overview of two national-scale projects (READI-Net and iTrackDNA)

Columbia River Basin AIS Team Meeting
June 2023

[1]Katy Klymus
[2]Margaret Hunter
[3]Caren C. Helbing
[4]Valerie S. Langlois
[3]Jacob J. Imbery
[4]Julie Couillard
[5]Adam Sepulveda

1 U.S. Geological Survey, Columbia Environmental Research Center, Columbia, MO, U.S.A.
2 U.S. Geological Survey, Wetland and Aquatic Research Center, Gainesville, FL, U.S.A.
3 Department of Biochemistry & Microbiology, University of Victoria, Victoria, British Columbia, Canada
4 Institut National de la Recherche Scientifique, Quebec City, Quebec, Canada
5 U.S. Geological Survey, Northern Rocky Mountain Science Center, Bozeman, MT, U.S.A

- **U.S. Geological Survey (USGS)** is a United States federal agency within the Department of the Interior.

- Conducts data collection, monitoring, and scientific understanding of the nation's water, energy, mineral and **biological** resources as well as natural hazards monitoring (i.e. earthquakes, flooding)

- **Provide quality scientific information to the public and policy makers** (https://www.usgs.gov/)

ECALS – eDNA Calibration Study

Invasive Carp Regional Coordinating Committee

Objective: Understand eDNA detections of invasive carps in the Chicago Area Waterways System

Aimed to:

- Develop new markers
- Understand shedding and degradation rates of carp eDNA
- Assess likelihood of alternative vectors or sources of carp eDNA signal

U.S. Fish and Wildlife Service's bighead and silver carp eDNA monitoring program

- Monitoring the Great Lakes, Illinois, Upper Mississippi and Ohio river systems
- In conjunction with numerous state agencies
- One of the largest and most well developed eDNA monitoring programs in the U.S.

USGS

USGS labs involved in eDNA research

USGS

Transitioning eDNA science from research to management

- Wildlife and natural resource managers are still hesitant to use eDNA tools:
  Can eDNA tell me if a species is present in an area?
  How trustworthy is that data?

- Reasons for limited uptake are many but can be distilled to:
  1. poor communication about the state of the science
  2. lack of trust in the accuracy and reliability of the data
  3. limited integration of decision-makers in the research and development process (e.g., Darling 2015).

Transitioning eDNA science from research to management

- Wildlife and natural resource managers are still hesitant to use eDNA tools:
  Can eDNA tell me if a species is present in an area?
  How trustworthy is that data?

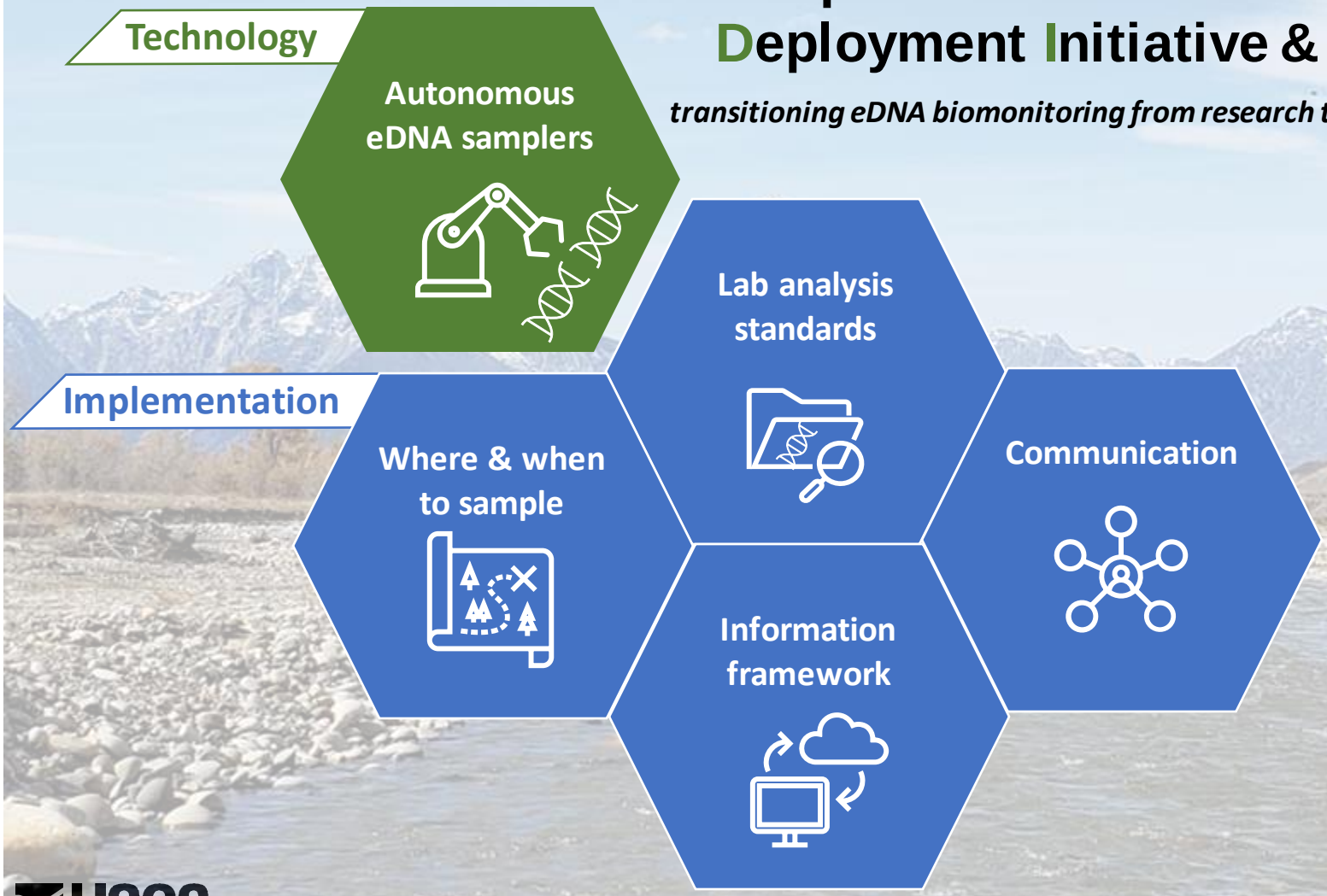
- Reasons for limited uptake are many but can be distilled to:
  1. poor communication about the state of the science
  2. lack of trust in the accuracy and reliability of the data
  3. limited integration of decision-makers in the research and development process (e.g., Darling 2015).

