# Metagenomic approach for eDNA sequencing with Oxford Nanopore MinION to detect elusive Alaskan species in a mesocosm

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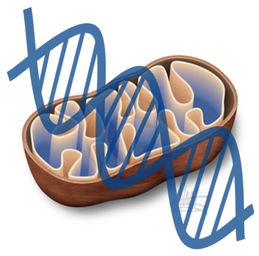
**We assess the possibility of processing species monitoring surveys through eDNA with Oxford Nanopore MinION and a metagenomic sequencing approach. We use a mesocosom (fish tank where two live individuals of Alaskan chubs (*Couesius plumbeus*) for this study along with Halibut DNA as an internal control. Detection is possible despite a low sensitivity. Low variance of the detection rates is encouraging for further improvements.**


**Mitochondrial DNA for target:**
- Macrobial eDNA in mitochondria and small cells (1),
- Target enlargement by >10 times,
- Abundance: in killifish, 0.4-2% of total nucleotide pool (2,3).




**Oxford Nanopore Technology:**
- Low-input barcoded metagenomic chemistry,
- minION: portability for field sequencing,
- real-time collection of data for rapid-response actions.

**Monitoring surveys of elusive species:**
- logistical efforts,
- financially costly,
- invasive for fragile species and ecosystems.

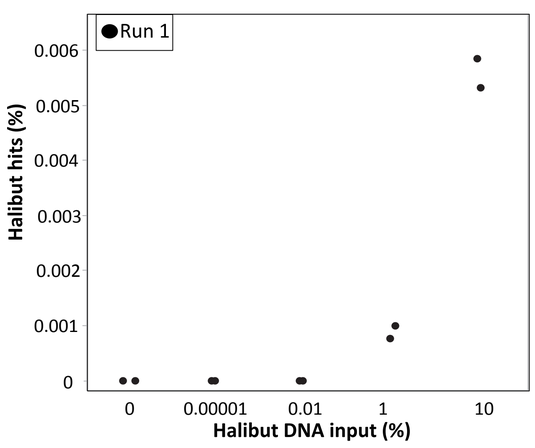

**Environmental DNA (eDNA):**
- 2 methods: qPCR and metabarcoding,
- Species specific,
- extensive batteries of controls,
- obstacle for non-model organisms,
- Inaccessible to the public,
- Restricted from the field: analysis and response delays.

**Perspectives:**
- Development of a method optimized for detection limits,
- Assessment of success rate,
- Assessment of the optimized methodology in the field.

## TESTING THE LIMITS OF DETECTION

**eDNA extracted from fish tank water are spiked with Halibut DNA as an internal control. Experiment set in duplicate. Library prepared with the low-input barcoded kit, RPB004.**

| Halibut input | Barcode | yield (bp) | reads (n) | Halibut hits (n) | Couesius hits (n) |
|---|---|---|---|---|---|
| 0% | 2 | 696,841,147 | 234,680 | 0 | 1 |
| | 8 | 950,739,754 | 411,583 | 0 | 0 |
| 0.00001% | 3 | 365,966,387 | 136,086 | 0 | 0 |
| | 9 | 757,419,597 | 270,119 | 0 | 1 |
| 0.01% | 4 | 911,533,874 | 332,850 | 0 | 0 |
| | 10 | 439,116,212 | 151,480 | 0 | 1 |
| 1% | 5 | 594,001,062 | 200,443 | 2 | 0 |
| | 11 | 668,766,201 | 260,830 | 2 | 0 |
| 10% | 6 | 772,033,970 | 342,137 | 20 | 0 |
| | 12a | 894,976,379 | 300,873 | 16 | 1 |
| | Total: | 7,051,394,583 | 2,641,081 | | |

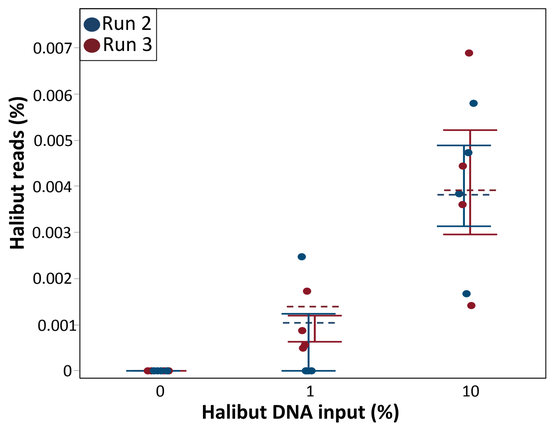



- Control spiking detected down to 1%: 50 pg or 16 pg/ul Halibut,
- Halibut detection correlates with internal control spiking,
- Duplicates show a low variance of detection,
- Fish species (*Couesius plumbeus*) from the fish tank is detected.

