

-A glimpse of future monitoring - eDNA



**A** REA  
52



**BIO ID**

Marine & Molecular Ecological Consultancy

# AREA

# 52



Siobhan Atkinson  
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Bernie Ball  
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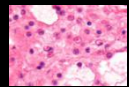
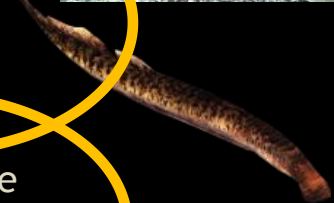
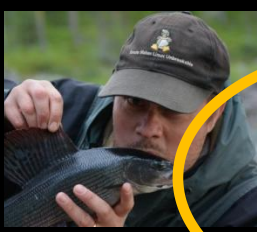
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An Roinn Talmhaíochta,  
Bia agus Mara  
Department of Agriculture,  
Food and the Marine

Ronan Carlsson  
Lab manager



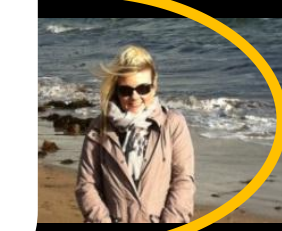
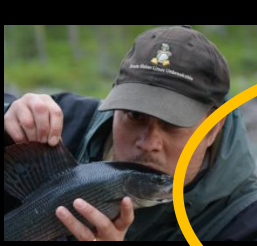
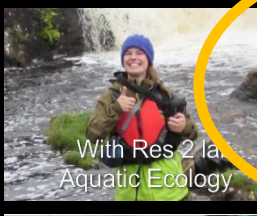
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Managing Director



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Director





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Jens Carlsson  
Director



UCC  
Coláiste na hOllscoile Corcaigh, Éire  
University College Cork, Ireland

KANTURK & DISTRICT TROUT ANGLERS ASSOCIATION

Local Authority Waters Programme  
vibrant communities | catchment assessment | healthy waters

Triturus

SuirCAN ENVIRONMENTAL

IRISH SPECIMEN FISH COMMITTEE

ATLANTIC SALMON TRUST

epa

Comhairle Cathrach & Contae Phort Láirge  
Waterford City & County Council

KFO

Marine Institute  
Foras na Mara

APEM

EAO  
European Association of Fish Producers Organisations

Iascach Intire Éireann  
Inland Fisheries Ireland

OIRIEL RIVER CATCHMENTS AND COASTAL ASSOCIATION

## eDNA

**Environmental DNA (eDNA) is any DNA in the form of sloughed biological detritus (such as mucous, faeces, blood, skin cells, or gametes) that has been separated from an organism and is freely present in the environment**



# Why eDNA?

**-Non-invasive**





# Why eDNA?

- Non-invasive
- Cost effective



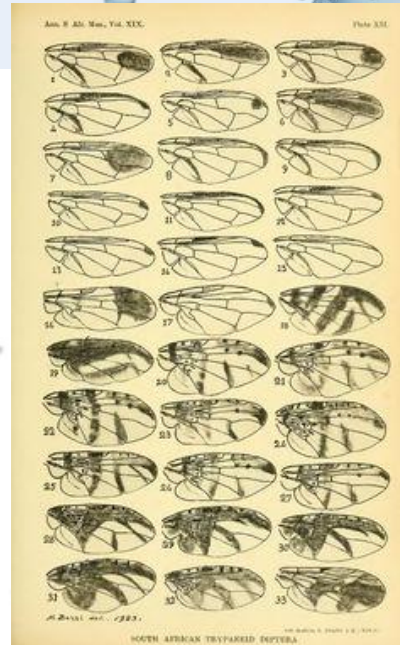
# Why eDNA?

- Non-invasive
- Cost effective
- Samples are relatively easy to take (e.g. taking a water sample vs electrofishing)



# Why eDNA?

- Non-invasive
- Cost effective
- Samples are relatively easy to take (e.g. taking a water sample vs electrofishing)
- Does not rely on taxonomic expertise to ID species



# Why eDNA?

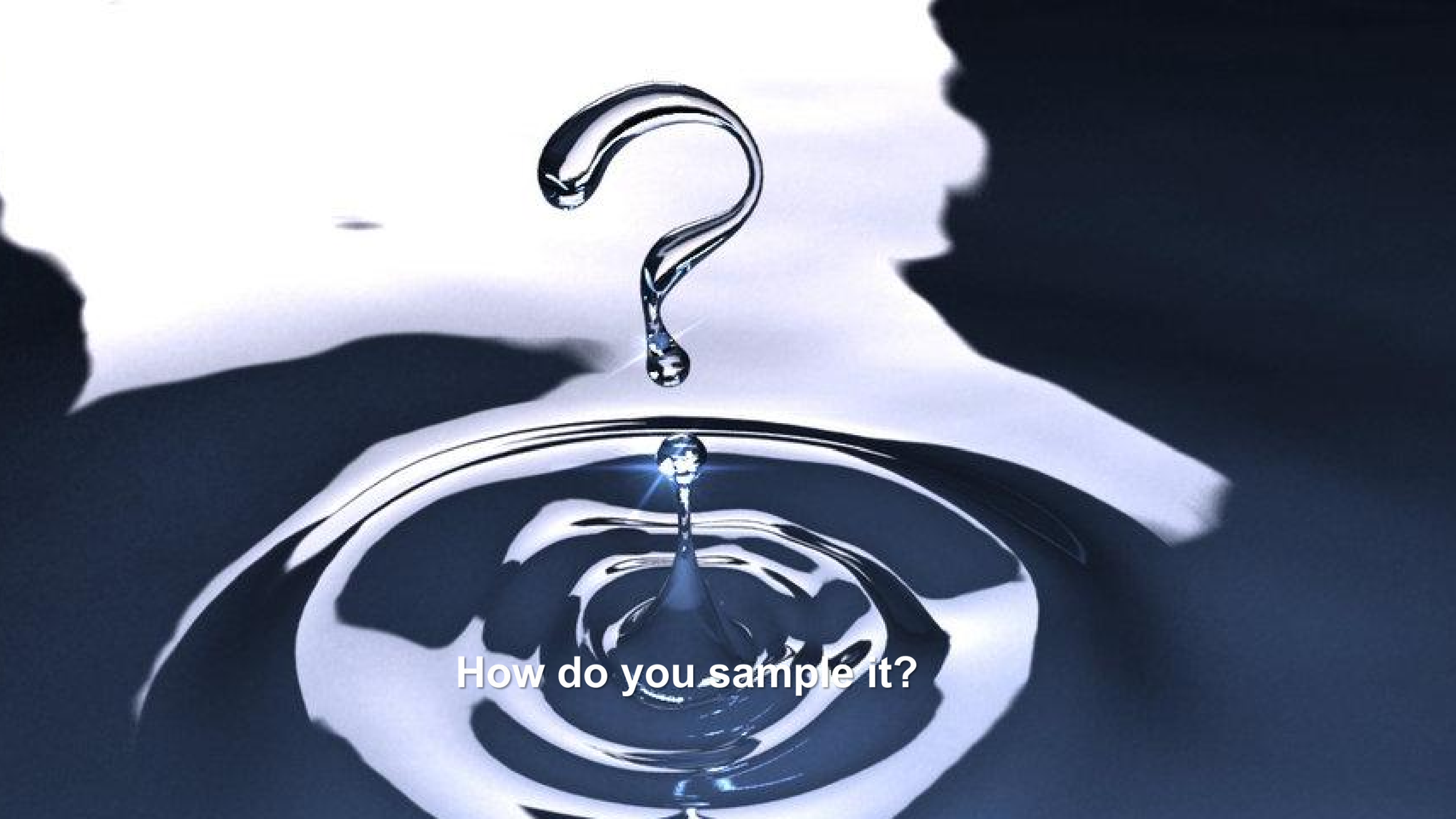
- Non-invasive
- Cost effective
- Samples are relatively easy to take (e.g. taking a water sample vs electrofishing)
- Does not rely on taxonomic expertise to ID species
- Can be used at any life stage



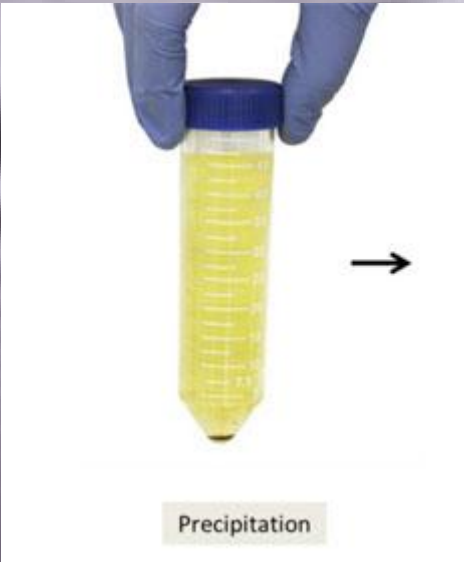
# Why eDNA?

- Non-invasive
- Cost effective
- Samples are re taking a water s
- Does not rely o
- species
- Can be used at
- Easy to take replicates/coverage





How do you sample it?



**DNA precipitation**

**DNA filtration**

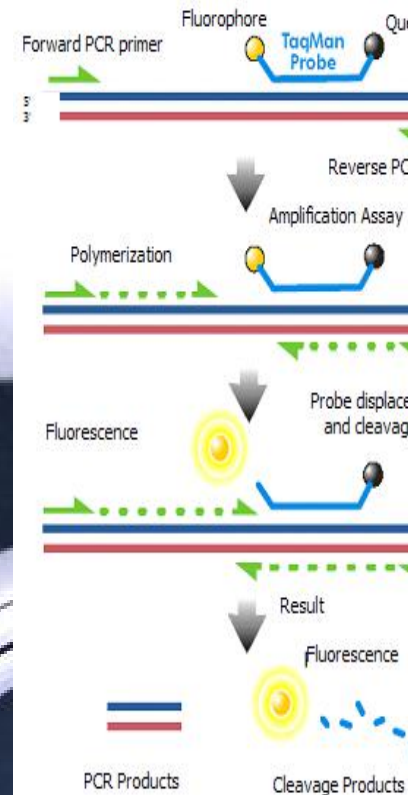




How to detect it?