- **Lack of standard methods can lead to uncertainty or concern over quality of results and subsequent interpretation. (#2 above)**

READI-Net

Rapid eDNA Assessment and Deployment Initiative & Network

transitioning eDNA biomonitoring from research to actionable science

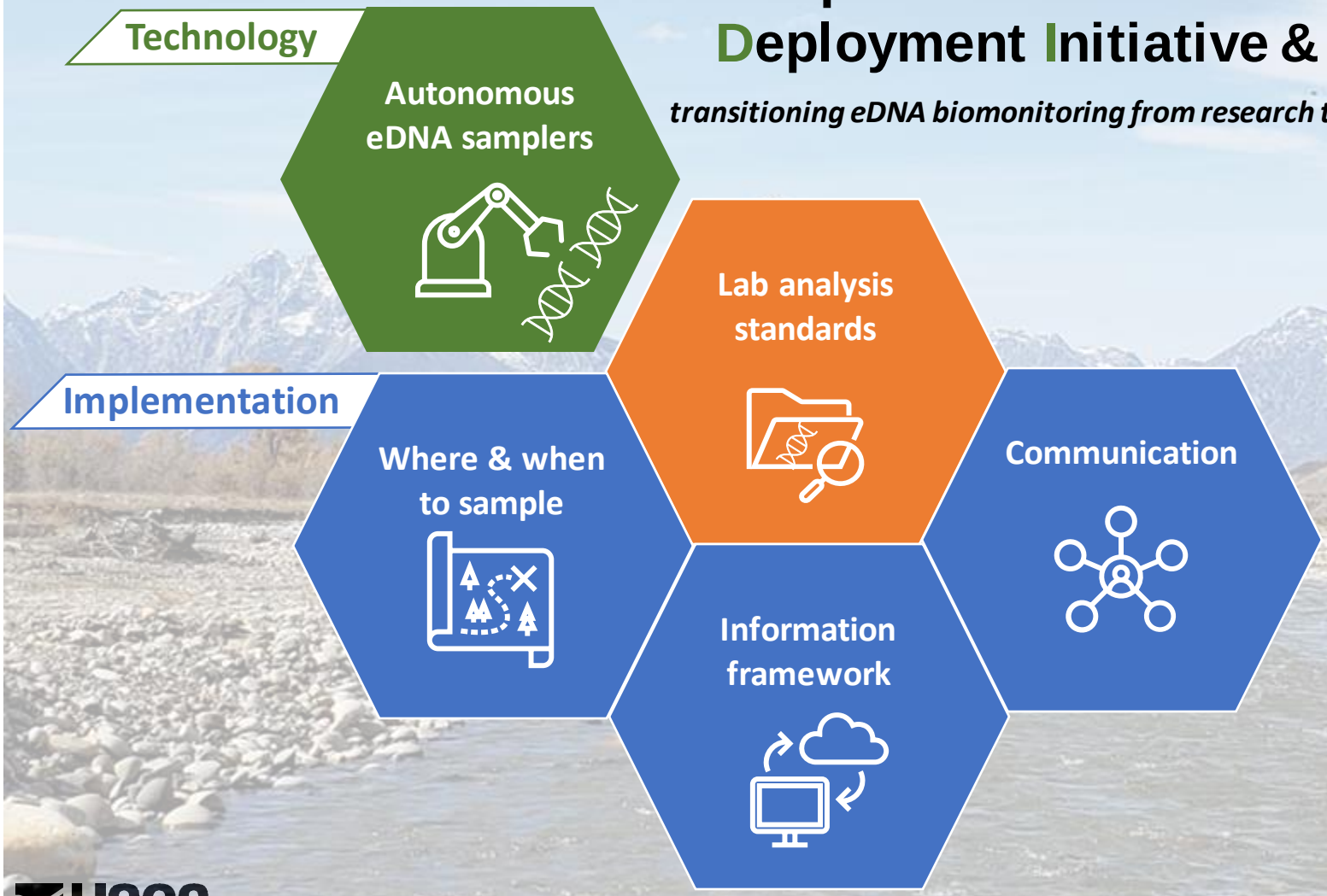


USGS

READI-Net

Rapid eDNA Assessment and Deployment Initiative & Network

transitioning eDNA biomonitoring from research to actionable science



USGS

READI-Net

Rapid eDNA Assessment and Deployment Initiative & Network

Goal: Develop standards and guidelines for eDNA analysis workflow among labs that will be participating in the READI-Net network

- Assess repeatability and reproducibility within and across labs via **interlaboratory calibration** studies and the development of within and between lab **proficiency testing**
- Create a **standards and guideline document** for lab analysis of READI-Net samples based off of NAS eDNA database standards

USGS

READI-Net

Rapid eDNA Assessment and Deployment Initiative & Network

NAS:

USGS Nonindigenous Aquatic Species (NAS) Database (https://nas.er.usgs.gov/)

Incorporation of eDNA data into this database required the development of standards and guidelines (Ferrante et al. 2022)

- Collection, Sample Processing, Contamination (Collection, Extraction, PCR), PCR Assay (Validation, Optimization, Standard curve), Reporting

iTrackDNA – Tracking at-risk and invasive species with confidence: Opportunities and challenges of eDNA approaches