## TESTING THE REPRODUCIBILITY

**A second library is prepared with barcodes spiked within the limits of detection. Each spike is set in 4 replicates. The library is ran on 2 flow cells to assess the effect of sequencing depth on the reproducibility of detection.**

| Halibut input | Barcode | yield (bp) | reads (n) | Halibut hits (n) | Couesius hits (n) |
|---|---|---|---|---|---|
| 0% | 9 | 195,717,621 | 62,486 | 0 | 0 |
| | 10 | 141,536,046 | 48,095 | 0 | 1 |
| | 11 | 158,574,203 | 53,551 | 0 | 0 |
| | 12a | 252,942,817 | 83,755 | 0 | 0 |
| 1% | 1 | 148,497,797 | 52,736 | 0 | 0 |
| | 2 | 184,314,450 | 69,253 | 0 | 0 |
| | 3 | 131,608,208 | 49,463 | 0 | 0 |
| | 4 | 233,893,642 | 80,901 | 2 | 0 |
| 10% | 5 | 180,042,916 | 63,444 | 3 | 0 |
| | 6 | 161,409,106 | 59,826 | 1 | 0 |
| | 7 | 222,491,787 | 78,163 | 3 | 0 |
| | 8 | 191,711,649 | 68,993 | 4 | 0 |
| | Total: | 2,202,740,242 | 770,666 | | |



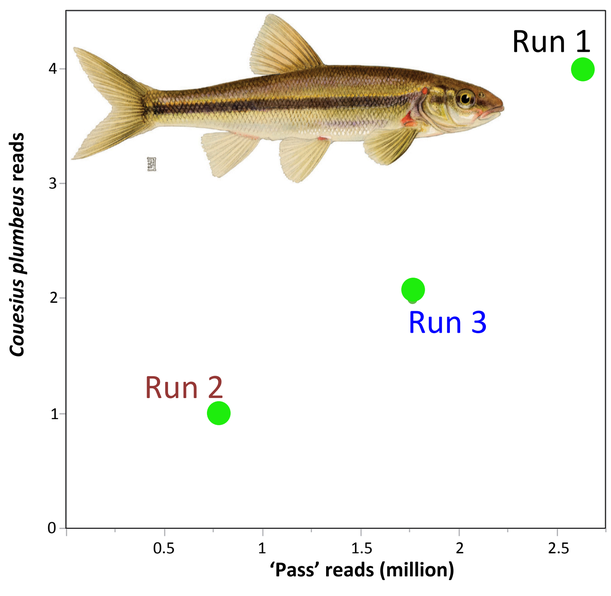

| Halibut input | Barcode | yield (bp) | reads (n) | Halibut hits (n) | Couesius hits (n) |
|---|---|---|---|---|---|
| 0% | 9 | 512,272,712 | 155,771 | 0 | 0 |
| | 10 | 326,694,036 | 106,770 | 0 | 0 |
| | 11 | 343,723,890 | 111,868 | 0 | 0 |
| | 12a | 475,728,832 | 151,780 | 0 | 0 |
| 1% | 1 | 331,566,073 | 114,696 | 1 | 0 |
| | 2 | 485,125,456 | 177,881 | 1 | 0 |
| | 3 | 317,761,543 | 115,847 | 2 | 0 |
| | 4 | 602,958,516 | 203,078 | 1 | 0 |
| 10% | 5 | 414,557,260 | 141,332 | 2 | 1 |
| | 6 | 380,589,369 | 138,780 | 5 | 0 |
| | 7 | 553,885,865 | 188,746 | 13 | 1 |
| | 8 | 451,802,134 | 157,719 | 7 | 0 |
| | Total: | 5,196,665,686 | 1,764,268 | | |