# Primer and probe design



Primer Express 3.0

TaqMan<sup>®</sup> MGB Quantification # 1

| Seq                        | Primer / Probe | Order    |        |         |           |          |        |
|----------------------------|----------------|----------|--------|---------|-----------|----------|--------|
| Candidate Primers & Probes |                |          |        |         |           |          |        |
| #                          | Prd Start      | Prd Len. | Prd To | Prd 35C | Rev Start | Rev Len. | Rev To |
| 1                          | 1241           | 15       | 53     | 40      | 1095      | 13       | 59     |
| 2                          | 1040           | 19       | 58     | 58      | 1099      | 22       | 59     |
| 3                          | 1040           | 19       | 58     | 58      | 1100      | 22       | 59     |
| 4                          | 1040           | 19       | 58     | 58      | 1100      | 22       | 59     |
| 5                          | 1040           | 19       | 58     | 58      | 1100      | 22       | 59     |

Location

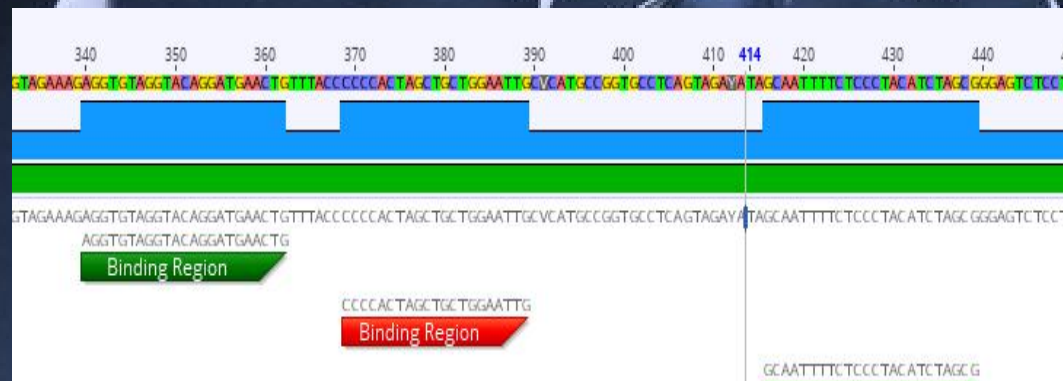
Secondary Structure

| Primer         | Length | Main | Self Dimers | Cross Dimers                |
|----------------|--------|------|-------------|-----------------------------|
| Forward Primer | 15     |      |             | None Stable Structure Found |
| Reverse Primer | 19     |      |             |                             |
| Probe          | 17     |      |             |                             |

Forward Primer: TTGCCCGCGCATT

Reverse Primer: BAAGCCCTGCACTGCAAA

Probe: TGAGGACTCTGATGCCG



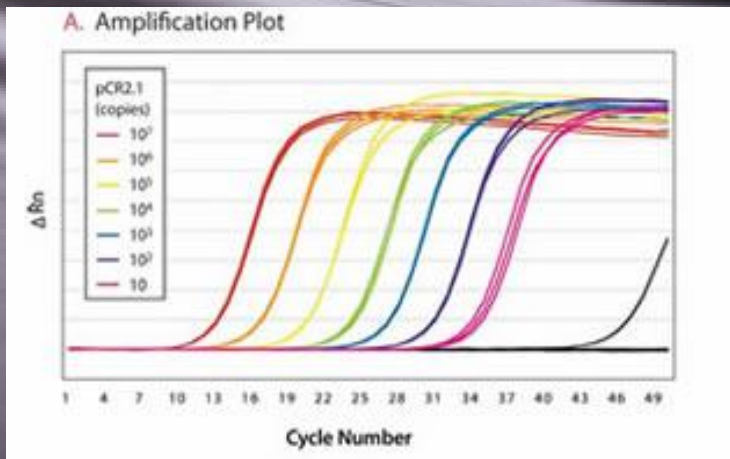
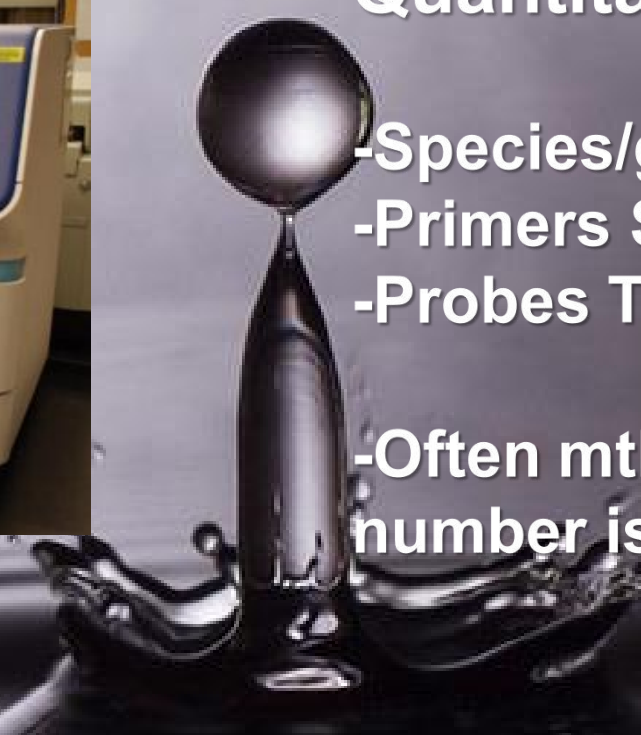
In silico primer and probe design for qPCR MGB probe based TaqMan qPCR assay.

# Quantitative (q/dd)PCR



- Species/group specific
- Primers SYBR Green
- Probes TaqMan/MGB

-Often mtDNA as copy number is high



-F  
-q

Journal of **FISH BIOLOGY**

Journal of Fish Biology (2015)  
doi:10.1111/jfb.12781, available online at wileyonlinelibrary.com

**BRIEF COMMUNICATION**

**An eDNA assay for Irish *Petromyzon marinus* antrutta and field validation in running water**

M. S. GUSTAVSON\*, P. C. COLLINS\*, J. A. FINARELLI\*, D. F. R. Ó. CONCHÚIR†, G. D. WIGHTMAN‡, J. J. KING‡, D. T. GAIR†, K. WHELAN||, J. E. L. CARLSSON\* AND J. CARLSSON\*

\*Area 52 Research Group, School of Biology & Environment Science and E. University College Dublin, Belfield, Dublin, Ireland. †Inland Fisheries Ireland.

Mar Biol (2017) 164:112  
DOI 10.1007/s00227-017-3141-x

**METHOD**

**Development of biodiversity of devil ray**

Laura M. Garga<sup>a</sup>, Jeanette E. L. Carlsson<sup>a</sup>

Received: 4 December 2017 | Revised: 15 February 2018 | Accepted: 3 April 2018  
DOI: 10.1002/aqc.2931

**SHORT COMMUNICATION**

**A quantitative PCR-based environmental DNA assay for detecting Atlantic salmon (*Salmo salar* L.)**

Siobhán Atkinson<sup>1,2</sup> | Jeanette E.L. Carlsson<sup>2</sup> | Bernard Ball<sup>2</sup> | Damian Egan<sup>1</sup> | Mary Kelly-Quinn<sup>1</sup> | Ken Whelan<sup>1,3</sup> | Jens Carlsson<sup>2</sup>

Received: 6 June 2018 | Revised: 17 October 2018 | Accepted: 19 November 2018  
DOI: 10.1002/ece3.4777

**ORIGINAL RESEARCH**

**Identifying spawning sites and other critical habitat in lotic systems using eDNA "snapshots": A case study using the sea lamprey *Petromyzon marinus* L.**

Fiona S. A. Bracken<sup>1</sup> | Sean M. Rooney<sup>2</sup> | Mary Kelly-Quinn<sup>3</sup> | James J. King<sup>2</sup> | Jens Carlsson<sup>1</sup>

Received: 22 November 2016 | Accepted: 16 April 2017  
DOI: 10.1002/aqc.2788

**SHORT COMMUNICATION**

**A qPCR MGB probe based eDNA assay for freshwater pearl mussel**

Siobhán Atkinson<sup>1,2</sup> | Jeanette E.L. Carlsson<sup>2</sup> | Bernard Ball<sup>2</sup> | Damian Egan<sup>1</sup> | Mary Kelly-Quinn<sup>1</sup> | Ken Whelan<sup>1,3</sup> | Jens Carlsson<sup>2</sup>

**WILEY**

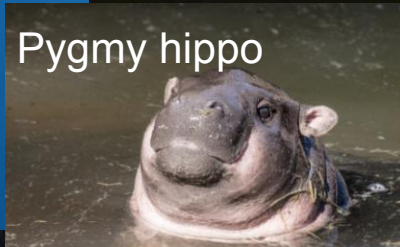
**assay for the threatened *Potamobius pallipes***

Siobhán Atkinson<sup>1,2</sup>, Mary Kelly-Quinn<sup>1,6</sup>

<sup>1</sup>College Dublin, Dublin, Ireland  
<sup>2</sup>Earth Institute, University College Dublin, Dublin, Ireland