Caren HELBING Project Shared Leader

Valerie LANGLOIS Project Shared Co-Leader

Jérôme DUPRAS Project Co-Leader & GE^{3}LS Leader

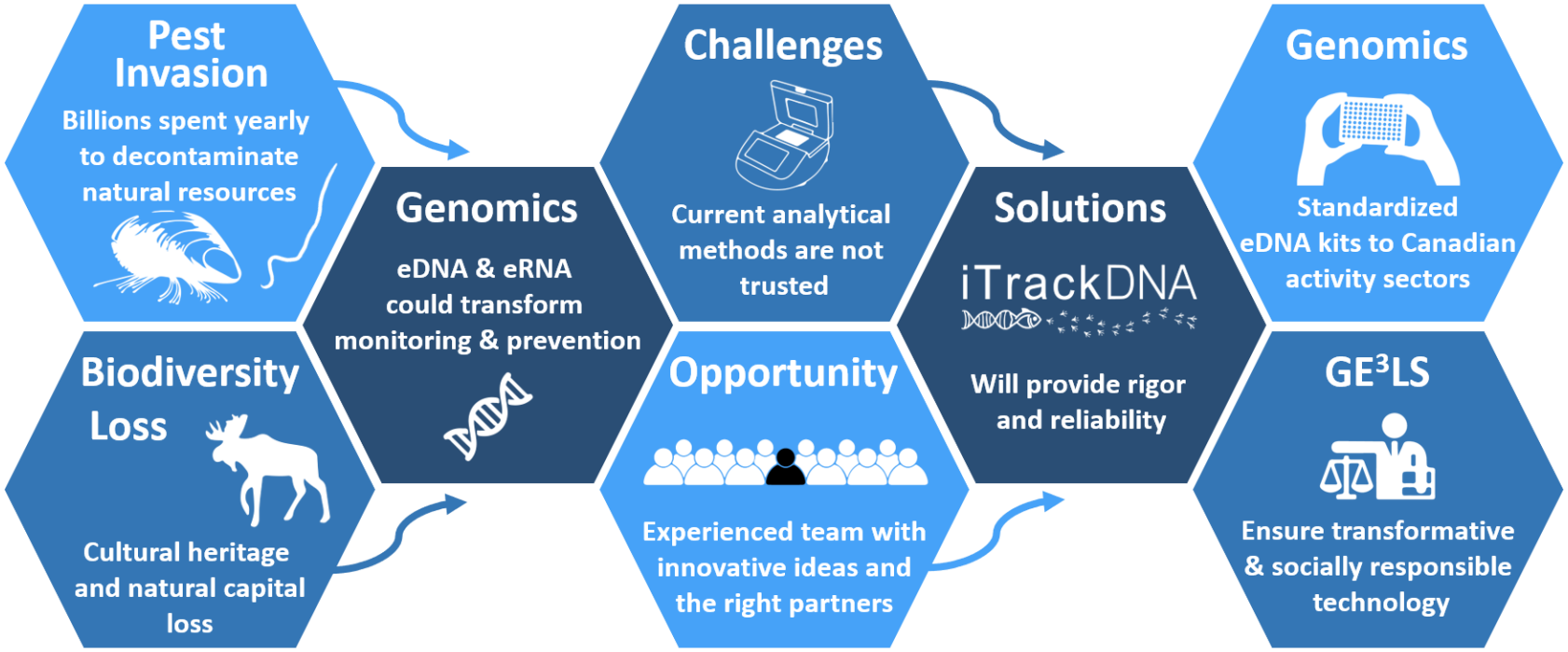
Louis BERNATCHEZ Project Co-Leader

iTrackDNA

GenomeCanada GenomeBritishColumbia GenomeQuébec

