

The Influence of Study Variables on eDNA Survey Results: Redside Dace in Irvine Creek, Ontario, Canada

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Agenda

- 1) Objective
- 2) Background
- 3) Site Selection
- 4) Approach
- 5) Results
- 6) Lessons Learned

Objective

- eDNA to detect Redside Dace
- Historic population
- Approaching end of regulatory protection
- eDNA permitted under ESA and SARA

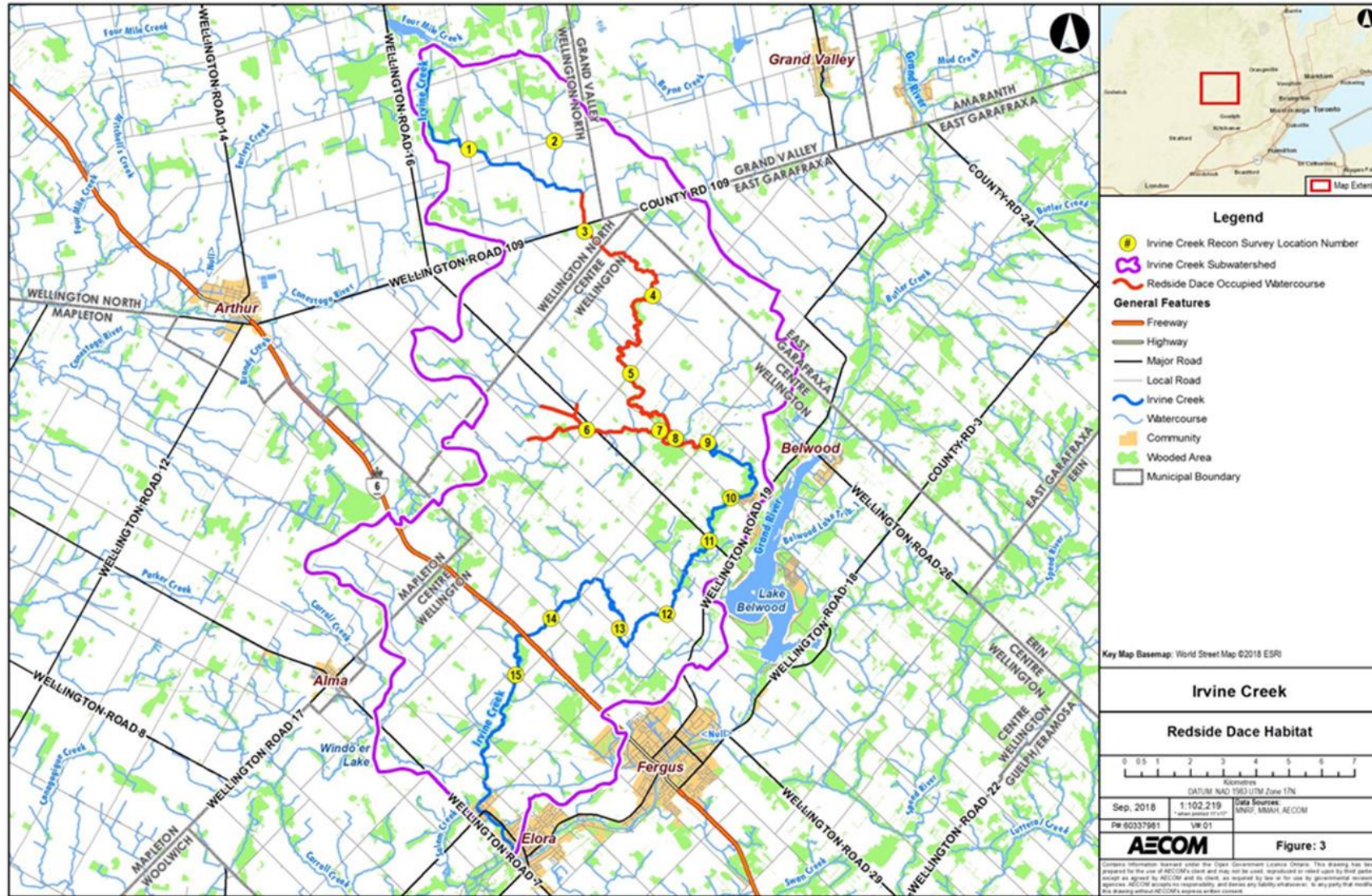


Project Background

- SAR permits
- Bridge rehab Irvine Creek
- Township of Centre Wellington
- Degraded habitat / altered regimes



Irvine Creek Overview



Site Selection & Sampling

- 15 sites
- Historically sampled sites
- Focus on sites with better quality habitat
- Provide spatial coverage
- Various times of year (Fall / Spring)
- eDNA analysis: Precision Biomonitoring Inc.