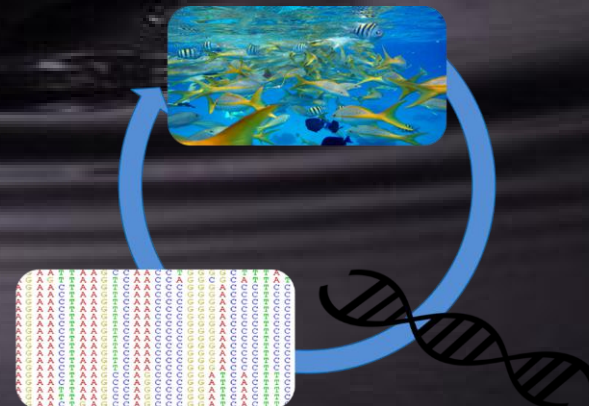


-Filter  
-qPCR using MGB Probes



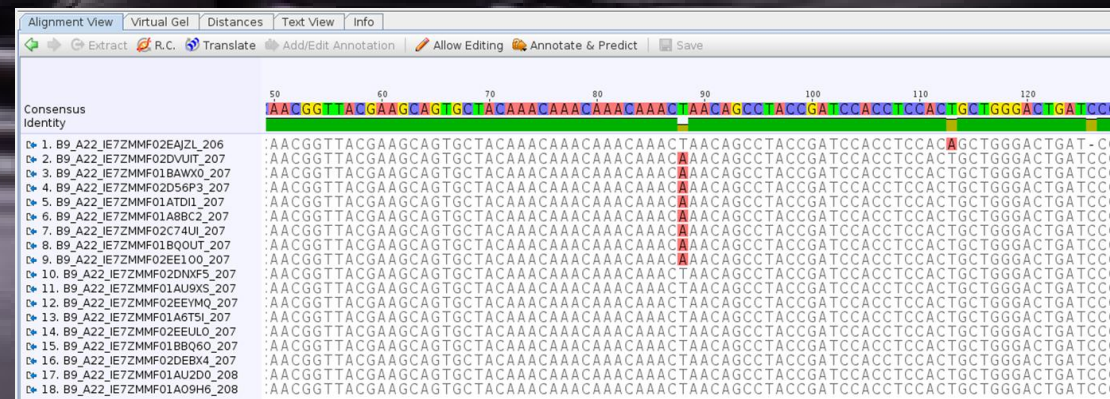
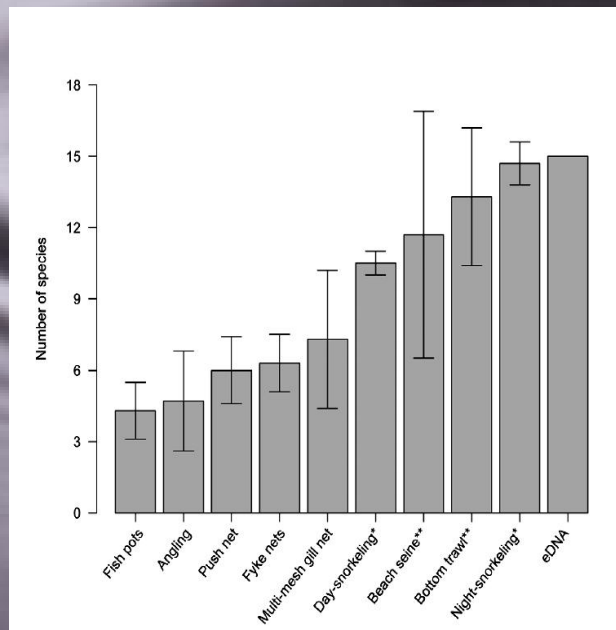
# Metabarcoding

Sequence pooled DNA to detect presence (quantify) organisms



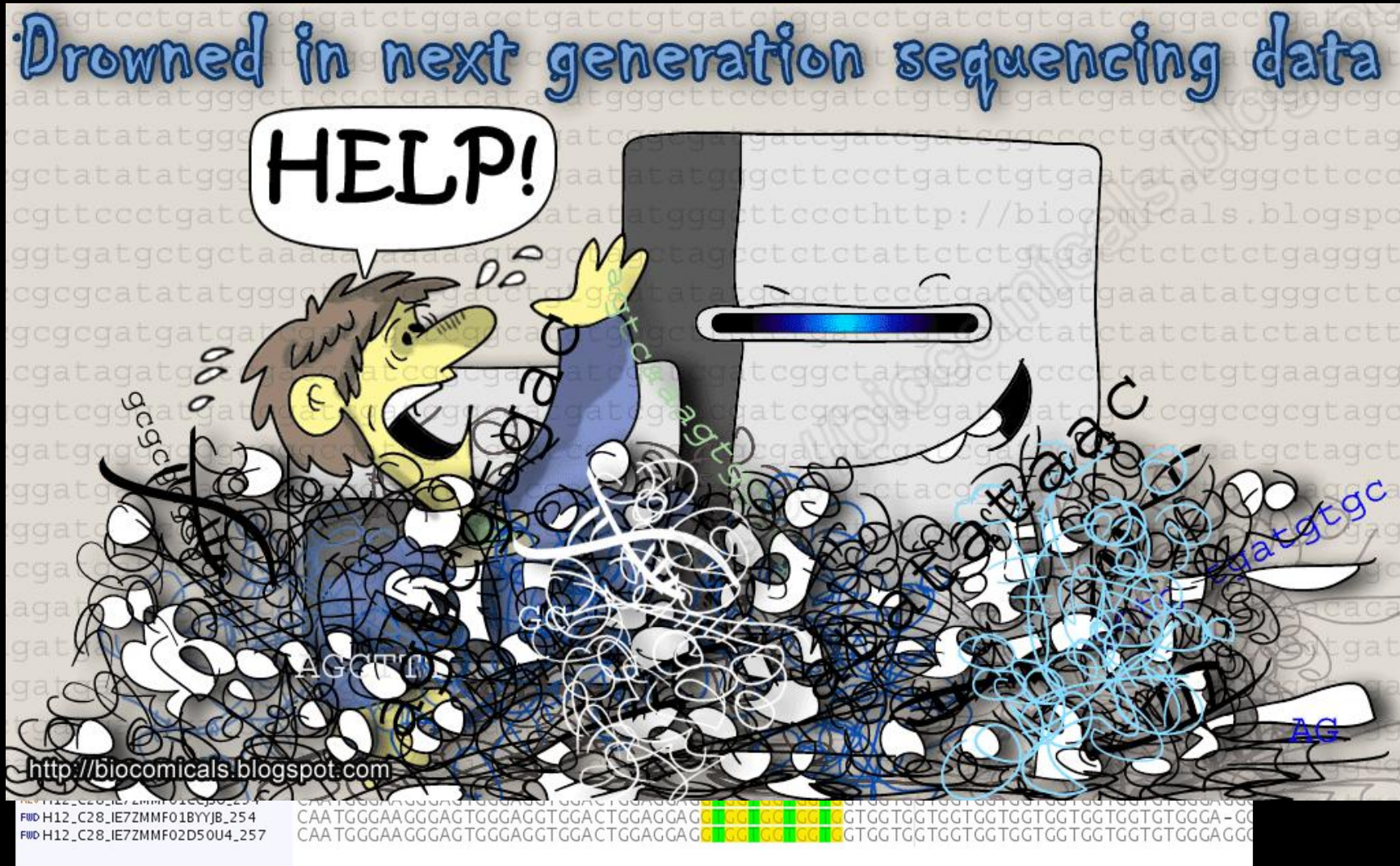
# Metabarcoding

- using “universal” primers to amplify and sequence the DNA of many species simultaneously
- Next generation sequencing
- Qualitative data – presence/absence
- Quantitative data??





# Bioinformatics





# Issues 1

Serious problem...

Quantification using reads/species?

What if?



# Issues 1

Serious problem...

Quantification using reads/species?

What if?

Holy crap! -the ocean is dominated by whale sharks and krill...