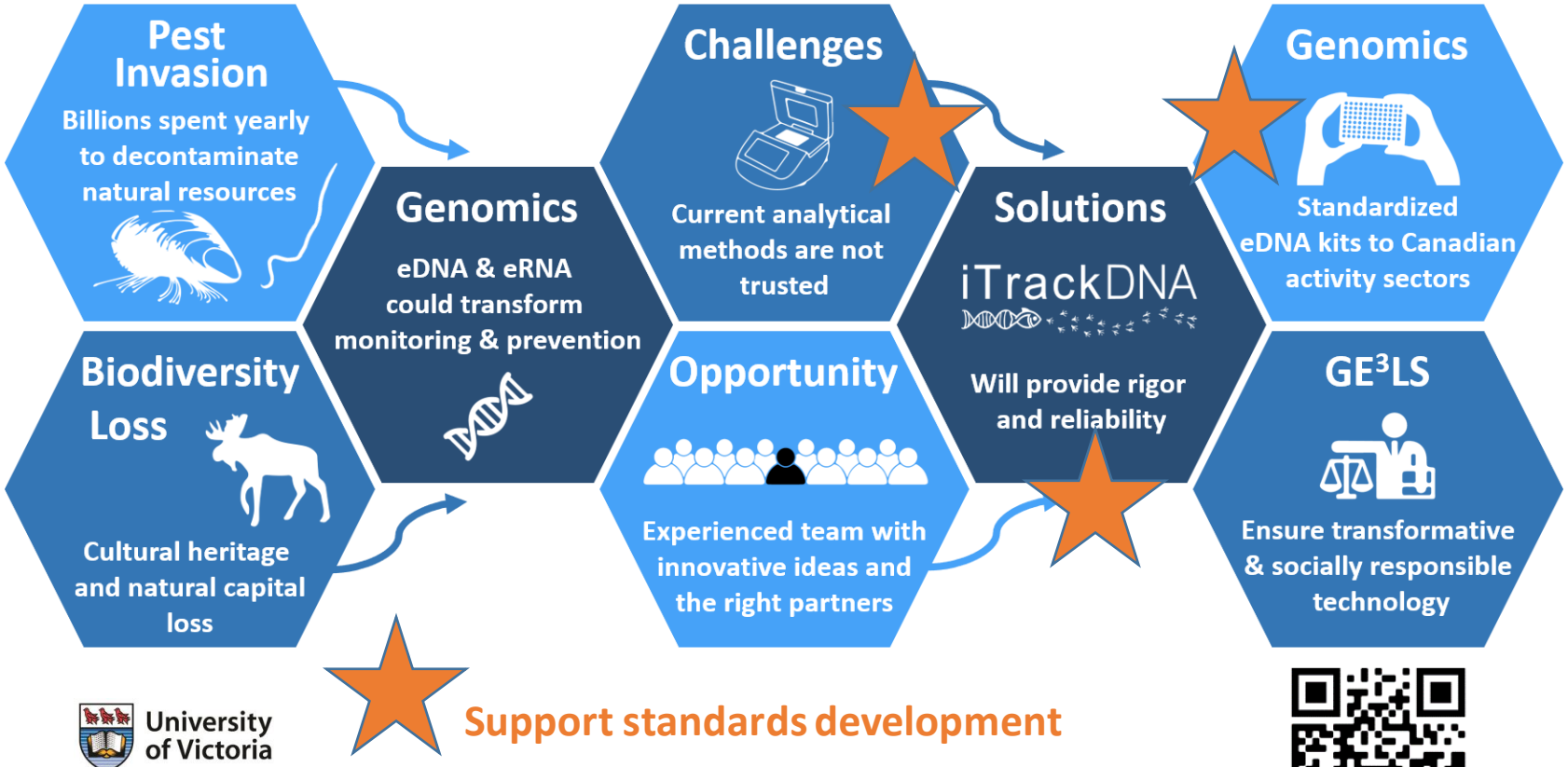
iTrackDNA

Tracking at-risk and invasive species with confidence: Opportunities and challenges of eDNA approaches



iTrackDNA

Tracking at-risk and invasive species with confidence: Opportunities and challenges of eDNA approaches