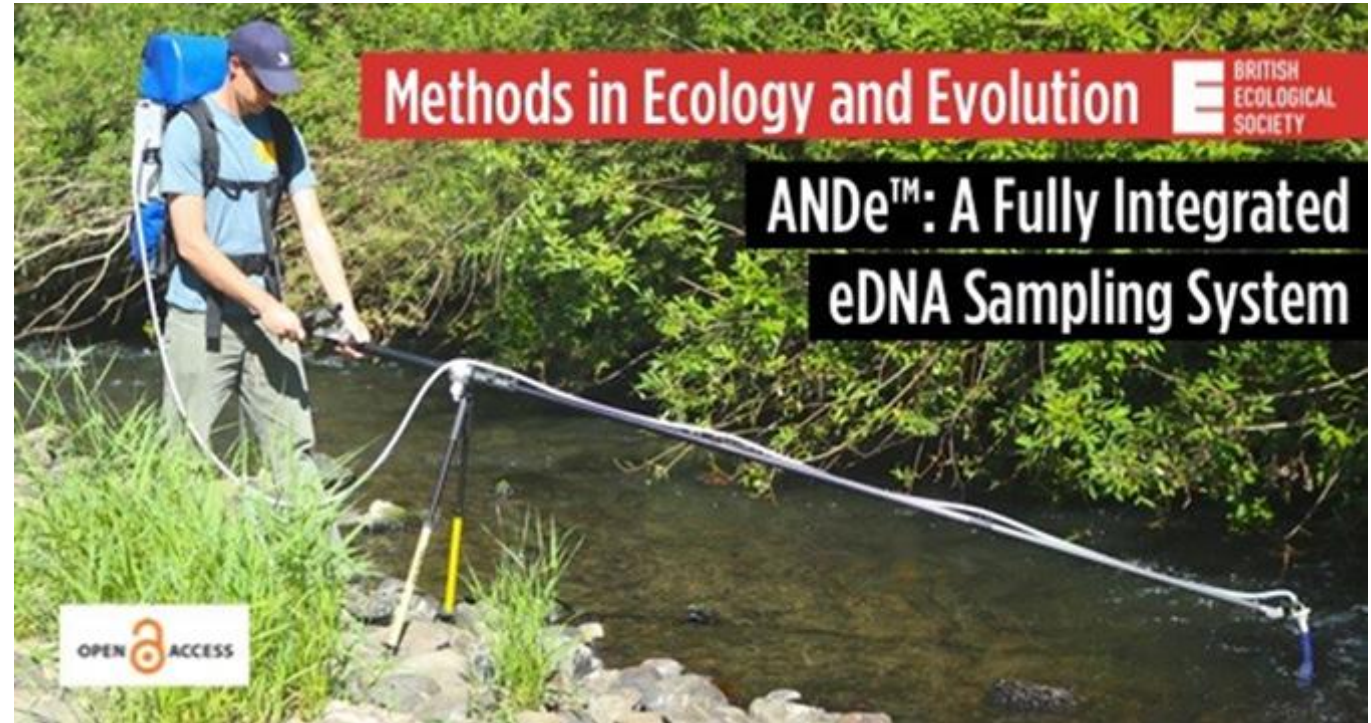
Approach

- Assay verification
- Positive control site
- eDNA inhibition
- Understanding of target organism
- Supporting data
- Lab QA/QC (IPCs, NTCs, PAC's, quantification of total eDNA capture)



Approach: Equipment

- Cross contamination
- Sample integrity
- Sample collection & Pumps
- Thermocyclers
- Extraction & preservation



Approach: Conventional Methods



- Seine netting selected
- 3 of the 15 sites sampled
- Highest probability sites
- 3 hauls / site
- Blocknets used



Fall Sampling 2018: Variables

- In-Lab Magnetic Induction Cyclor (MIC) thermocycler
- 6 technical replicates
- 2 L samples
- Filter pore sized increased
- Grab sample
- Low flow conditions