# Issues 2

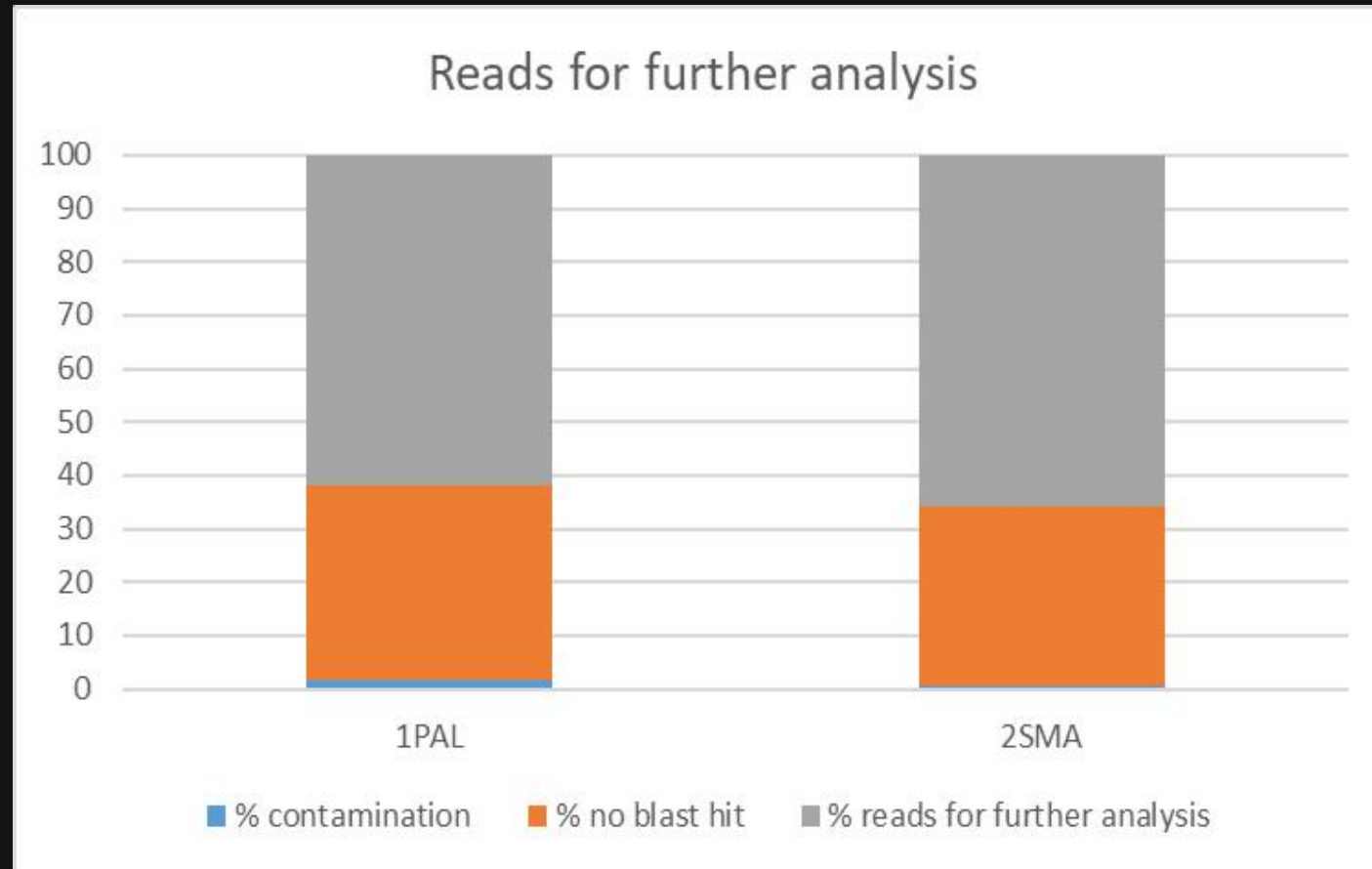
**Serious problem...**

Most or many of the primers used for metabarcoding will amplify **YOU** and **EVERYONE ELSE** taking part in sampling or working in the lab - human contamination...

**Would be nice with a human blocker...**

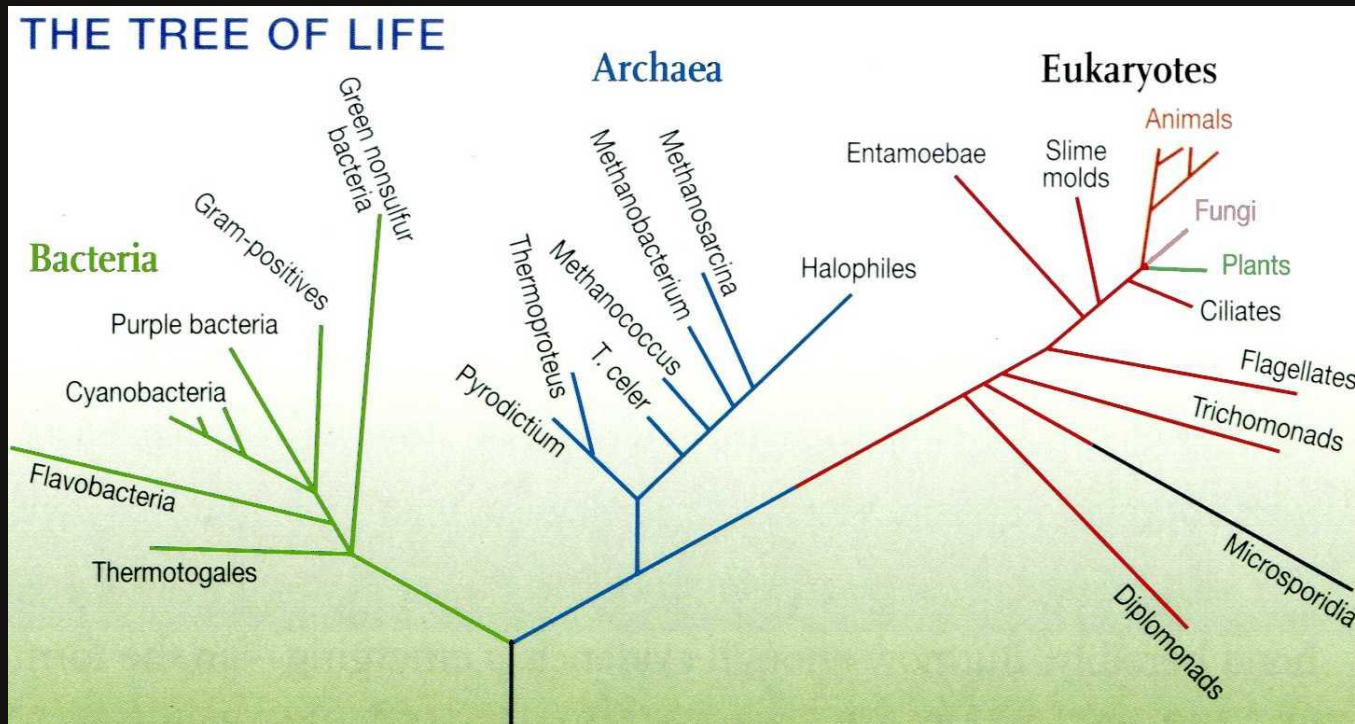


# Issues 3



# Biodiversity

- ~ 1 million known species
- ~ 8.7 million estimated (eukaryotes)
- Microbial????

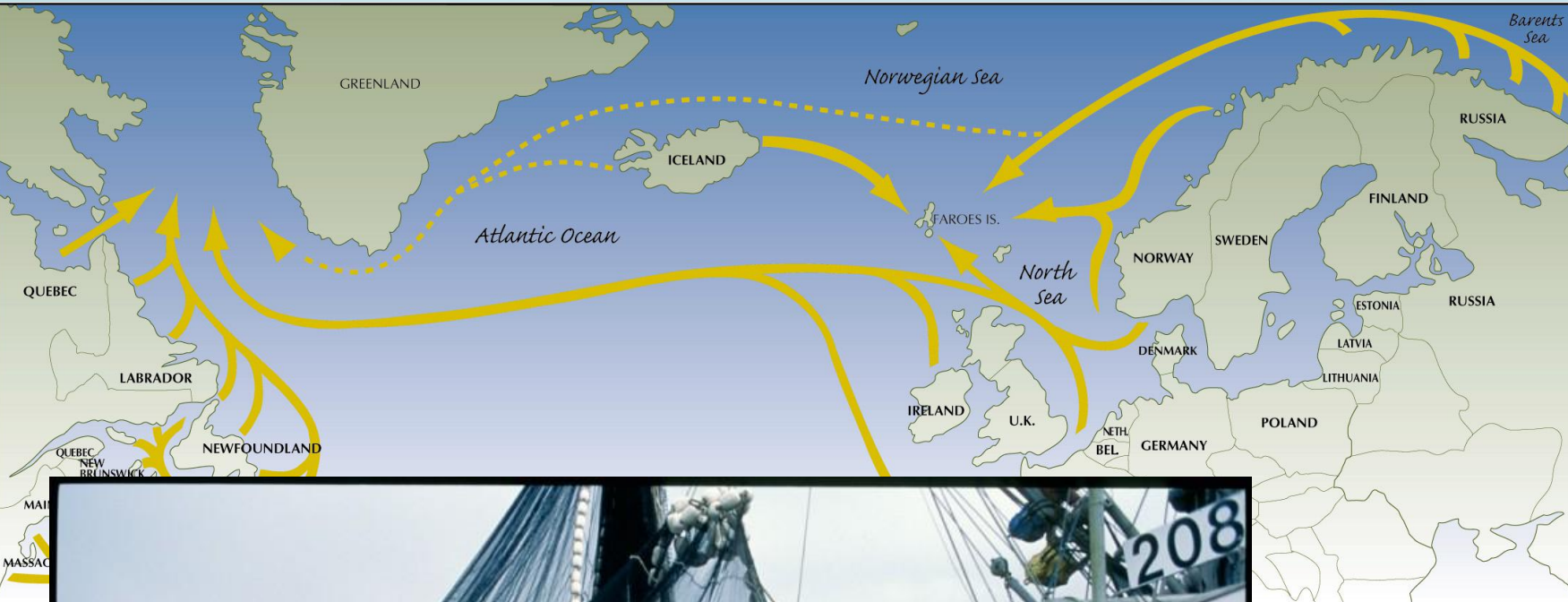


- <6000 species of mammals
- ~7000 species of amphibians
- ~20,000 species of bird & reptiles
- ~30,000 species of fish