University of Victoria

iTrackDNA – Interlab calibration studies

Purpose: To establish lab protocols and set lab performance baselines

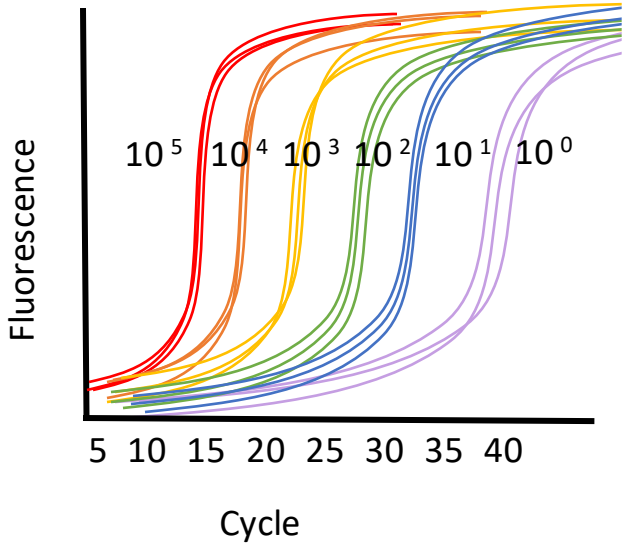
Phase 1: Assess variation between labs in running standard curves for qPCR/ dPCR

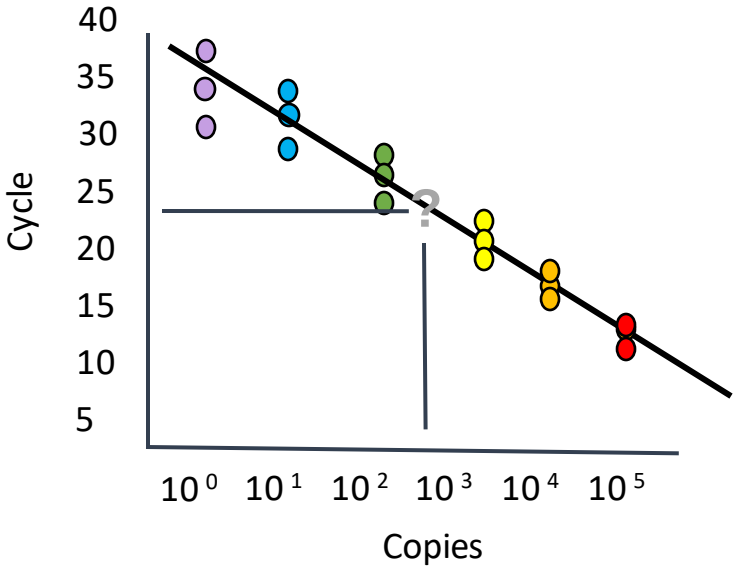
Methods:

- Each lab ran multiple replicates of standard qPCR standard curves using the same assay
- All reagents and standards were provided by a central lab
- Variables: labs prepare plates with provided serial dilutions, different qPCR thermocycler
- Assessment: LOD and LOQ of each labs curve was measured

iTrackDNA

iTrackDNA – Interlab calibration studies





iTrackDNA

iTrackDNA – Interlab calibration studies

- Five-fold serial dilutions ranging from 62,500 to 500 copies/reaction (n=8 technical replicates) and 100 to 0.032 copies/reaction (n=24) were prepared in bulk plus 24 NTCs and aliquoted for distribution to participating labs
- Each lab ran three plates of the serial dilutions and used eLOWQuant (Lesperance et al. 2021) to calculate LOD and LOQ

Plate 1

	1	2	3	4	5	6
A	32,150	6,250	1,250	250	50	50
B	32,150	6,250	1,250	250	50	50
C	32,150	6,250	1,250	250	50	50
D	32,150	6,250	1,250	250	50	50
E	32,150	6,250	1,250	250	50	50
F	32,150	6,250	1,250	250	50	50
G	32,150	6,250	1,250	250	50	50
H	32,150	6,250	1,250	250	50	50