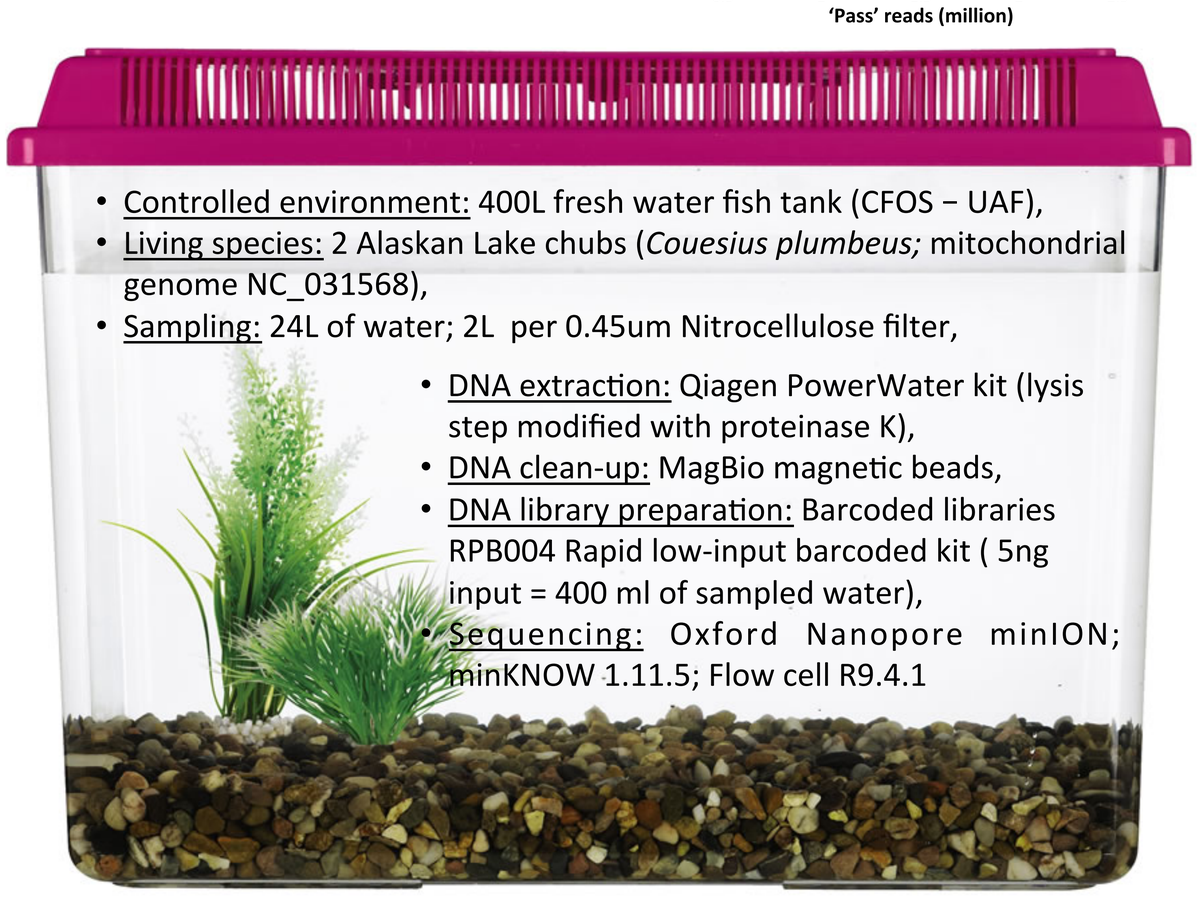
- Sequencing depth doesn't impact detection reproducibility,
- Low sensitivity of detection requires fair amounts of reads,
- 8 additional replicates of Halibut spiking consistent with run 1

## DETECTION OF *COUESIUS PLUMBEUS*

**Reads collected from the 3 runs are searched for mitochondrial hits of *Couesius plombeus*, the species of fish living in the fish tank.**

- **2 individuals of fish living in 400L water detected with low-input metagenomic sequencing approach and mitochondrial hit search,**
- **Detection success correlates sequencing depth.**



- Controlled environment: 400L fresh water fish tank (CFOS − UAF),
- Living species: 2 Alaskan Lake chubs (*Couesius plumbeus;* mitochondrial genome NC_031568),
- Sampling: 24L of water; 2L per 0.45um Nitrocellulose filter,
- DNA extraction: Qiagen PowerWater kit (lysis step modified with proteinase K),
- DNA clean-up: MagBio magnetic beads,
- DNA library preparation: Barcoded libraries RPB004 Rapid low-input barcoded kit ( 5ng input = 400 ml of sampled water),
- Sequencing: Oxford Nanopore minION; minKNOW 1.11.5; Flow cell R9.4.1

**BIOINFORMATIC:**
- Basecalling: ONT Albacore Sequencing Pipeline Software (version 2.2.7)
  - Quality filtering (qscore > 7)
- BLASTn (version 2.2.31+) search against custom mitochondrial database
  - word size 11 e-value cutoff 0.0001

**References:**
(1) Turner C. *et al*, 2014; *Meth. in Ecol. and Evol.*: 5 (7): 676-684.
(2) Hartmann N. *et al*, 2011; *Aging Cell*: 10 (5): 824-831.
(3) Valenzano D. *et al*, 2015; *Cell*: 163 (6): 1539-1554

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