Wildlife-related pathogen surveillance with Nanopore sequencing of eDNA samples: application of shotgun sequencing coupled with Nanopore Adaptive Sampling

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Surveillance for wildlife-related pathogens (WRP):

Management of Disease in Wild Mammals. 2009 : 187–213. PMCID: PMC7121720
doi: 10.1007/978-4-431-77134-0_10

Nature Public Health Emergency Collection

Wildlife Disease Surveillance and Monitoring

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Eur J Wildl Res (2016) 62:511–525
DOI 10.1007/s10344-016-1033-x

CrossMark

REVIEW

Infections shared with wildlife: an updated perspective

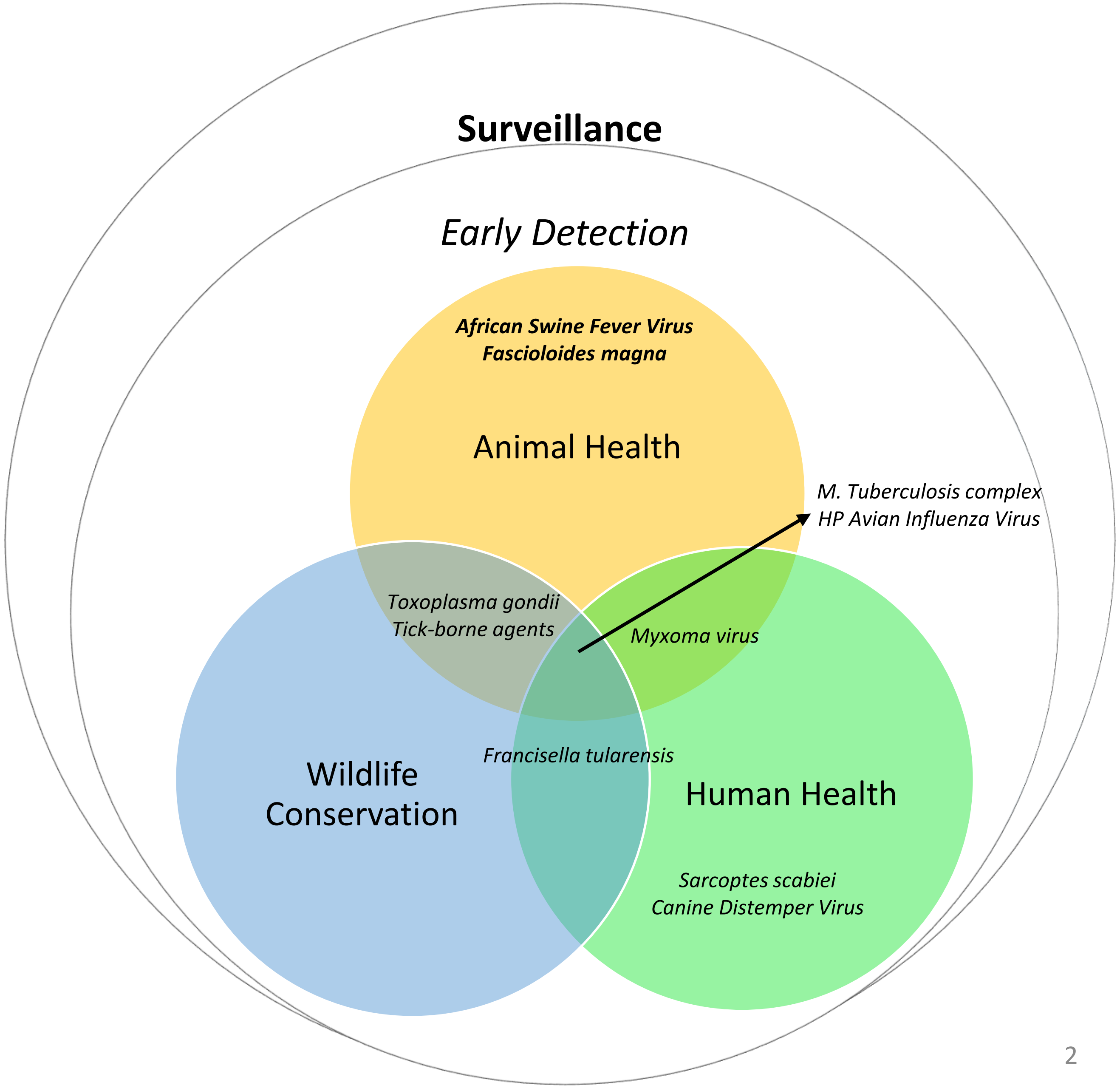
C. Gortázar[1] · J. F. Ruiz-Fons[1] · U. Höfle[1]

Eur J Wildl Res (2007) 53:241–256
DOI 10.1007/s10344-007-0098-y

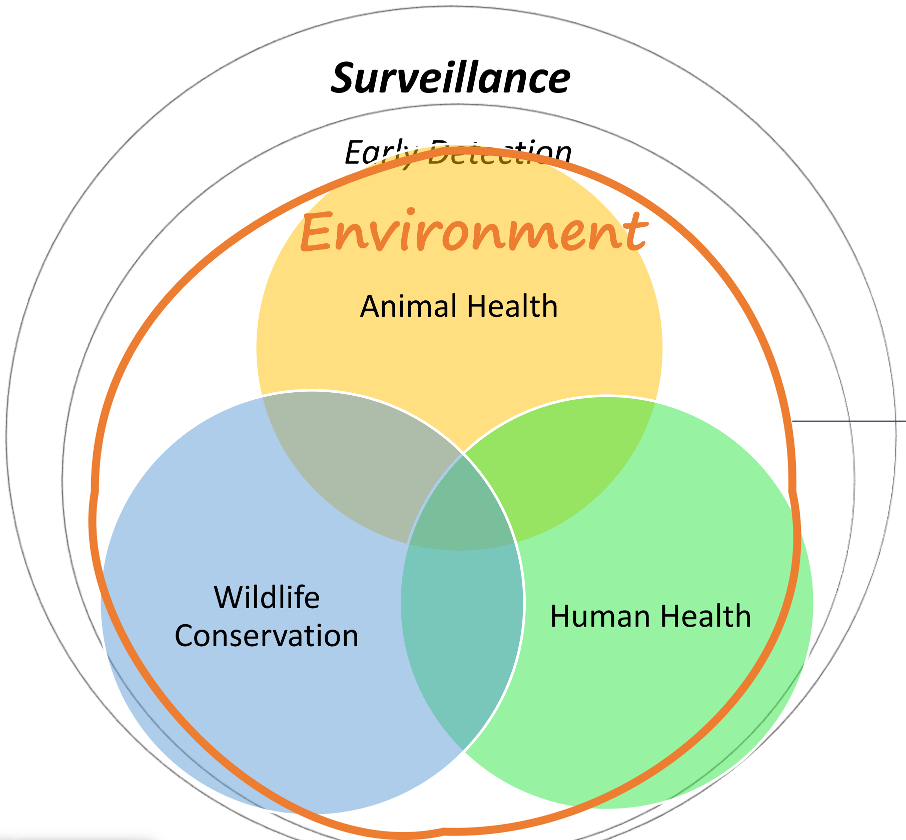
REVIEW

Diseases shared between wildlife and livestock: a European perspective

Christian Gortázar · Ezio Ferroglio · Ursula Höfle · Kai Frölich · Joaquín Vicente



Surveillance for wildlife-related pathogens:



Surveillance

Early Detection

Environment

Animal Health

Wildlife Conservation

Human Health

Figure 1. environmental DNA (eDNA) methods.

