The *Haplosporidium nelsoni* genome: an important tool to advance understanding and management of MSX disease in the eastern oyster

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DFO: MSX Science Research Funding

- completing an annotated chromosome-level assembly of the genome for *H. nelsoni*
- comparing the assembled genome with other *H. nelsoni* sequences to assess the parasite variability, and obtain insights into the origin of the 2024 Prince Edward Island (PEI) outbreak
- analyzing historical samples of Eastern oysters from PEI to search for previously undetected presence of MSX prior to outbreak


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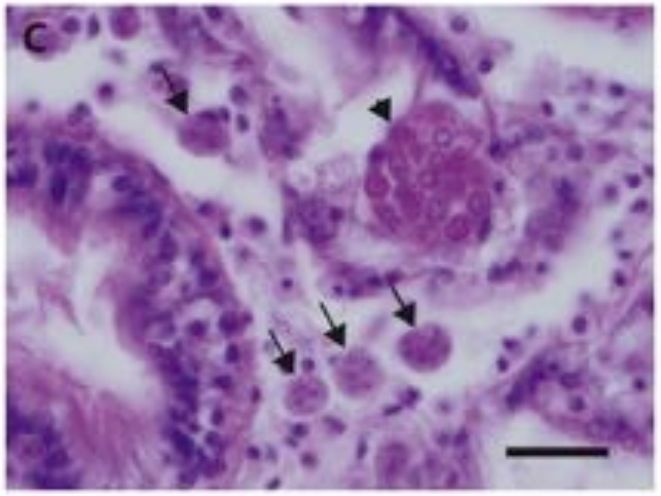
Multinucleate Sphere Unknown X (MSX)
Haplosporidium nelsoni



Haplosporidia protists: internal parasites of marine invertebrates, mainly molluscs

Major pathogens of concern: responsible for devastating marine disease epizootics

MSX disease: *Haplosporidium nelsoni*
Infects eastern oyster (*Crassostrea virginica*)

Complete life cycle not known; intermediate host suspected

Slows growth, high mortality rates (> 90% mortality observed following outbreaks)

Devastating impacts on wild and farmed oyster populations

Image from Arzul & Carnegie. 2015. New perspective on the haplosporidian parasites of molluscs. J. Invertebr. Pathol. 131:32-42 doi: 10.1016/j.jip.2015.07.014

Why is a reference genome useful?

In general …

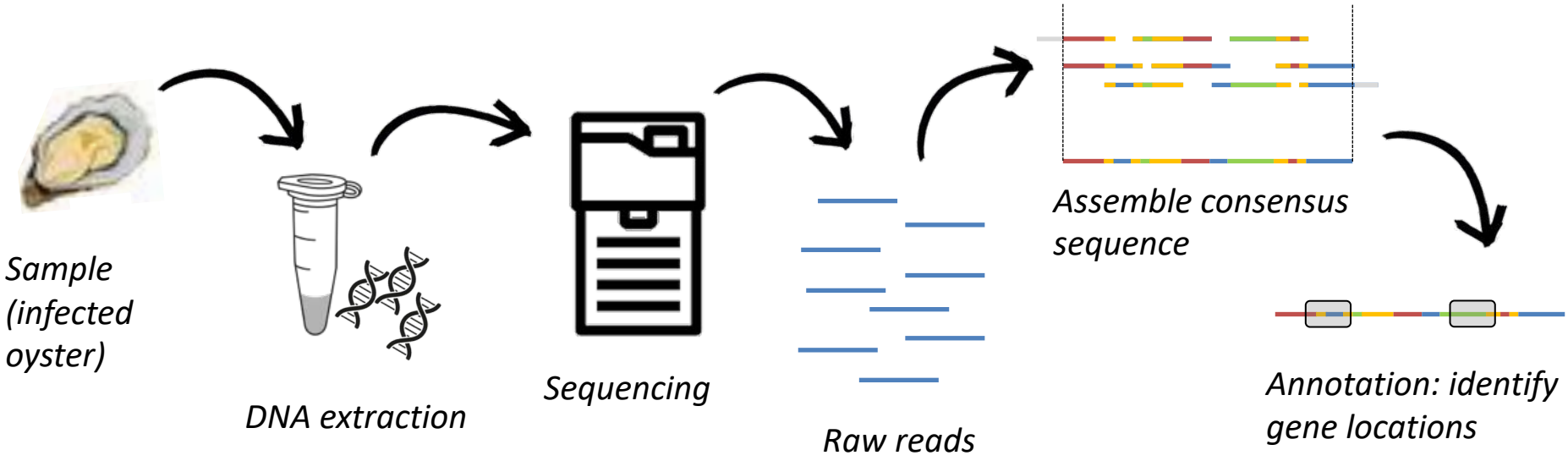
- a blueprint to align other DNA sequences to identify locations of genetic variation among individuals (e.g., SNP markers)
- identify specific gene functions