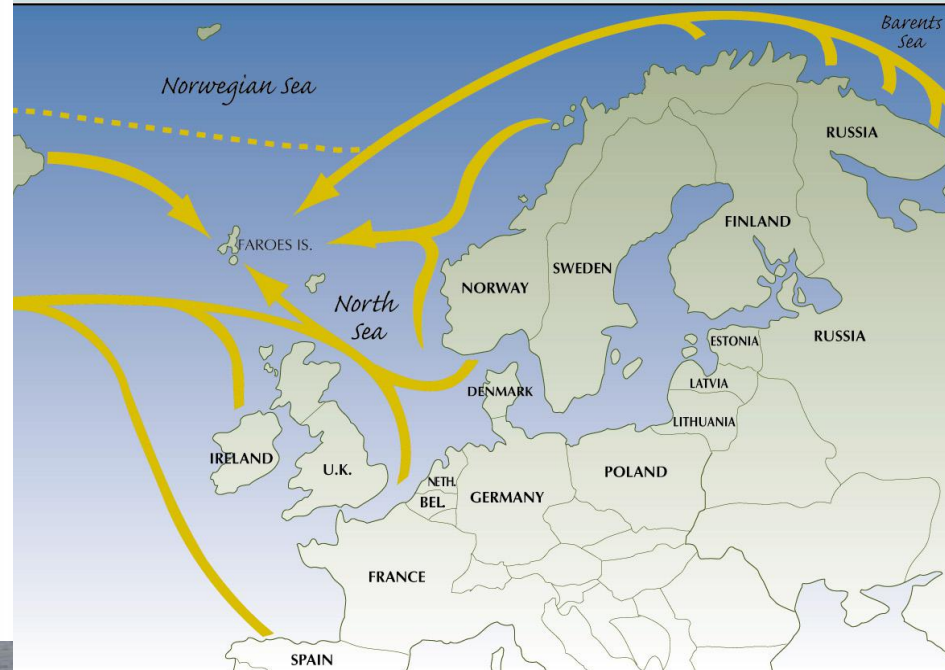
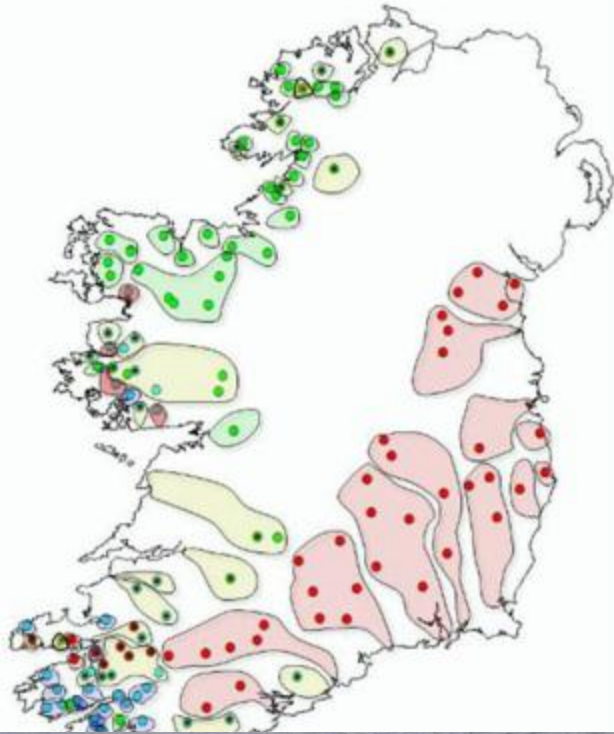
# Surveying for migration obstacles