Materials and Methods:

Study area and sample collection:

- **8 sampling points:**

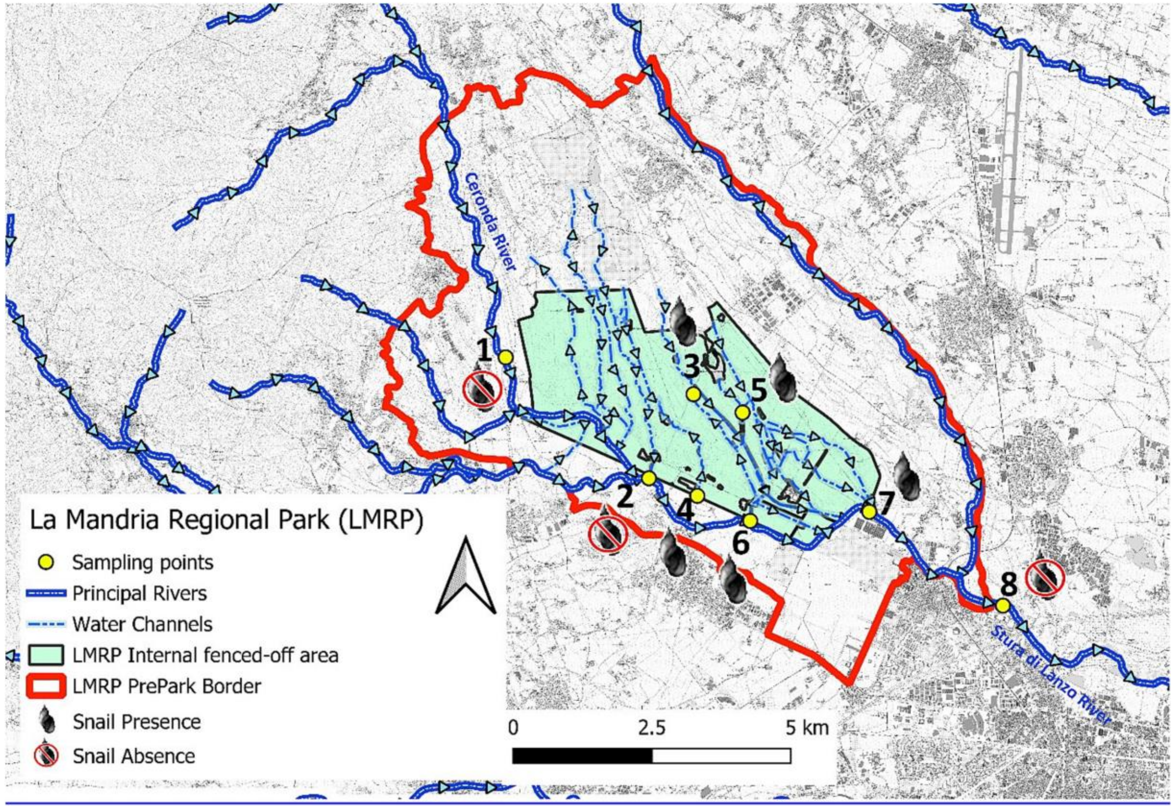


Figure 2. Sampling points. 15-30 Sept. 2022.

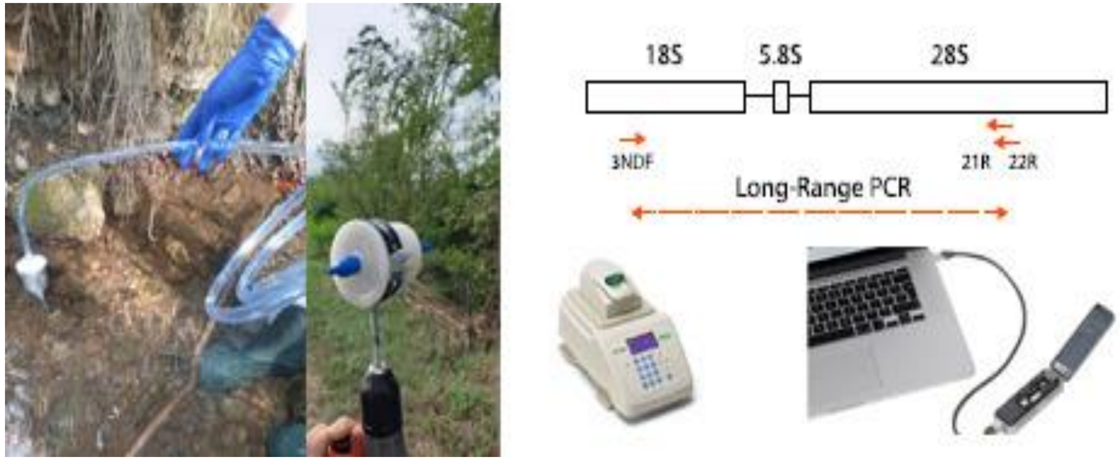


Figure 3. Sampling and laboratory procedures.

eDNA extraction:

DNA extraction with DNeasy Power Soil kit (Qiagen, Germany)

Metabarcoding:

3NDF,21R and 22R primers amplifying a ~4500 bps fragment of protist rDNA operon.

Library Preparation and Nanopore Sequencing:

Oxford Nanopore Technologies’ Native Barcoding Kit for genomic DNA
Equimolar eDNA input of 90 ng/sample

Nanopore Sequencing for WRP genomic surveillance

Materials and Methods:

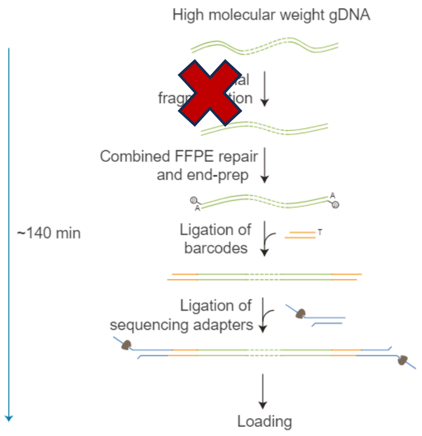
Metabarcoding:

Metabarcoding:

in vitro enrichment

Amplification

Sequencing



Metabarcoding:

Active surveillance

↓

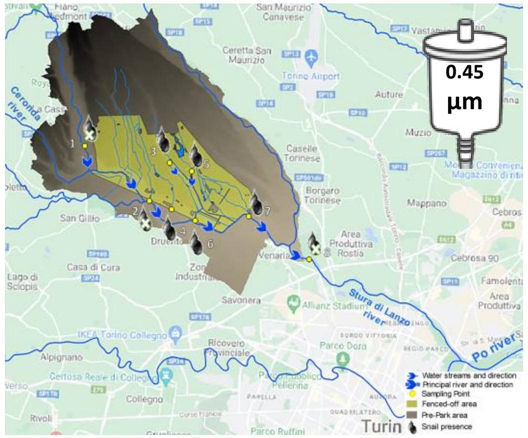
Basecalling and trimming [1]

↓

Clustering , Polishing and Consensus read acquisition [2]

↓

Taxonomy assignment by ncbi BLAST euk-nt [3]



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1. Guppy and Racon, **2**. minimap2

3. https://gitlab.c3s.unito.it/avarzand/blast

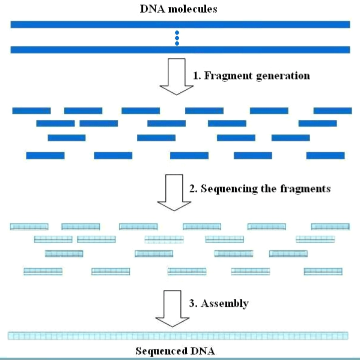
Nanopore Sequencing for WRP genomic surveillance

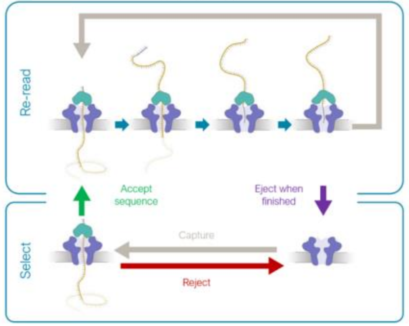
Materials and Methods:

Ligation sequencing and adaptive sampling:

Simultaneous ligation sequencing and Adaptive sampling (SLSAS)