The availability of a reference genome can improve our knowledge of basic aspects of *H. nelsoni* biology, much of which is unknown

- Strain variation and identification
- Population structure
- Identification of genes involved in infection
- Parasite adaptation, predictive models
- Identification of additional markers for accurate and rapid diagnostics (e.g., eDNA)

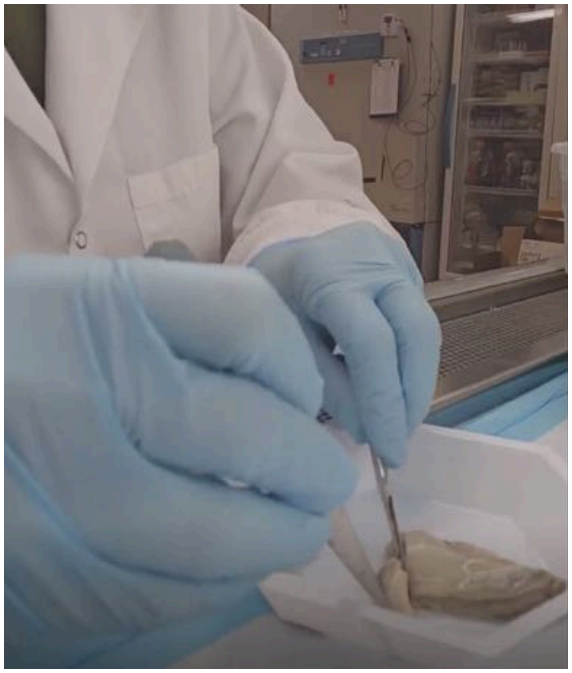
Genome Assembly

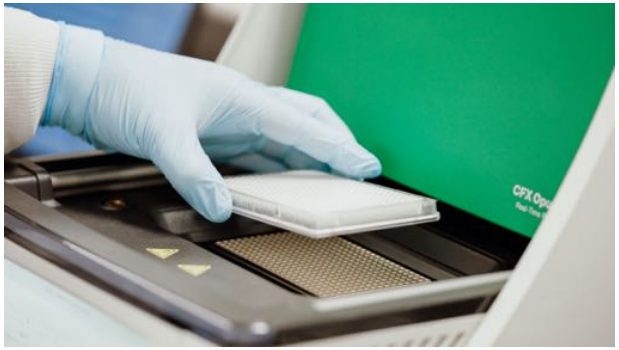
Reconstruction of the entire genome sequence from DNA sequence fragments
De novo assembly: no prior knowledge of length of genome or composition



H. nelsoni detection and DNA quantification: qPCR

Cross section of mantle, gill, and stomach of oysters
qPCR with *H. nelsoni* target gene primers (SSU)
10 samples with high *H. nelsoni* infection kept for further screening