Plate 2

	1	2	3	4	5	6	7	8	9	10	11
A	50	10	10	2	2	2	0.4	0.4	0.4	NTC	NTC
B	50	10	10	2	2	2	0.4	0.4	0.4	NTC	NTC
C	50	10	10	2	2	2	0.4	0.4	0.4	NTC	NTC
D	50	10	10	2	2	2	0.4	0.4	0.4	NTC	NTC
E	50	10	10	2	2	2	0.4	0.4	0.4	NTC	NTC
F	50	10	10	2	2	2	0.4	0.4	0.4	NTC	NTC
G	50	10	10	2	2	2	0.4	0.4	0.4	NTC	NTC
H	50	10	10	2	2	2	0.4	0.4	0.4	NTC	NTC

iTrackDNA

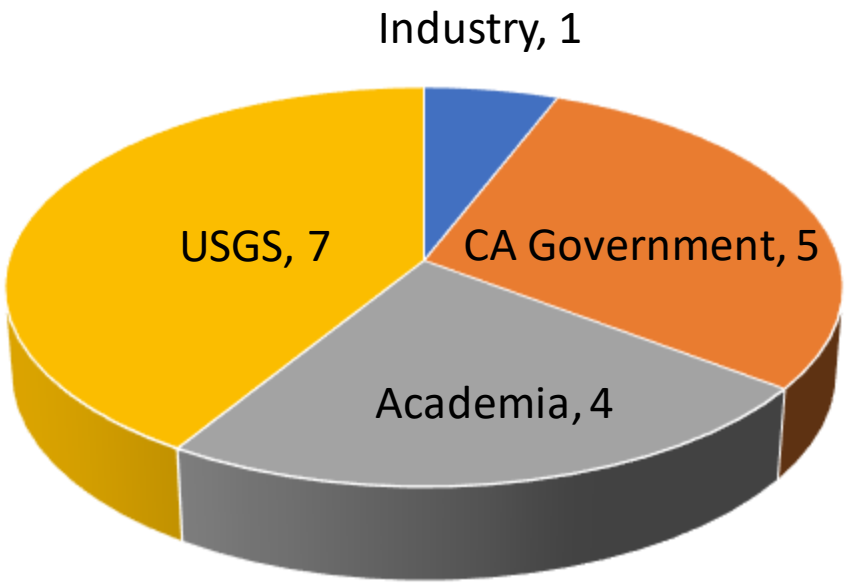
Plate 3

	1	2	ß	4	5	6	7	8
A	0.08	0.08	0.08	0.016	0.016	0.016	NTC	10
B	0.08	0.08	0.08	0.016	0.016	0.016	NTC	10
C	0.08	0.08	0.08	0.016	0.016	0.016	NTC	10
D	0.08	0.08	0.08	0.016	0.016	0.016	NTC	10
E	0.08	0.08	0.08	0.016	0.016	0.016	NTC	10
F	0.08	0.08	0.08	0.016	0.016	0.016	NTC	10
G	0.08	0.08	0.08	0.016	0.016	0.016	NTC	10
H	0.08	0.08	0.08	0.016	0.016	0.016	NTC	10

iTrackDNA – Interlab calibration studies

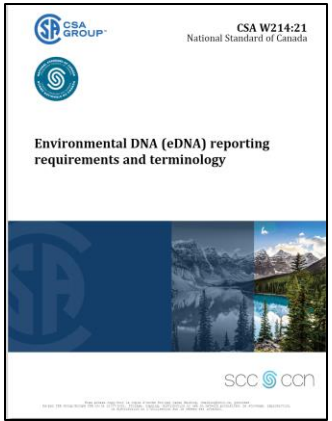
Results:

- Total number of participating labs: 17
- USGS READInet program: 7 labs
- Experimental design is consistent with draft Canadian Standards Association (CSA) eDNA standard