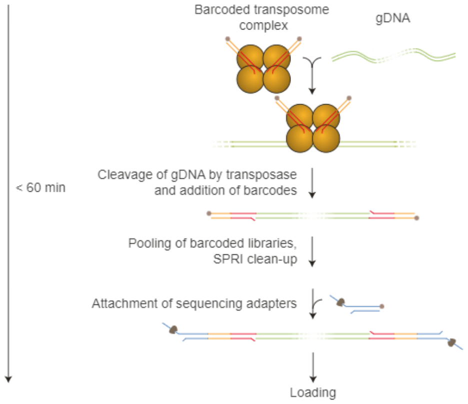
in silica enrichment

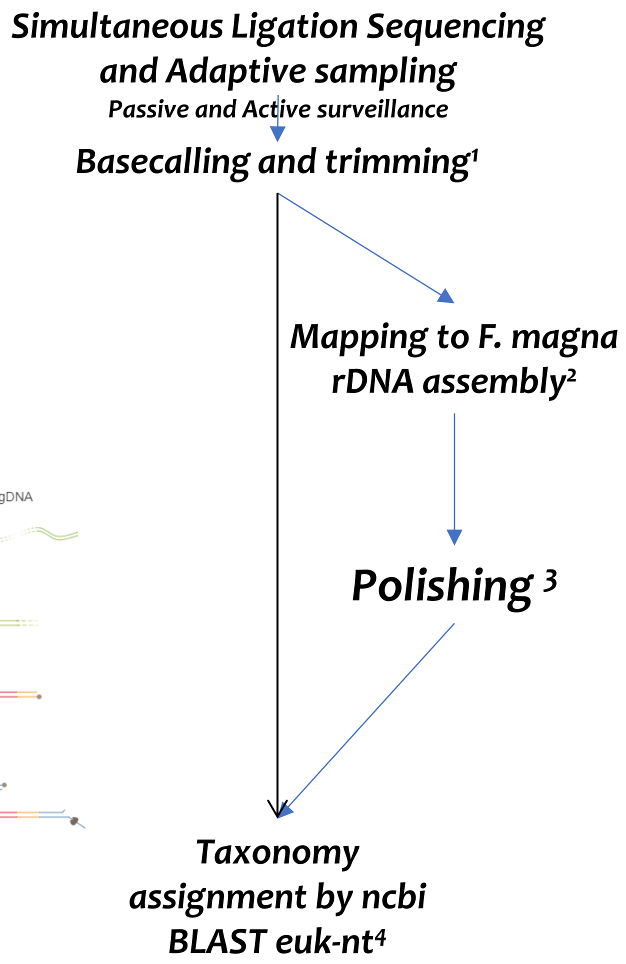




Available ribosomal 18s and 28s of Fascioloidae, Galba sp., Cervus elaphus, Sus scrofa, Radix sp. and etc were used for Adaptive Sampling.

Sequencing





Simultaneous Ligation Sequencing and Adaptive sampling

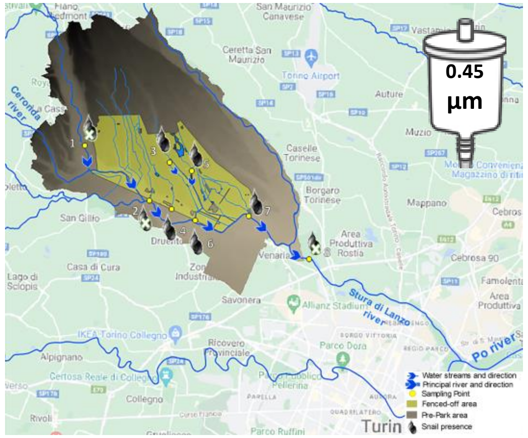
Passive and Active surveillance

Basecalling and trimming[1]

Mapping to F. magna rDNA assembly[2]

Polishing [3]

Taxonomy assignment by ncbi BLAST euk-nt[4]



1. Guppy and Racon, **2**. minimap2, **3**. Racon

4. https://gitlab.c3s.unito.it/avarzand/blast

Nanopore Sequencing for WRP genomic surveillance

Results:

Metabarcoding:

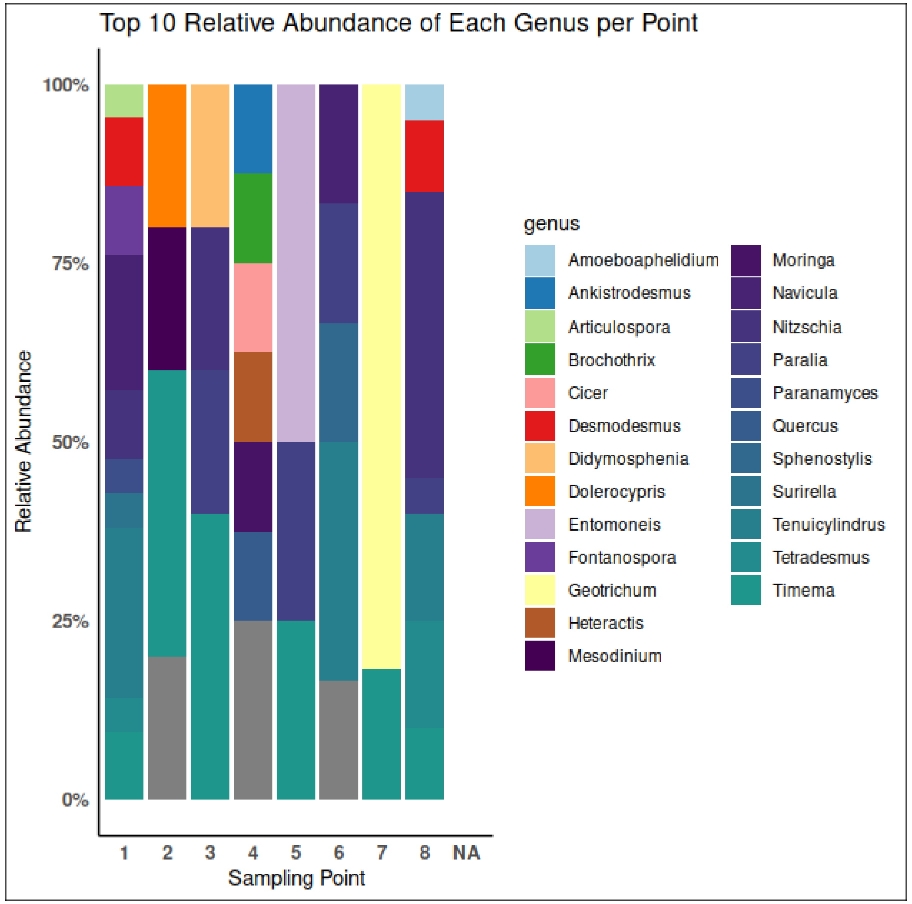
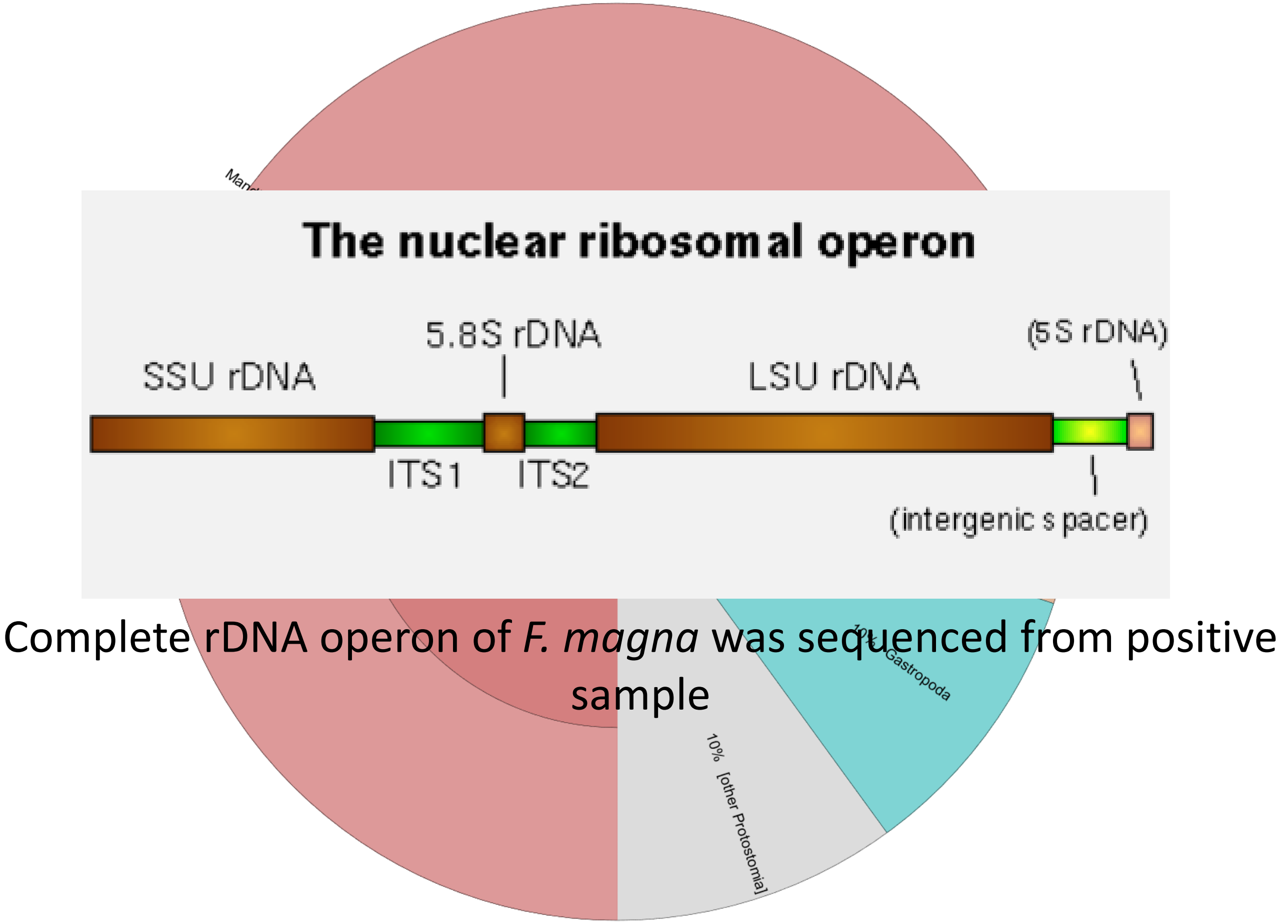