# Bycatch -as a tool?



Bycatch  
-as a tool?  
-constant sampling



Clade specific  
Corridors  
Temporal  
Distribution



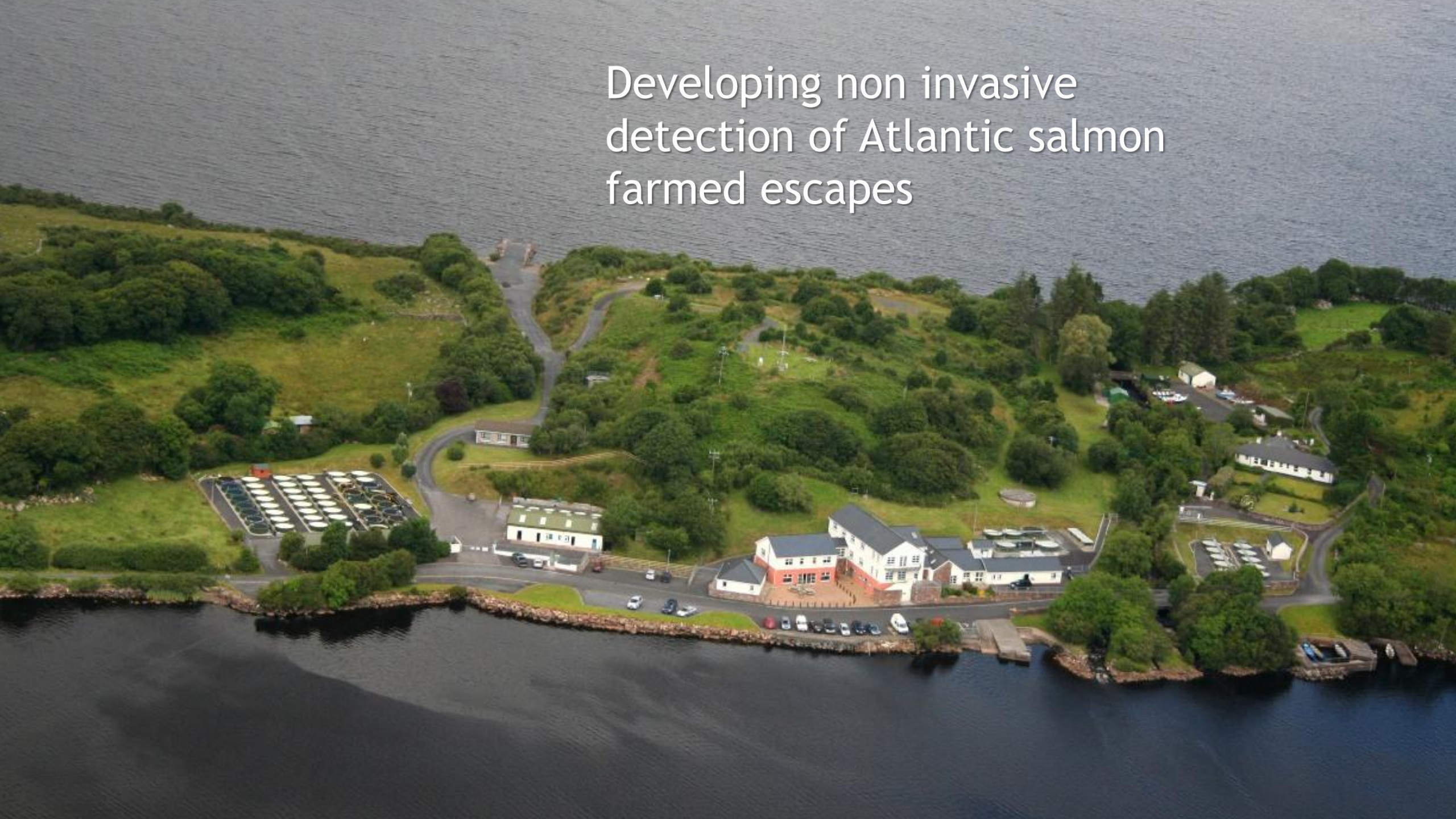
# Paleo records of Atlantic salmon



# Atlantic salmon farmed escapes



Developing non invasive  
detection of Atlantic salmon  
farmed escapes





Both wild and farmed Atlantic salmon introduced to experimental stream



Both wild and farmed Atlantic salmon introduced to experimental stream





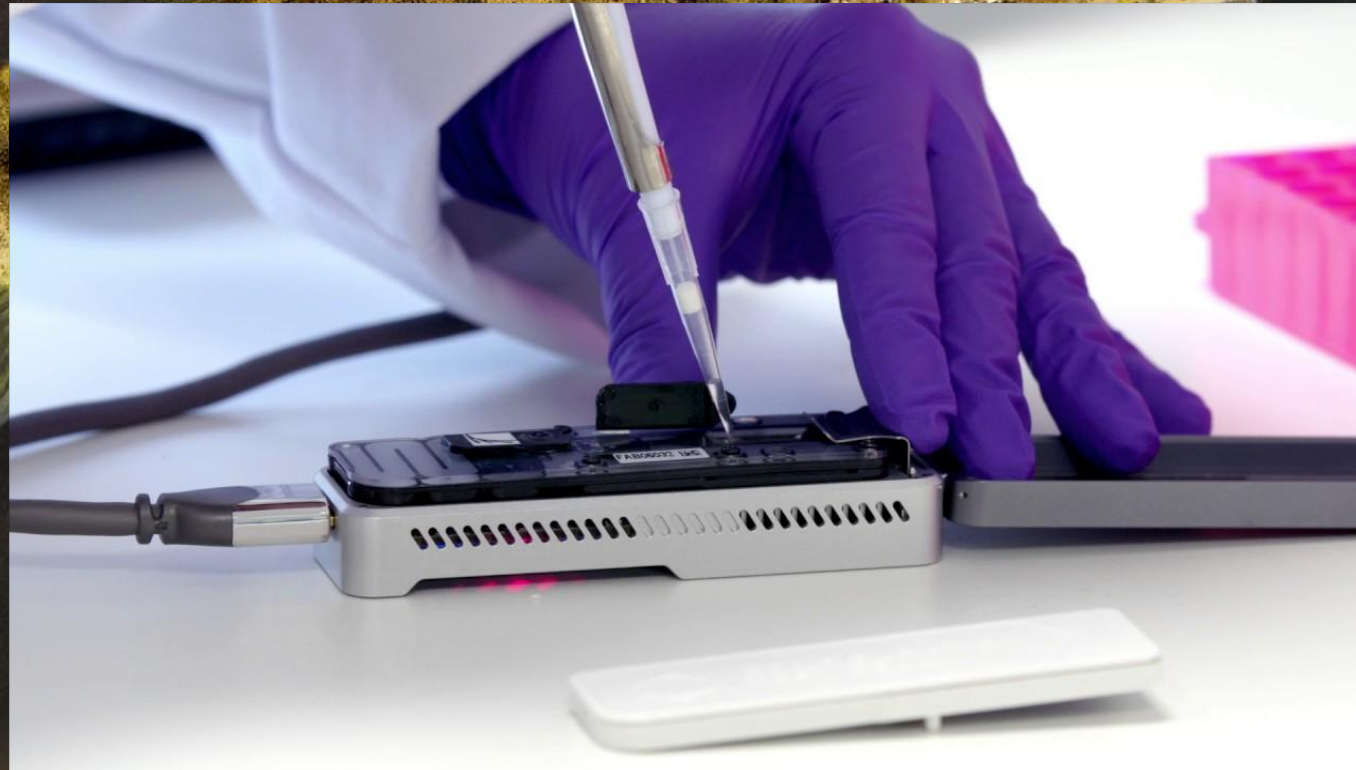
Wild samples - UCD

Farmed sampled - UCD

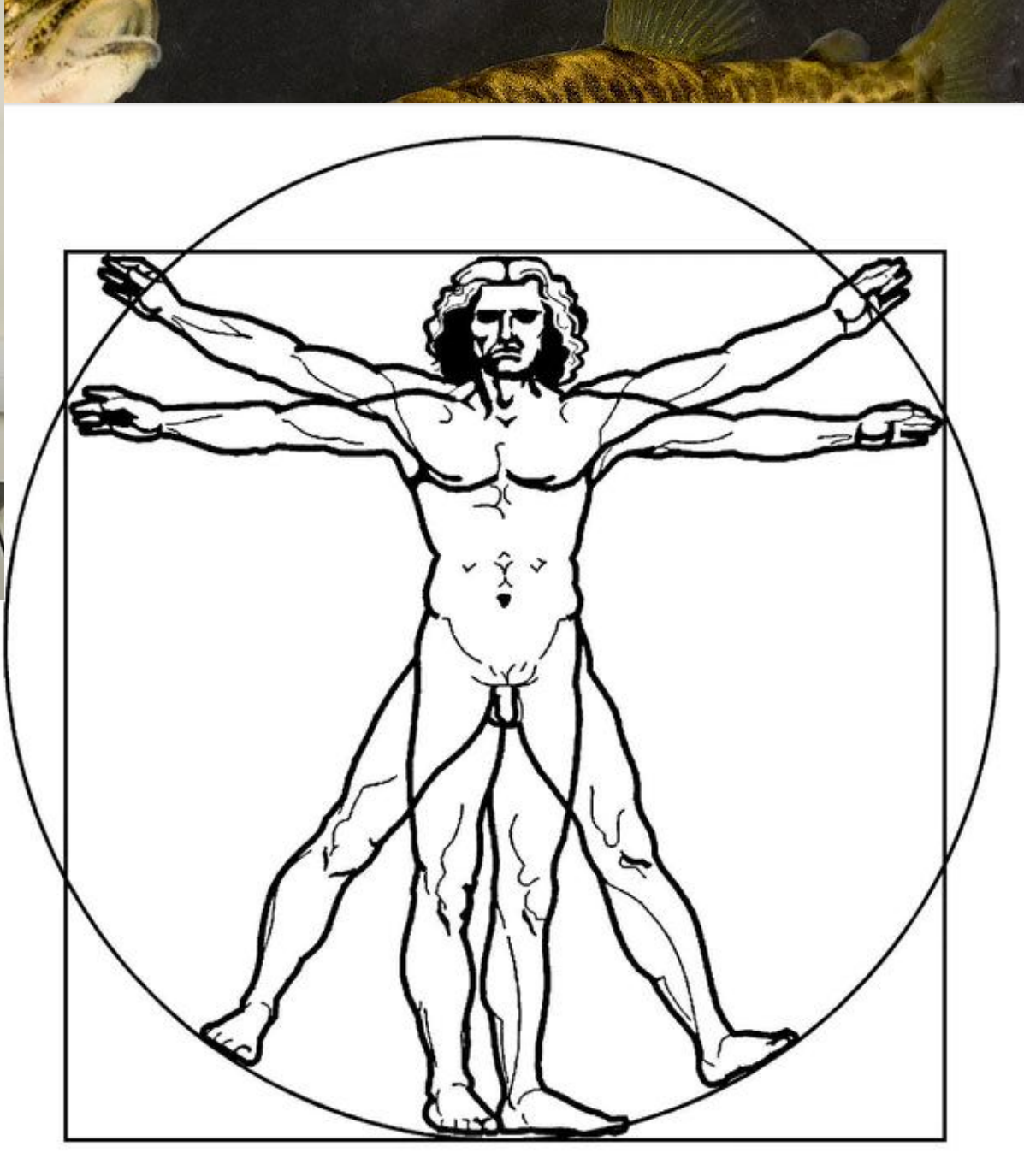
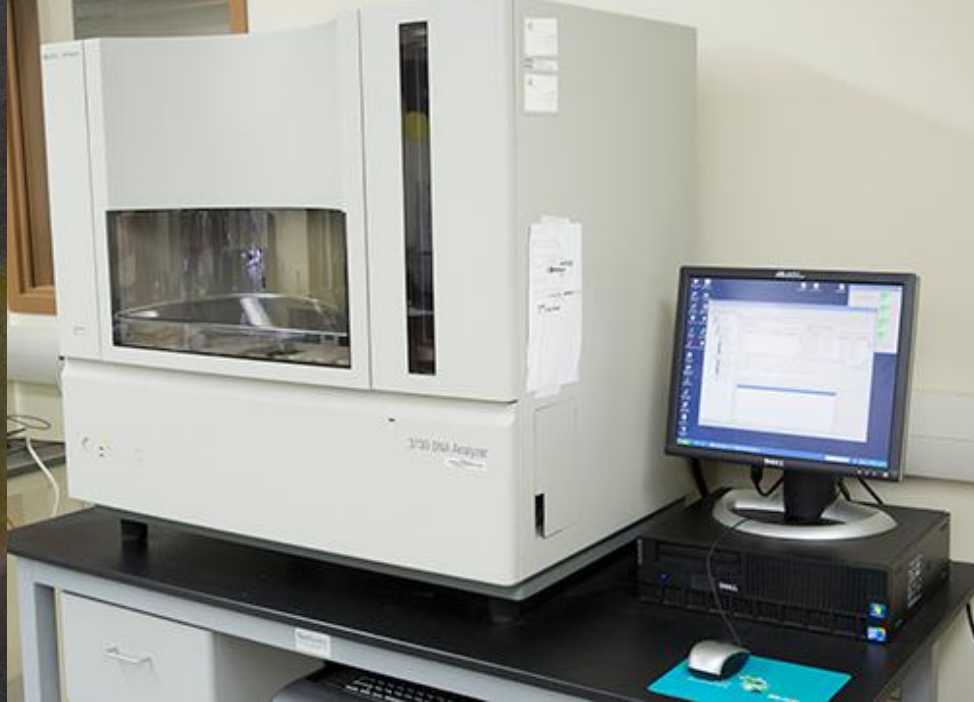
# Minion sequencer

-22Gb in 48h

-EUR900







## Human genome project

33Gb (10x) in 13 years

USD5 billion

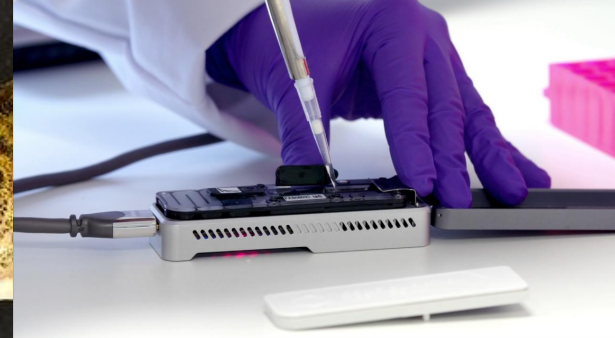
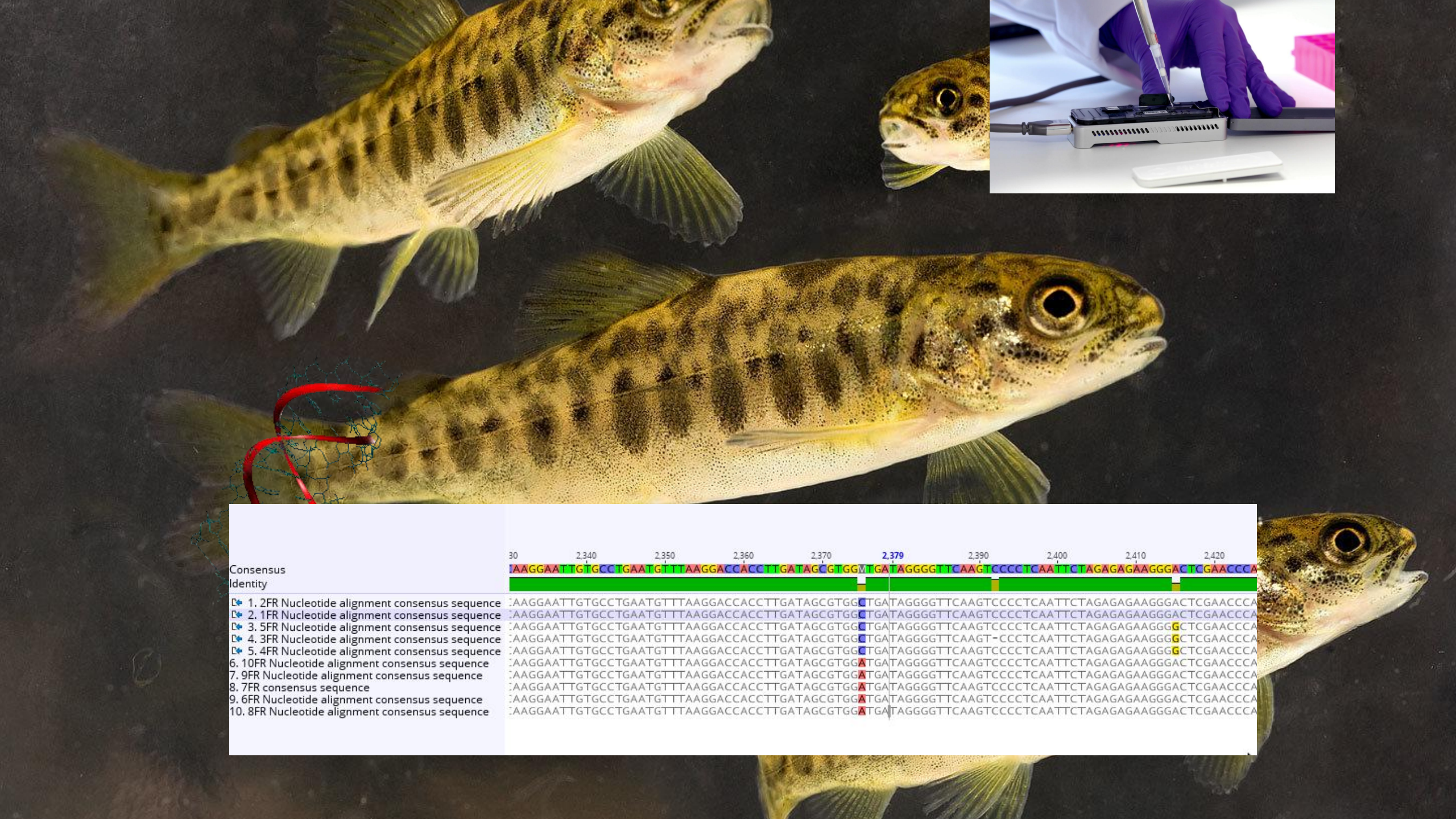
c. 300,000 bp/h