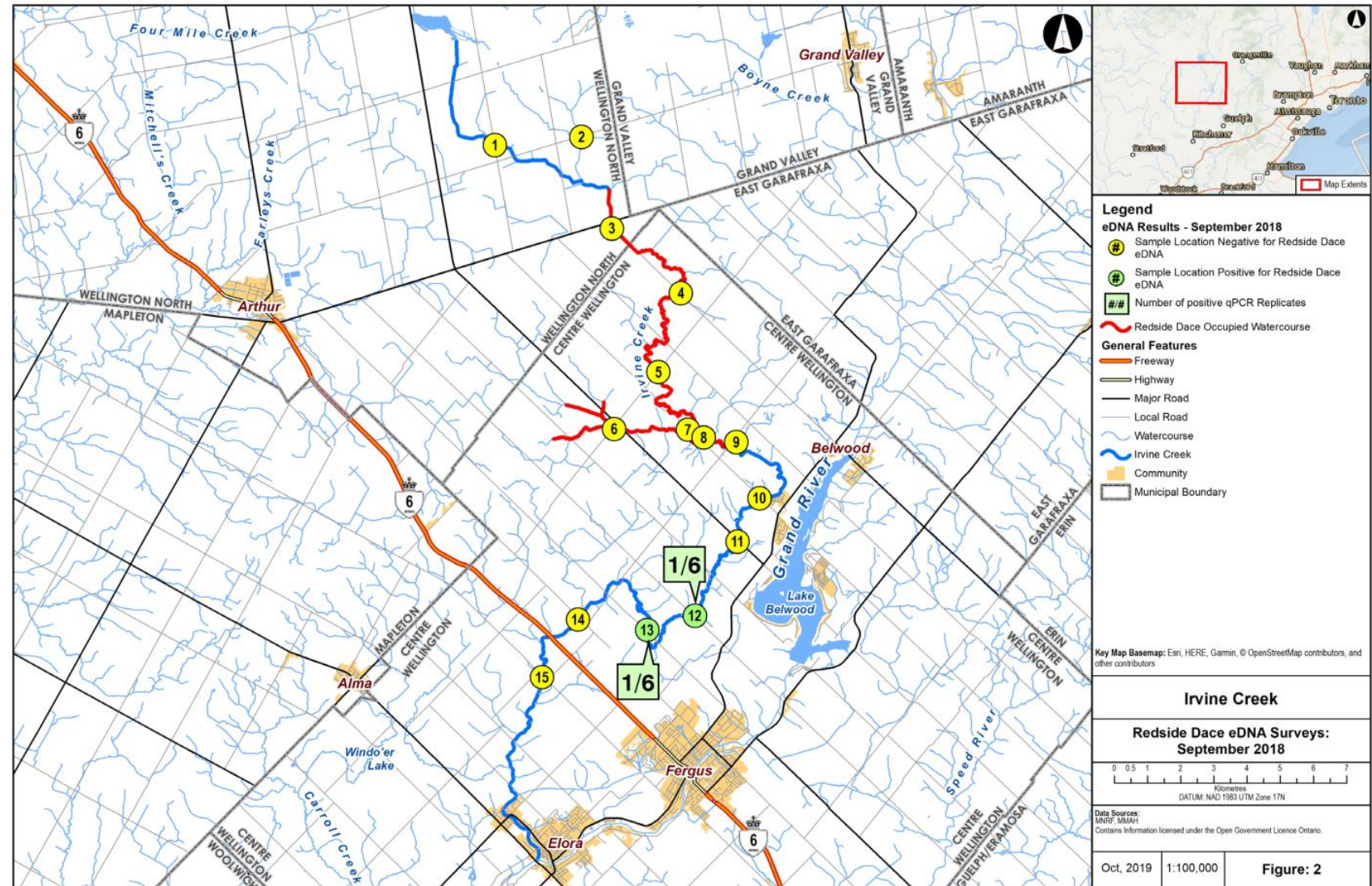
Refining the Process: 2019 Survey Variables

- CFX96 Real-Time Detection System (BioRad)
- Spawning activity (increased DNA shedding)
- Grab sampling vs. transect
- Altered pump speed
- Increased flow conditions