Figure 4. Top 10 abundant genera – each genera represent a consensus read from at least 20 aligned and clustered sequencing reads using Decona(Nijland et al., doi: https://doi.org/10.1101/2021.11.26.470087)workflow.



Complete rDNA operon of *F. magna* was sequenced from positive sample

Figure 5. Highest resolution achieved for targets (particularly *F. magna, and G. truncatula*)

Nanopore Sequencing for WRP genomic surveillance

Results:

Ligation sequencing and adaptive sampling:

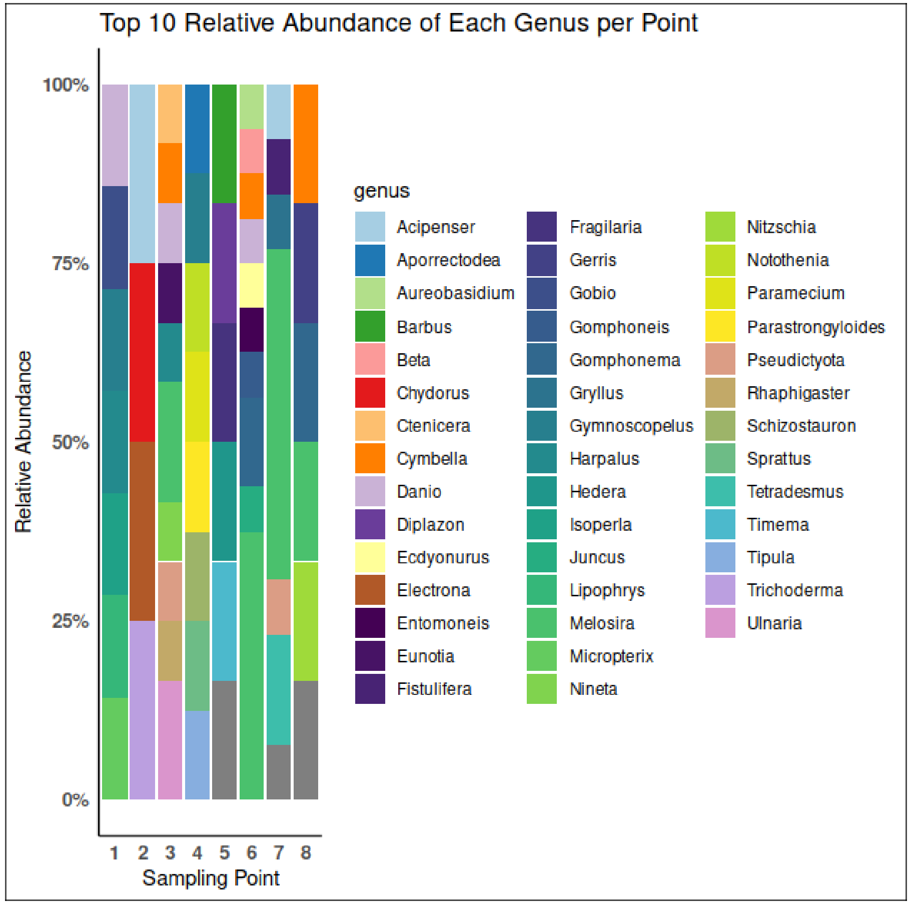


Figure 6. Top 10 abundant genera

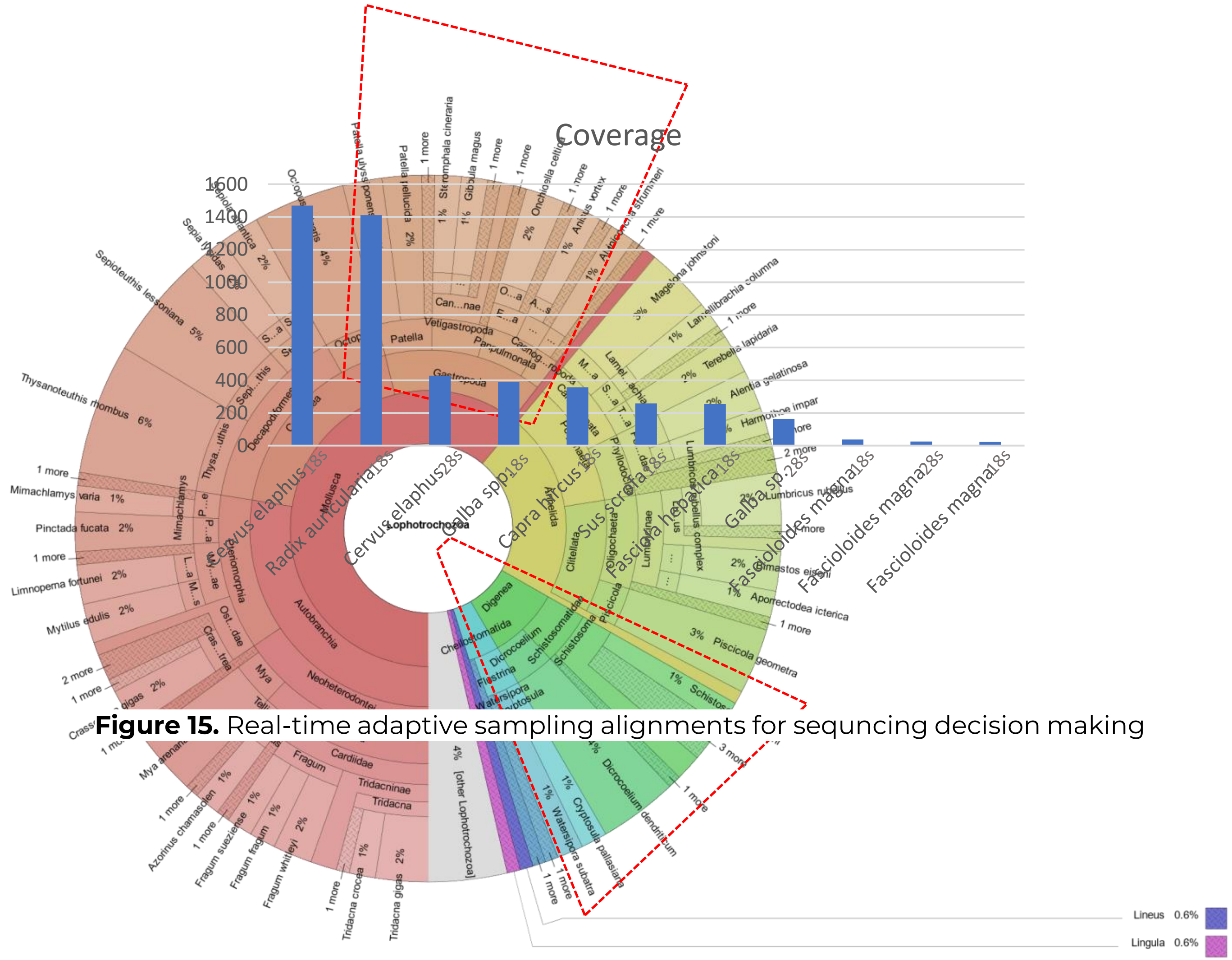


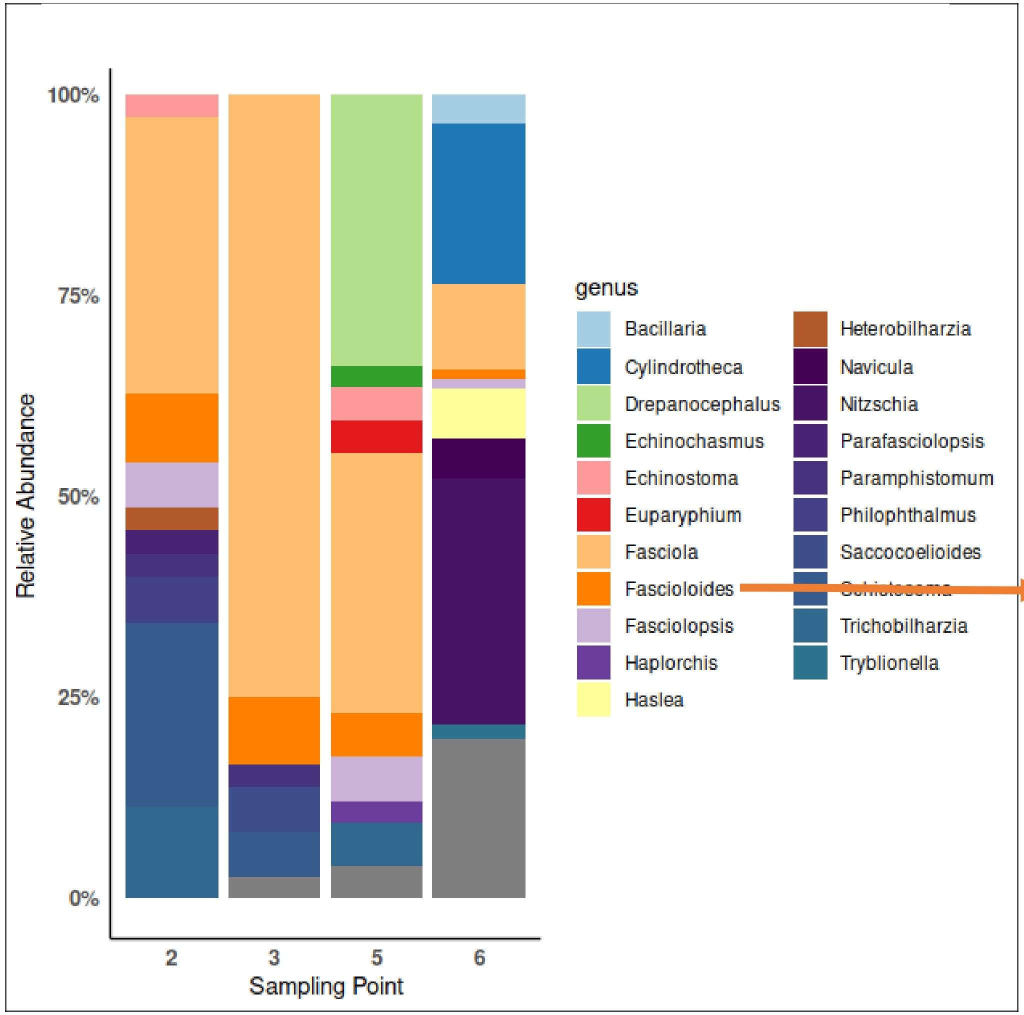
Figure 15. Real-time adaptive sampling alignments for sequncing decision making

Figure 7. Highest resolution representing both targets (*F. magna* and *G. truncatula*)

Nanopore Sequencing for WRP genomic surveillance

Results:

Ligation sequencing and adaptive sampling:



Sequences producing significant alignments

☑ select all *100 sequences selected*

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☑	Fascioloides magna small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S …	Fascioloides ma…	4375	4375	45%	0.0	100.00%	2920	EF051080.1
☑	Fasciola jacksoni haplotype Fj18S-1 from Sri Lanka 18S ribosomal RNA gene, partial sequence	Fasciola jacksoni	2579	2579	26%	0.0	99.65%	1980	ON661085.1
☑	Fasciola gigantica haplotype Fg18S-1 from Burkina Faso 18S ribosomal RNA gene, partial sequence	Fasciola gigantica	2573	2573	26%	0.0	99.57%	1980	ON661089.1
☑	Fasciola gigantica haplotype Fg18S-1 from Nigeria 18S ribosomal RNA gene, partial sequence	Fasciola gigantica	2573	2573	26%	0.0	99.57%	1980	ON661088.1

Figure 8. Results from reads successfully mapped to *F. magna* rDNA operon and polished with Racon(among results *only reads classified to F. magna* showed 100% similiarity)

Nanopore Sequencing for WRP genomic surveillance

Summary

Screening with native sequencing:

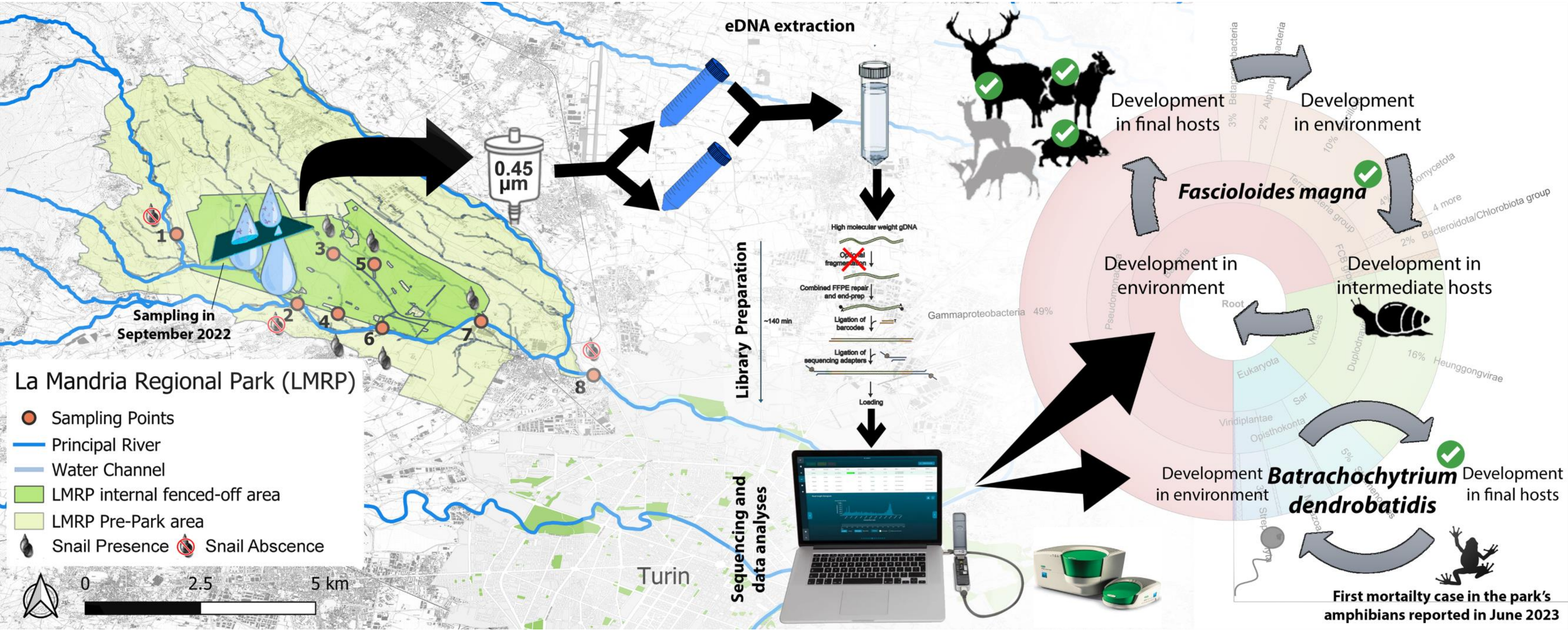


Figure 9. Graphic Abstract nanopore screening study

Nanopore Sequencing for WRP genomic surveillance

Methods and Materials

Screening with native sequencing:

- **eDNA sampling and extraction:**
  - Up to 20 L of water filtered with Waterra 0.45-micron filters.
  - DNA extraction with DNeasy Power Soil kit (Qiagen, Hilden, Germany)
- **Library Preparation and Nanopore Sequencing:**
  - Oxford Nanopore Technologies' Native Barcoding Kit for genomic DNA
  - Equimolar eDNA input of 180ng/sample
- **Taxonomy assignment :**
  - Kraken2 and megablast
- **Screening List:**
  - The Eukaryotic Pathogen, Vector and Host Informatics Resource (VEuPathDB68)
  - Autochthonous (Park's Fauna) or allochthonous (Alien Species) species indicated in La Mandria site-specific measures report
  - Involved species in *F. magna* lifecycle and gastropods found in this study
  - Enetwild: 50 zoonotic pathogens selected by One Health working group of EFSA for environmental detection.ù

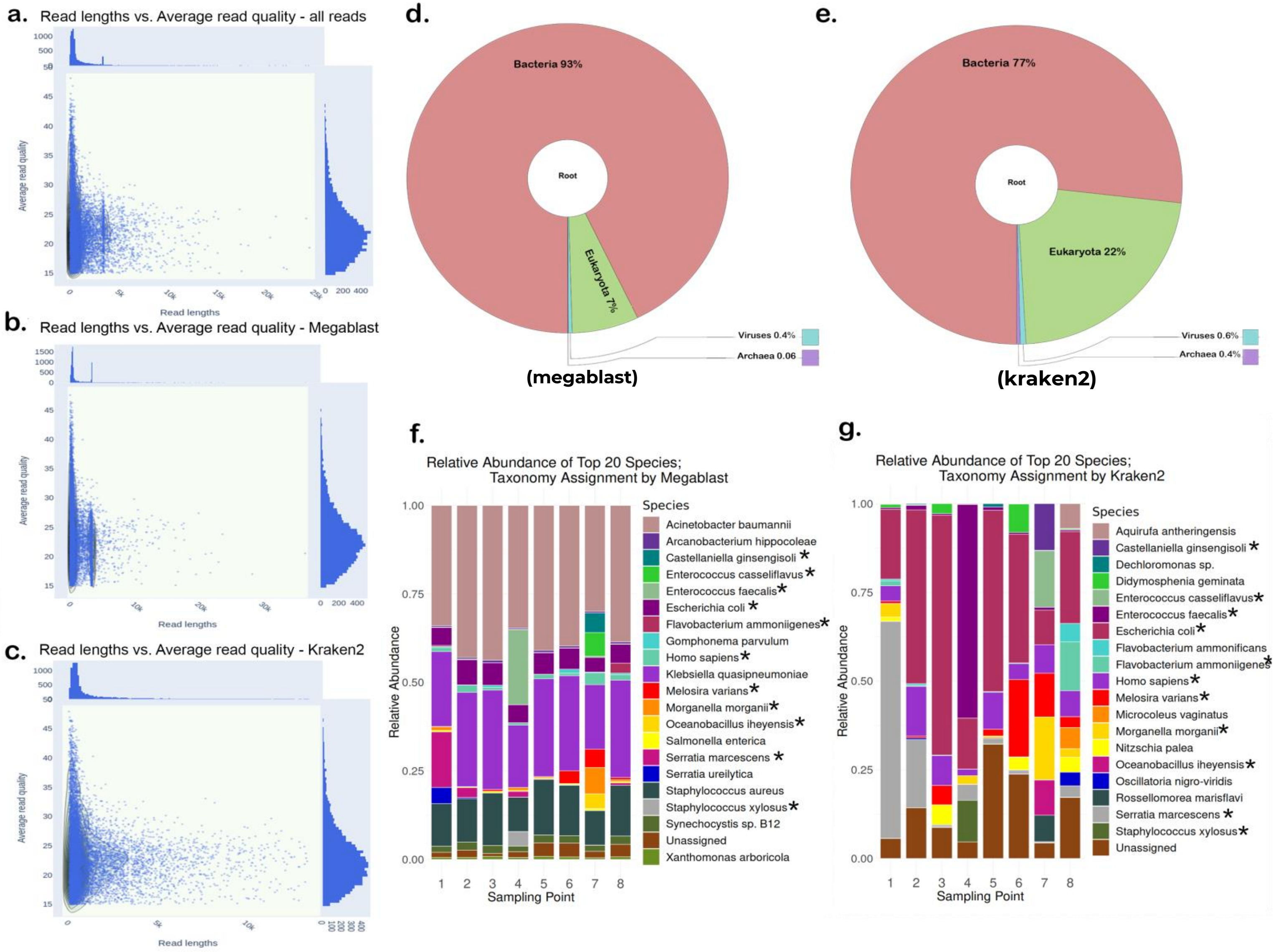
  - **Statistical analyses:**
  - Shannon and Simpson for sampling points
  - PCoA analyses
- **ddPCR for *B. dendrobatidis* detection**

Nanopore Sequencing for WRP genomic surveillance

Results

Screening with native sequencing:

Figure 10 Read length Vs. Average read quality for **a.** all unclassified reads, **b.** classified reads by megablast (after filtering) using NCBI *nt* database **c.** classified reads by kraken2 using NCBI *nt* database . **d.** and **e.** General distribution of identified taxa for megablast and kraken2. **f.** and **g.** Top 20 most abundant species identified by megablast and kraken2.(plot is visualized using NanoPlot)



a. Read lengths vs. Average read quality - all reads

b. Read lengths vs. Average read quality - Megablast

c. Read lengths vs. Average read quality - Kraken2

d.