c. 6bp/USD

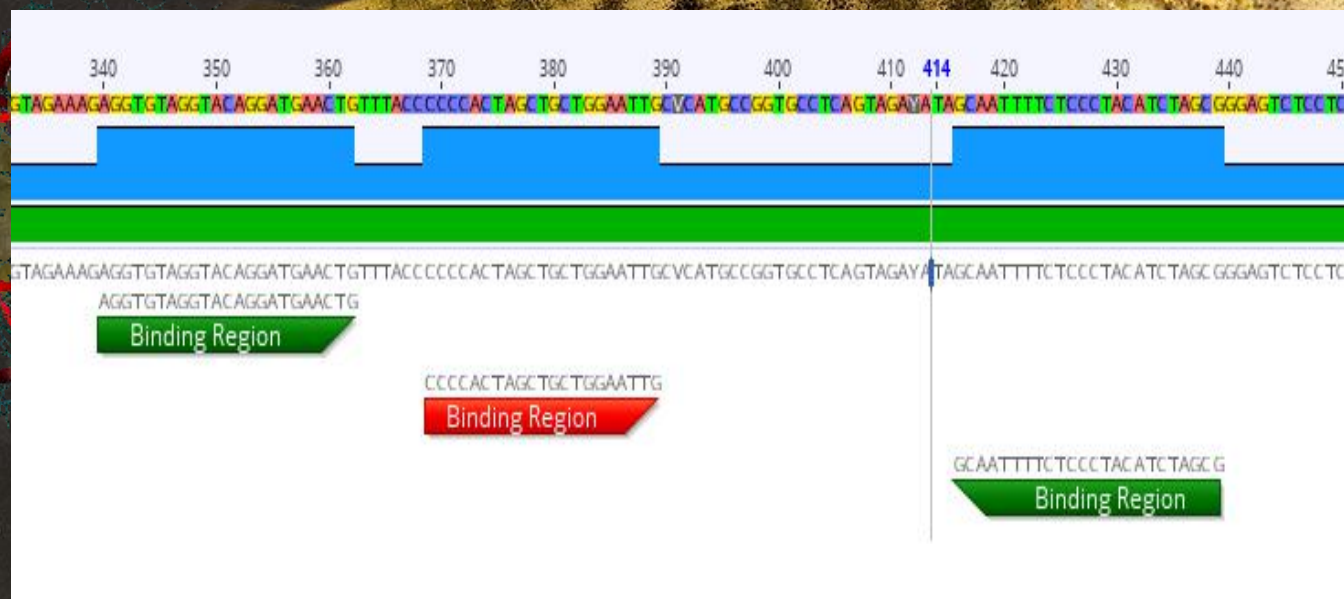
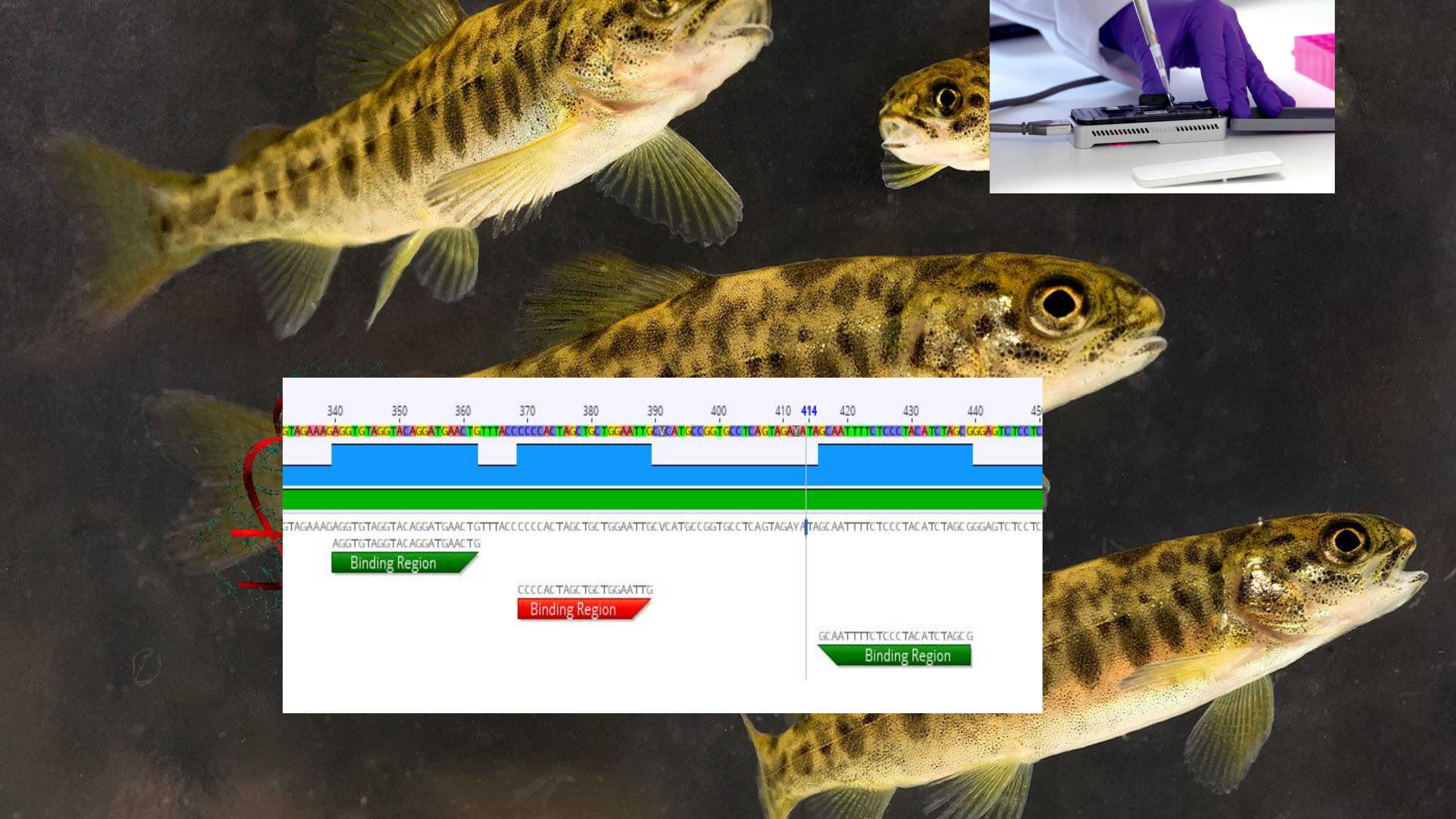
# Minion sequencer

- 22Gb in 48h
- EUR900
- c. 450,000,000 bp/h
- c. 22,000,000 bp/EUR





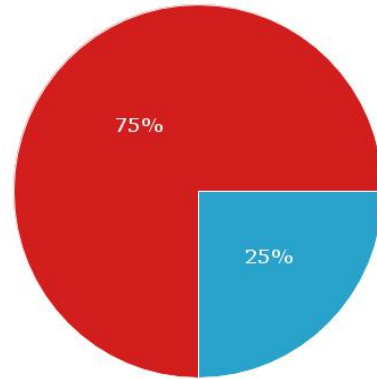
|   | 30  | 2,340 | 2,350 | 2,360 | 2,370 | 2,379 | 2,390 | 2,400 | 2,410 | 2,420 |  |
|---|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|--|
| Consensus Identity                              | :AAGGAAATTGTGCCCTGAATGTTAAGGACCACCTTGATAGCGTGGTGA TAGGGGTTCAAGTCCCCCAATTC TAGAGAGAAGGGACTCGAACC   |       |       |       |       |       |       |       |       |       |  |
| 1. 2FR Nucleotide alignment consensus sequence  | :AAGGAAATTGTGCCCTGAATGTTAAGGACCACCTTGATAGCGTGGCTGATAGGGGTTCAAGTCCCCCTCAATTC TAGAGAGAAGGGACTCGAACC |       |       |       |       |       |       |       |       |       |  |
| 2. 1FR Nucleotide alignment consensus sequence  | :AAGGAAATTGTGCCCTGAATGTTAAGGACCACCTTGATAGCGTGGCTGATAGGGGTTCAAGTCCCCCTCAATTC TAGAGAGAAGGGACTCGAACC |       |       |       |       |       |       |       |       |       |  |
| 3. 5FR Nucleotide alignment consensus sequence  | :AAGGAAATTGTGCCCTGAATGTTAAGGACCACCTTGATAGCGTGGCTGATAGGGGTTCAAGTCCCCCTCAATTC TAGAGAGAAGGGACTCGAACC |       |       |       |       |       |       |       |       |       |  |
| 4. 3FR Nucleotide alignment consensus sequence  | :AAGGAAATTGTGCCCTGAATGTTAAGGACCACCTTGATAGCGTGGCTGATAGGGGTTCAAGTCCCCCTCAATTC TAGAGAGAAGGGACTCGAACC |       |       |       |       |       |       |       |       |       |  |
| 5. 4FR Nucleotide alignment consensus sequence  | :AAGGAAATTGTGCCCTGAATGTTAAGGACCACCTTGATAGCGTGGCTGATAGGGGTTCAAGTCCCCCTCAATTC TAGAGAGAAGGGACTCGAACC |       |       |       |       |       |       |       |       |       |  |
| 6. 10FR Nucleotide alignment consensus sequence | :AAGGAAATTGTGCCCTGAATGTTAAGGACCACCTTGATAGCGTGGATGATAGGGGTTCAAGTCCCCCTCAATTC TAGAGAGAAGGGACTCGAACC |       |       |       |       |       |       |       |       |       |  |
| 7. 9FR Nucleotide alignment consensus sequence  | :AAGGAAATTGTGCCCTGAATGTTAAGGACCACCTTGATAGCGTGGATGATAGGGGTTCAAGTCCCCCTCAATTC TAGAGAGAAGGGACTCGAACC |       |       |       |       |       |       |       |       |       |  |
| 8. 7FR consensus sequence                       | :AAGGAAATTGTGCCCTGAATGTTAAGGACCACCTTGATAGCGTGGATGATAGGGGTTCAAGTCCCCCTCAATTC TAGAGAGAAGGGACTCGAACC |       |       |       |       |       |       |       |       |       |  |
| 9. 6FR Nucleotide alignment consensus sequence  | :AAGGAAATTGTGCCCTGAATGTTAAGGACCACCTTGATAGCGTGGATGATAGGGGTTCAAGTCCCCCTCAATTC TAGAGAGAAGGGACTCGAACC |       |       |       |       |       |       |       |       |       |  |
| 10. 8FR Nucleotide alignment consensus sequence | :AAGGAAATTGTGCCCTGAATGTTAAGGACCACCTTGATAGCGTGGATGATAGGGGTTCAAGTCCCCCTCAATTC TAGAGAGAAGGGACTCGAACC |       |       |       |       |       |       |       |       |       |  |