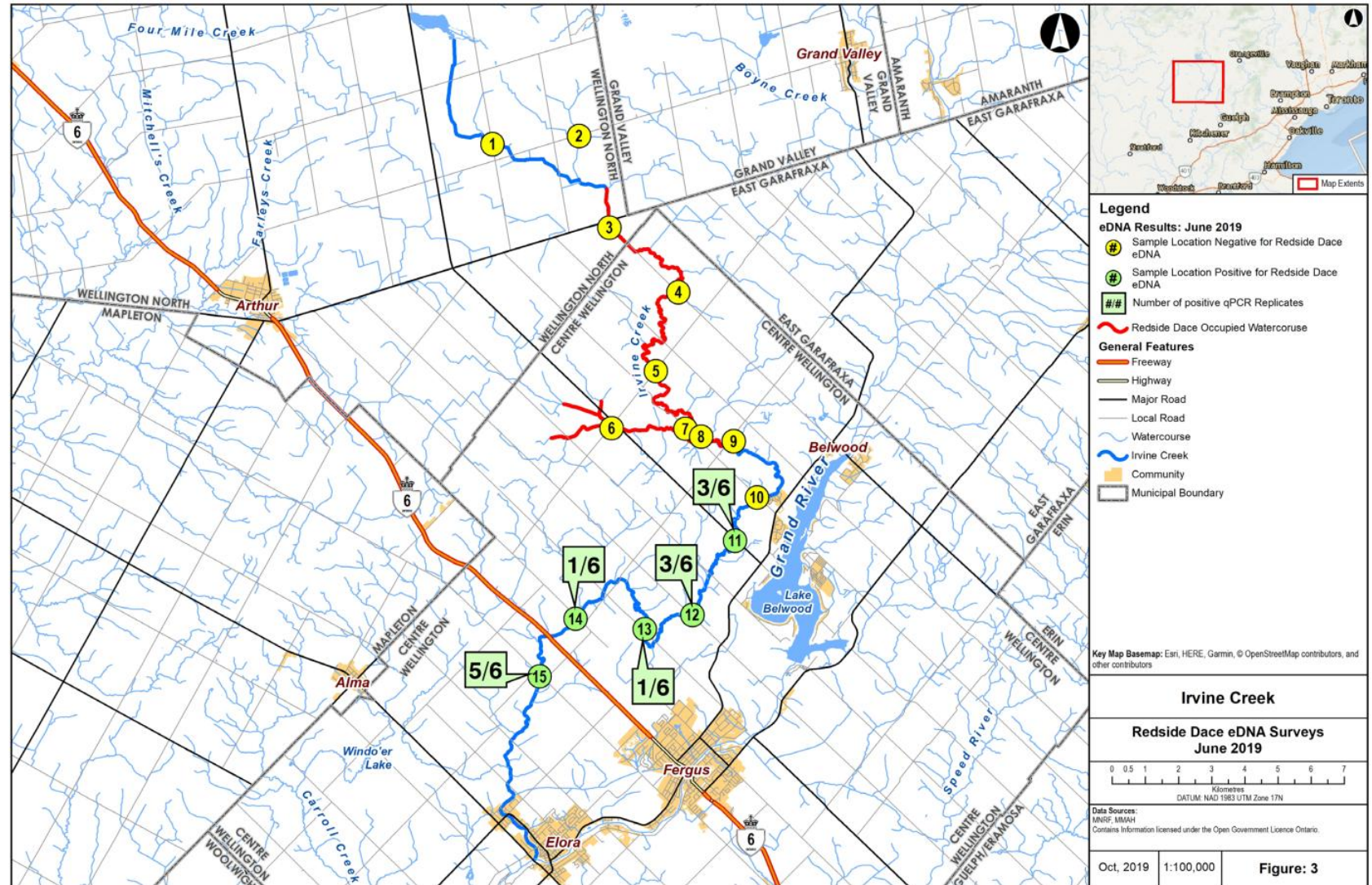
Fall 2018 Results

- Positive DNA detections at 2 sites
- 1/6 technical replicates
- Outside of historic reaches
- Better quality habitat, groundwater influence



Spring 2019 Results

- Positive DNA detections at 5 sites
- 1/6 to 5/6 technical replicates
- Outside of historic reaches
- Better quality habitat, groundwater influence



Lessons Learned

- Sample volume vs. sample area
- Transect vs. grab
- Equipment sensitivity
- eDNA capture vs. specimen capture
- Spatial increase from cost decrease



| Site No. | 2018 qPCR Summary (MIC PCR) | 2019 qPCR Summary (BioRad) |
|----------|--------------------------------|-------------------------------|
| Site 1 | 0/6 | 0/6 |
| Site 2 | 0/6 | 0/6 |
| Site 3 | 0/6 | 0/6 |
| Site 4 | 0/6 | 0/6 |
| Site 5 | 0/6 | 0/6 |
| Site 6 | 0/6 | 0/6 |
| Site 7 | 0/6 | 0/6 |
| Site 8 | 0/6 | 0/6 |
| Site 9 | 0/6 | 0/6 |
| Site 10 | 0/6 | 0/6 |
| Site 11 | 0/6 | 3/6 |
| Site 12 | 1/6 | 3/6 |
| Site 13 | 1/6 | 1/6 |
| Site 14 | 0/6 | 1/6 |
| Site 15 | 0/6 | 5/6 |

Lessons Learned

- Maximize confidence
- Agency acceptance
- Responsible development
- Establishing a benchmark
- Increasing demand
- Need for expedited sampling
- Advancing acceptance



Thank you!

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