(megablast)

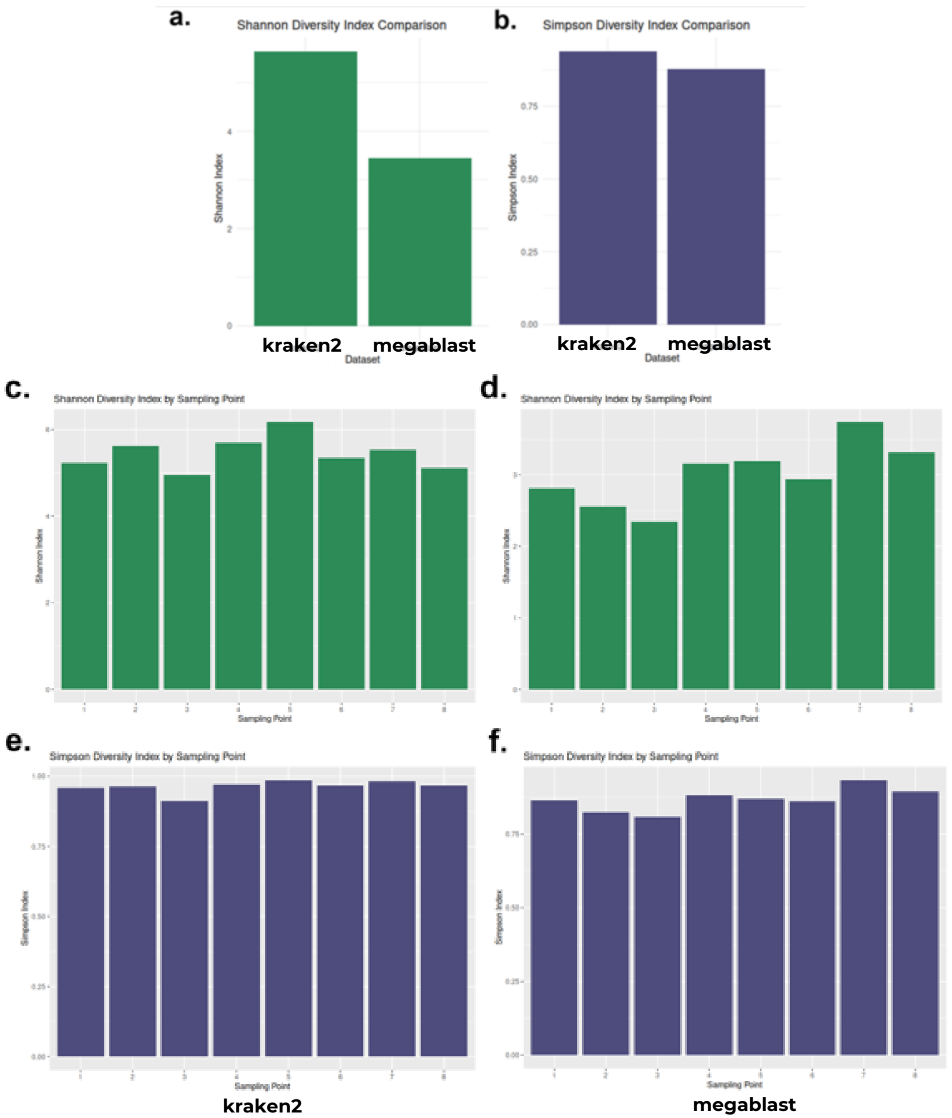
e.

(kraken2)

f. Relative Abundance of Top 20 Species; Taxonomy Assignment by Megablast

g. Relative Abundance of Top 20 Species; Taxonomy Assignment by Kraken2

Screening with native sequencing:



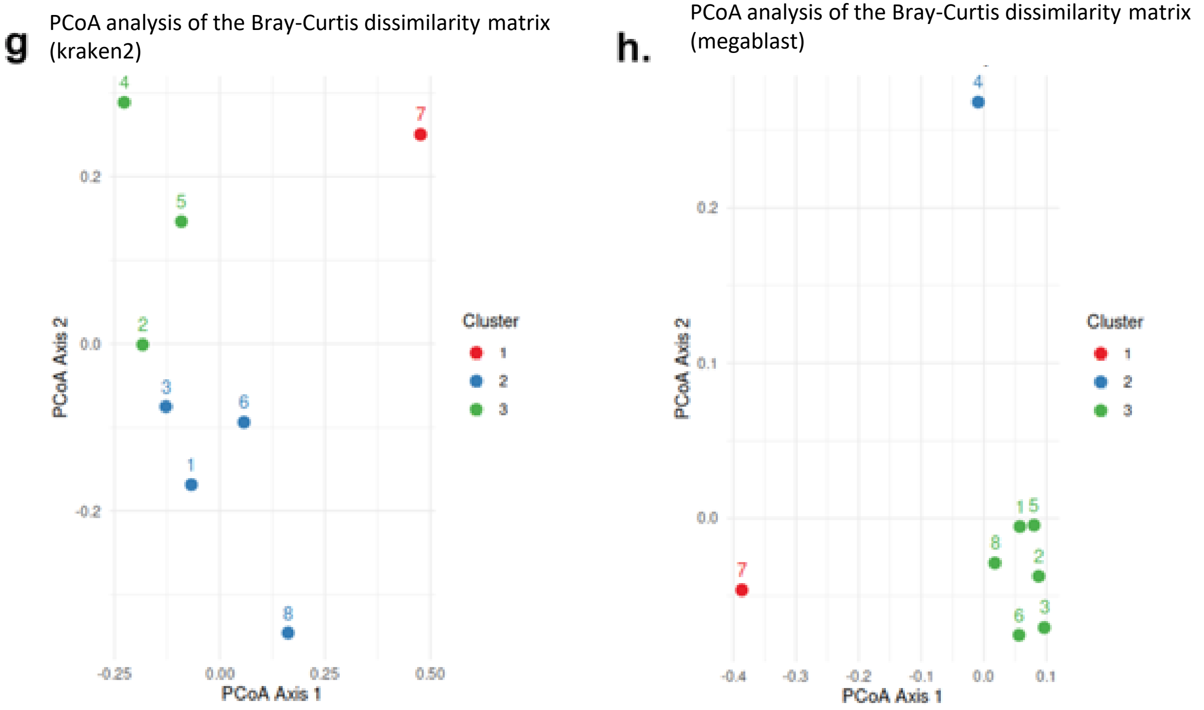


Figure 11. Comparison of Shannon (a) and Simpson (b) diversity indices between classified reads by megablast and Kraken2. Comparison of Shannon (c&d) and Simpson (e&f) diversity indices of classified reads kraken2 (c&e) and megablast (d&f) among different sampling points. Diversity clusters of classified reads by kraken2 (g) and megablast (h)

Nanopore Sequencing for WRP genomic surveillance

Results

Screening with native sequencing:

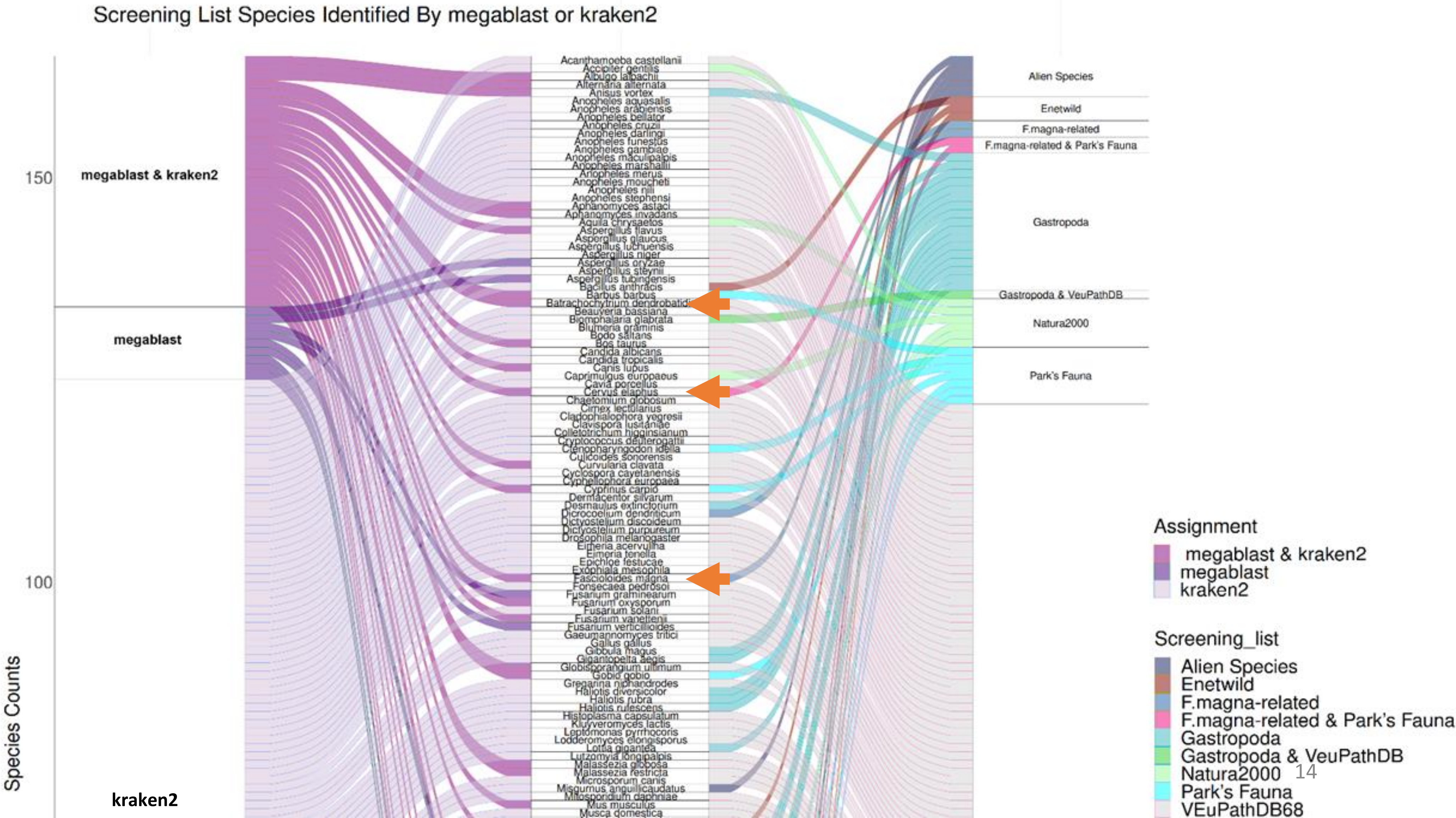


Figure 12. Identified species by both taxonomy assignment tools and their relation to different subsets of the screening list.

Nanopore Sequencing for WRP genomic surveillance

Results

Screening with native sequencing:

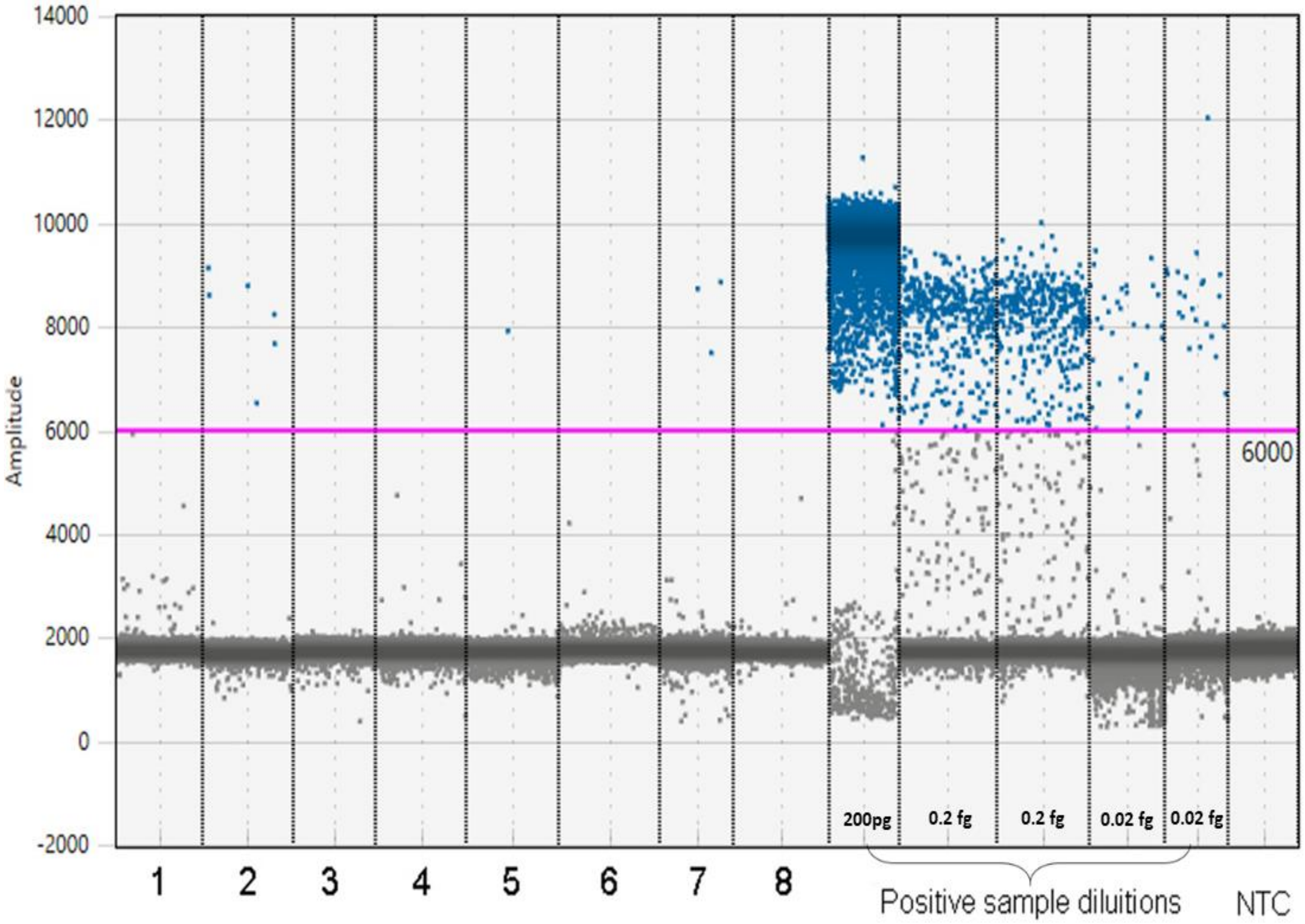


Figure 13. 1- 8 are ddPCR results for pooled sample. The positive samples' concentration range was from 200 picograms to 0.02 femtograms. The amplitude threshold to call positive droplets was considered at 6000, based on positive sample results and in agreement with Bio-Rad's specialist consults.

Nanopore Sequencing for WRP genomic surveillance

Discussion and Conclusion

- **Metabarcoding of long eDNA fragments showed lower efficiency in the detection of target sequences and is inherently limited to the choice of primers.**

- **eDNA simultaneous SLS and AS sequencing in the enrichment for the selected species; however, the identification was through mapping strategy and not de novo assembly.**

- **eDNA simultaneous SLS and AS sequencing is a real-time combined Active and Passive surveillance approach which provides information for possible unknown disease/pathogen.**

- **Nanopore native sequencing captures both overall biodiversity and low-abundance but ecologically significant species.**

- **The study reaffirmed the presence of F. magna and identified several potential hosts in its lifecycle. However, F. magna DNA was only one sampling point. The presence of B. dendrobatidis was confirmed downstream in the park. This finding is critical, as an outbreak in La Mandria park was only discovered 6 months later.**

- **Nanopore native sequencing is a valuable, efficient tool for biodiversity and pathogen detection, providing key insights into wildlife health and ecosystem dynamics.**

Thank you for your attention.

I thank the people of my Ph.D. in particular:

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