Proportion FARMED

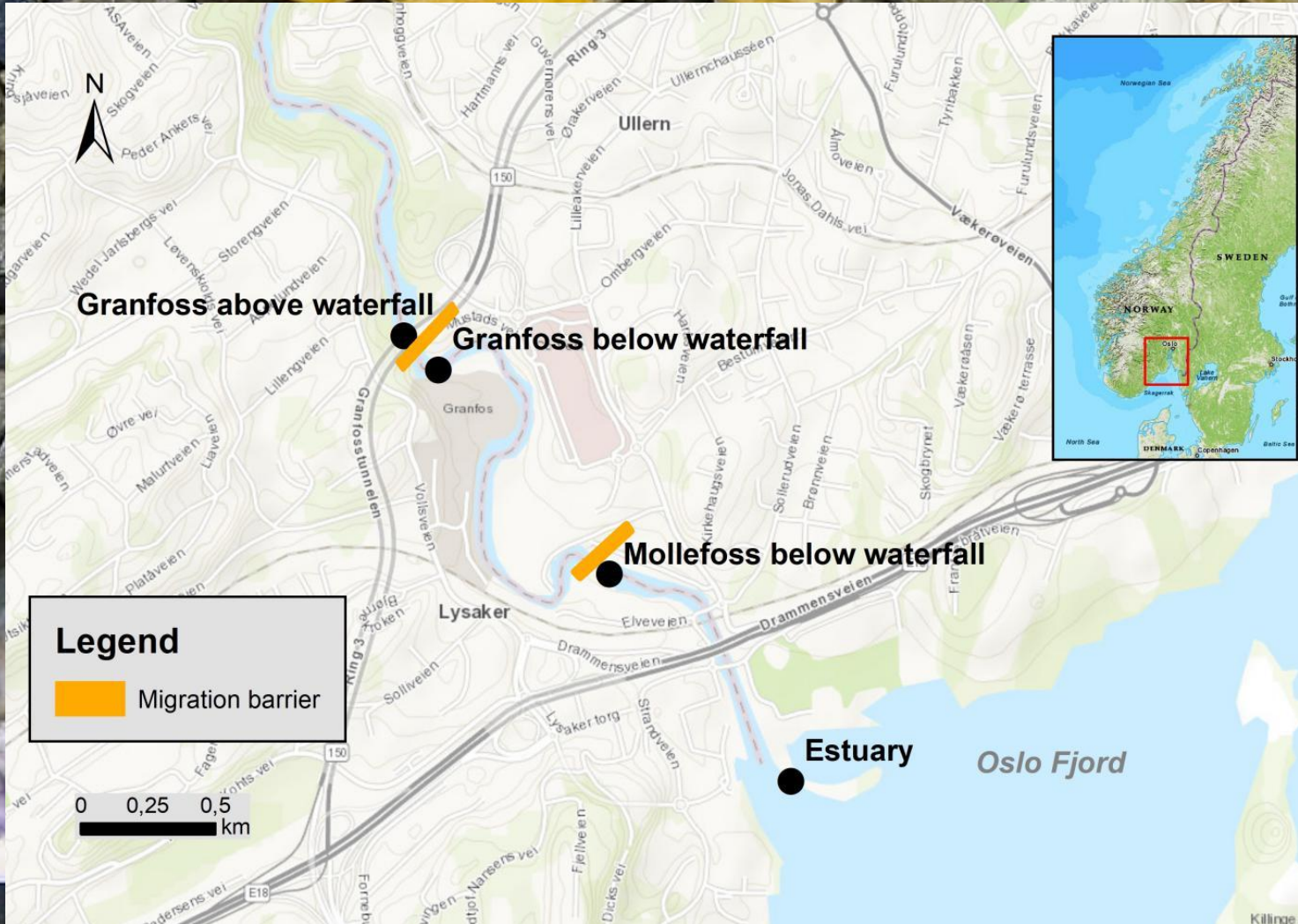


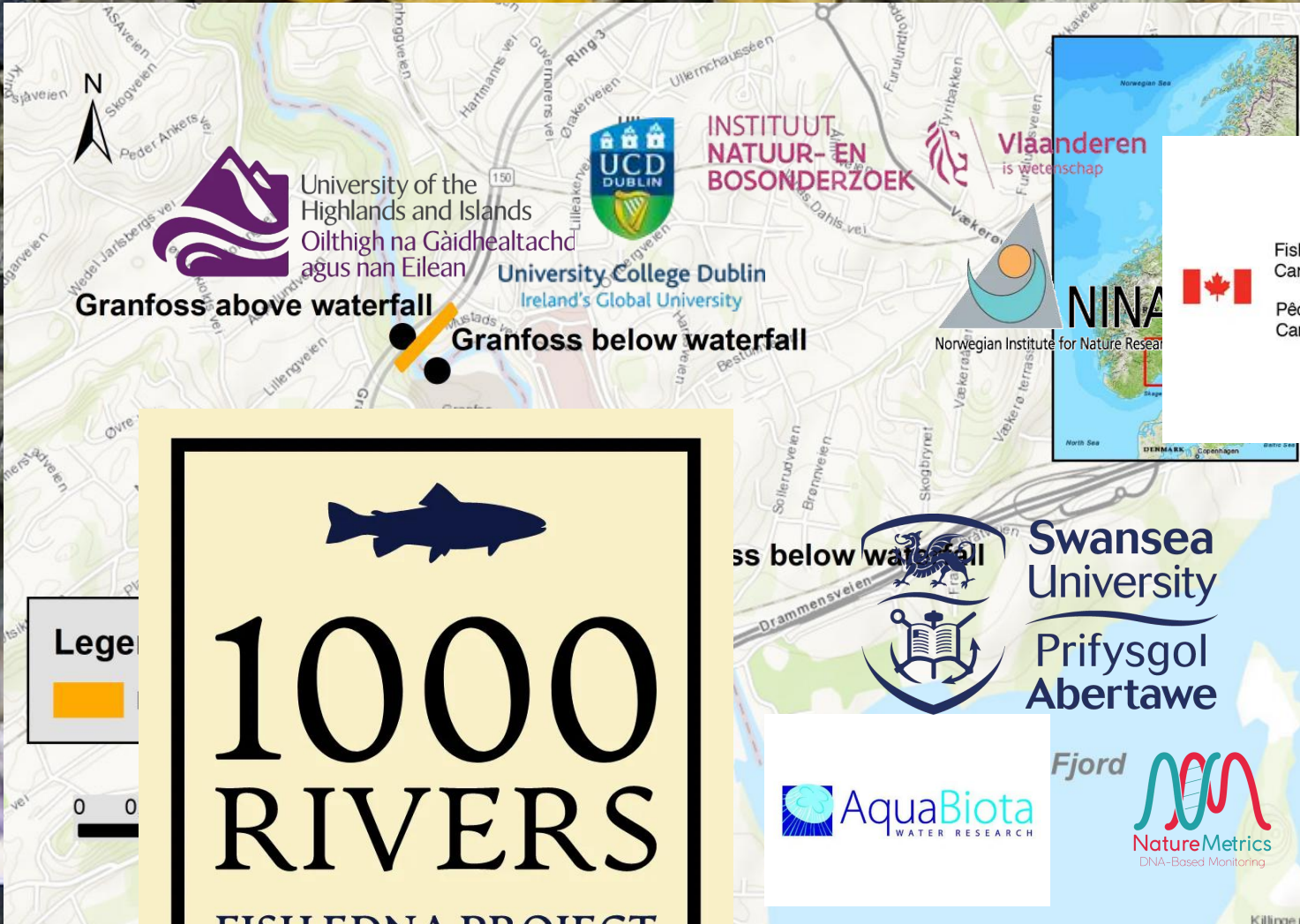
Proportion WILD



Pink salmon







**1000 RIVERS**  
FISH EDNA PROJECT

<https://1000rivers.net>

**INRA**  
SCIENCE & IMPACT

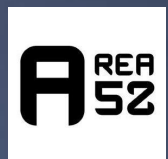
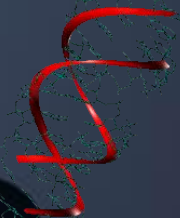
**UNIVERSITY OF HULL**



<https://sites.google.com/view/lab-area52/home>



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