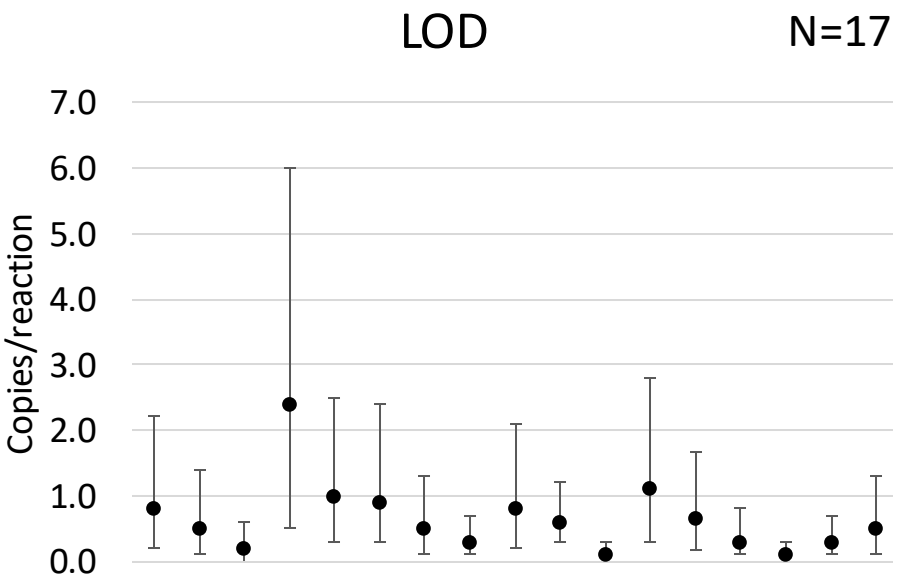


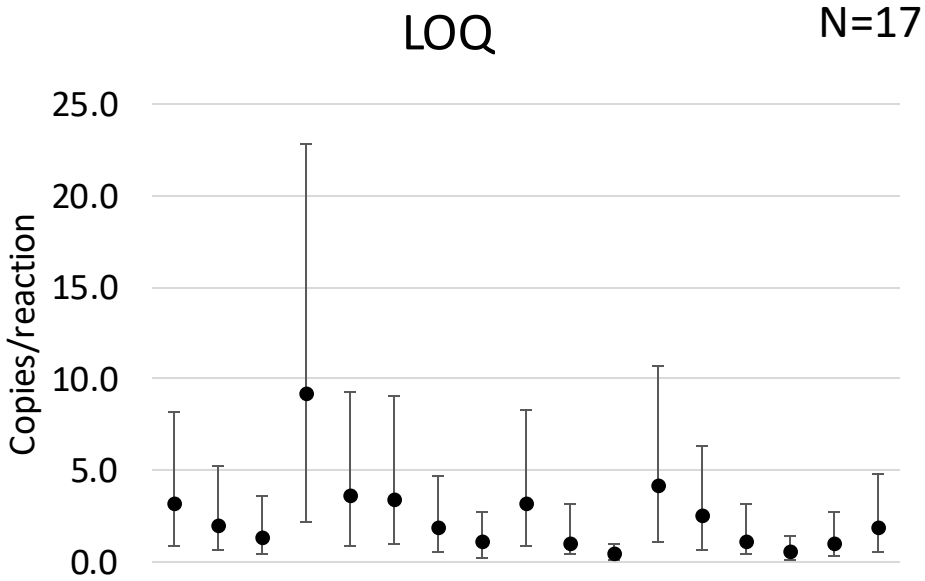
iTrackDNA

First CSA Group national eDNA standard



iTrackDNA – Interlab calibration studies LOD and LOQ Results





Statistic	LOD (copies/rxn)	LOD 95% CI Lower	LOD 95% CI Upper	LOQ (copies/rxn)	LOQ 95% CI Lower	LOQ 95% CI Upper
Median±MAD	0.5±0.3	0.4±0.2	0.8±0.6	1.9±1.2	1.3±0.9	2.9±2.0
MAX	2.40	1.90	3.60	9.20	7.00	13.60
MIN	0.10	0.10	0.20	0.40	0.30	0.60

iTrackDNA

iTrackDNA – Interlab calibration studies Phase 2

Methods:

- Multiple assays
- Labs will purchase reagents
- Labs will prepare own standard curves given a specific protocol

Results: June 2023

Future Phases:

- Different enzyme/ mastermixes
- Use of tRNA
- Extraction methods

iTrackDNA

Conclusions

- Lack of method standardization may contribute to the lack of implementation by managers

- There are a numerous guidelines and standards being produced by various entities (examples: CSA, eDNA Society, USDA Genomics, NAS, Aquanet, Finland, Australia)

- We are utilizing these to develop standards specifically for the READinet and iTRACK programs in order to:

1. Understand what variables most strongly influence lab variation
    - Phase 1 iTRACK: the 17 labs had very low variation in LOD and LOQ, suggesting good lab technique and instrumentation differences were not problematic

2. Act as a demonstration to the management community.

USGS

Acknowledgements

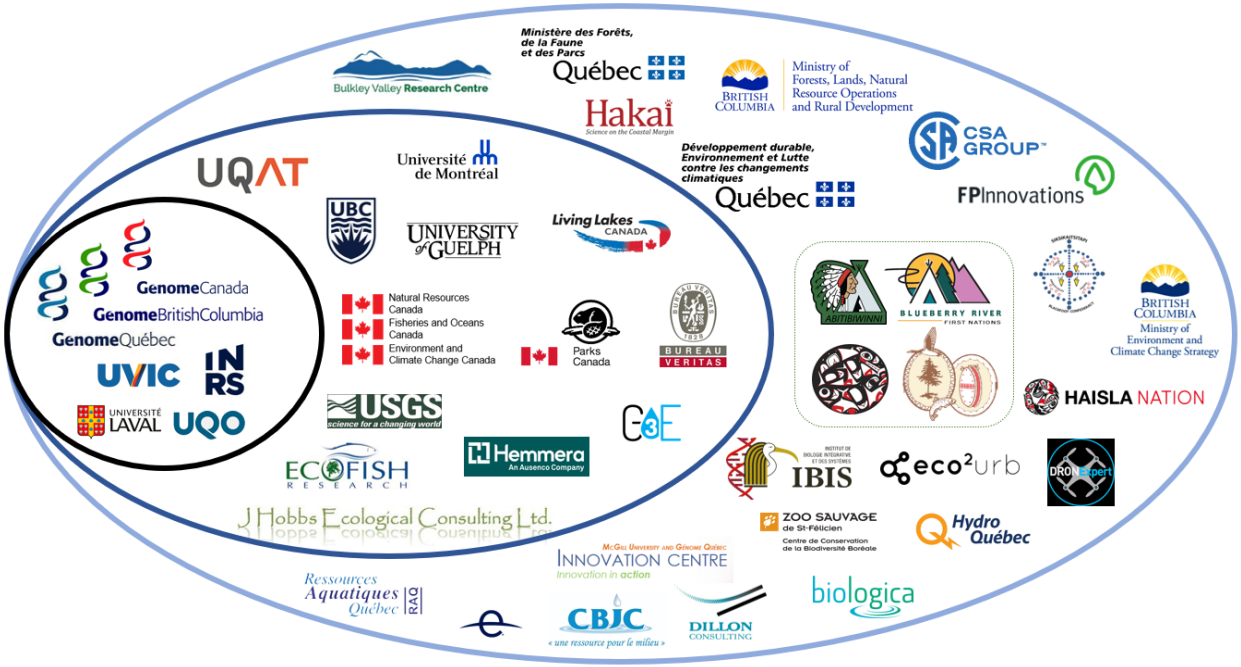
READI-net

Elliot Barnhart
Sara Eldridge
Stephen Spear
Chris Merkes
Tariq Tajjioui
Murulee Byappanahalli

Ashley Spoljaric
Patrick Hutchins
David Pilliod
Amanda Boone
John Stechly
Caitlin Beaver

Devin Jones
Nathan Thompson
Megan Voshage

iTRACK



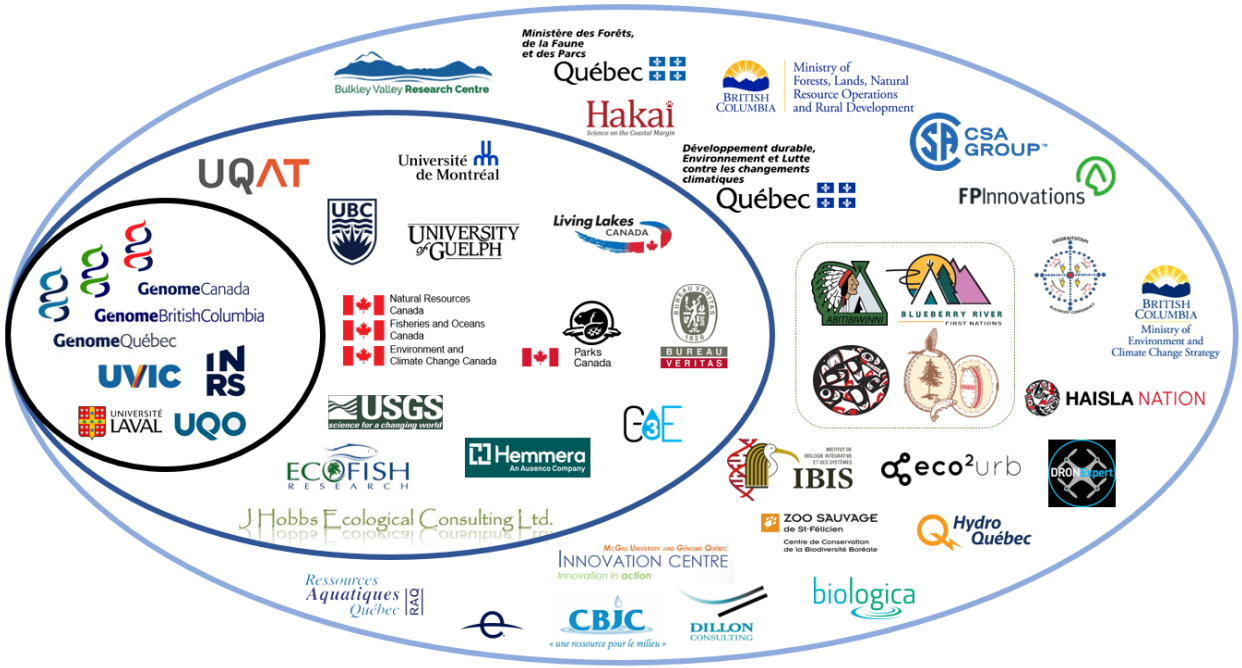
USGS

Funding

READI-net

This project is one among a set of coordinated projects funded by (or in part by) the Bipartisan Infrastructure Law through the US Department of the Interior to advance a nationally coordinated Early Detection and Rapid Response Framework.

iTRACK



USGS

Questions?

Katy Klymus
kklymus@usgs.gov

USGS