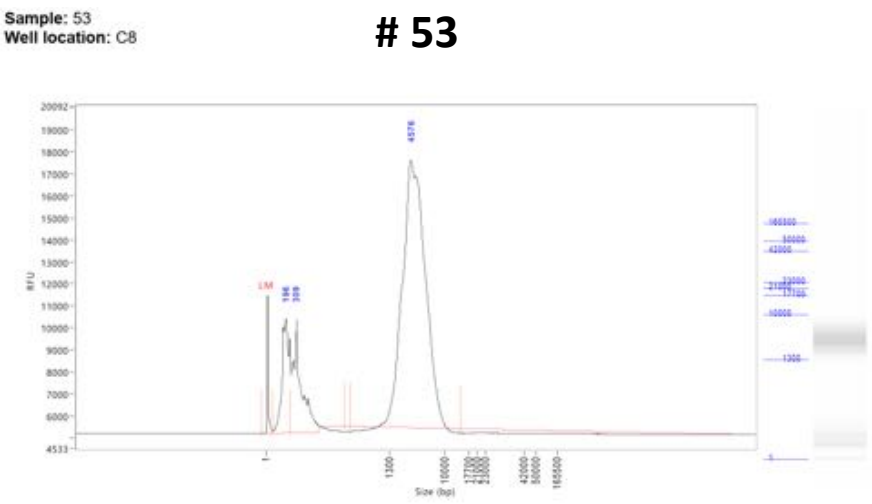


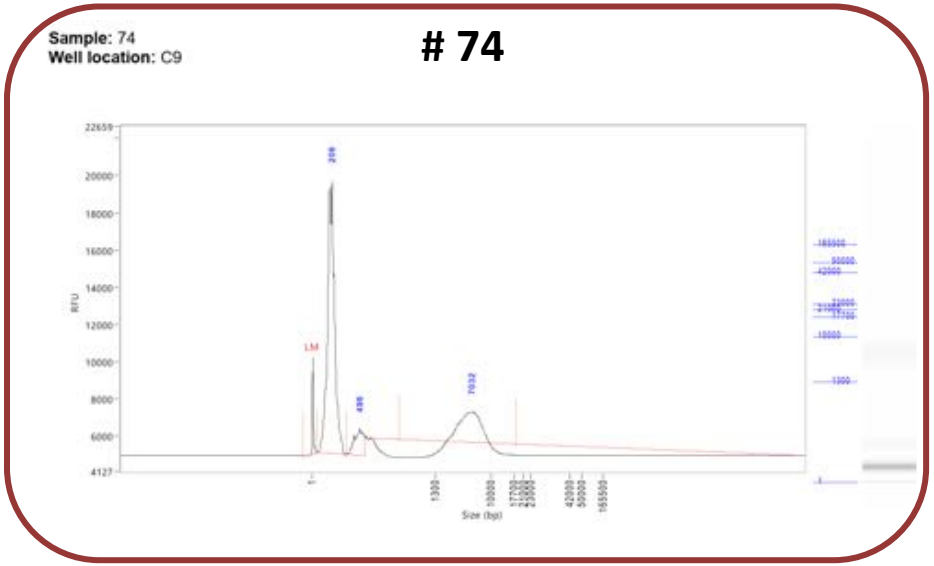
H. nelsoni detection and DNA quantification: qPCR

Sample ID	Ef1a Cq	TubulinB Cq	Haplo Cq	Delta Cq Haplo-Ef1a	Delta Cq Haplo-TubulinB
2	16.003	16.382	11.5	-4.503	-4.882
3	14.167	14.667	12.2	-1.967	-2.467
7	13.853	14.144	12.6	-1.253	-1.544
10	15.263	15.797	11.3	-3.963	-4.497
12	13.991	14.418	11.2	-2.791	-3.218
14	16.061	16.453	12.2	-3.861	-4.253
17	14.136	14.772	11.7	-2.436	-3.072
53	15.462	15.624	10.8	-4.662	-4.824
74	16.665	17.093	10.7	-5.955	-6.933
86	14.877	15.413	12.3	-2.577	-3.113

H. nelsoni DNA quality: Femto Pulse gDNA

DNA fragment size distribution





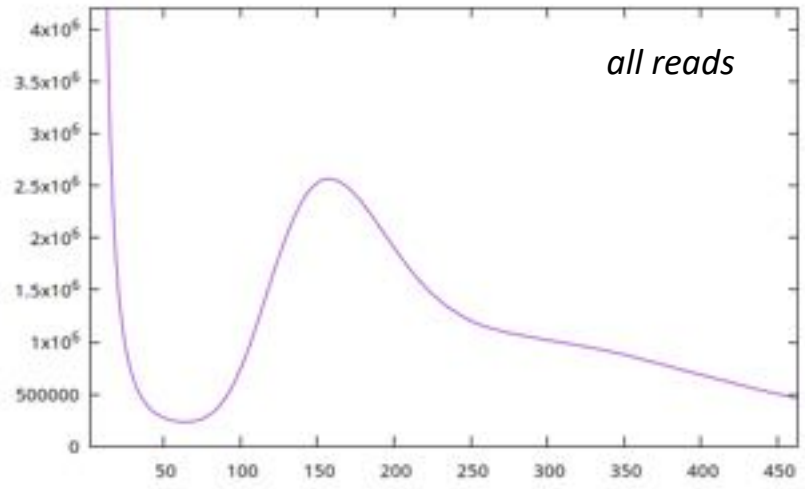
- average size ~5000-7000 bp
- Ok for short read sequencing, not for long read sequencing

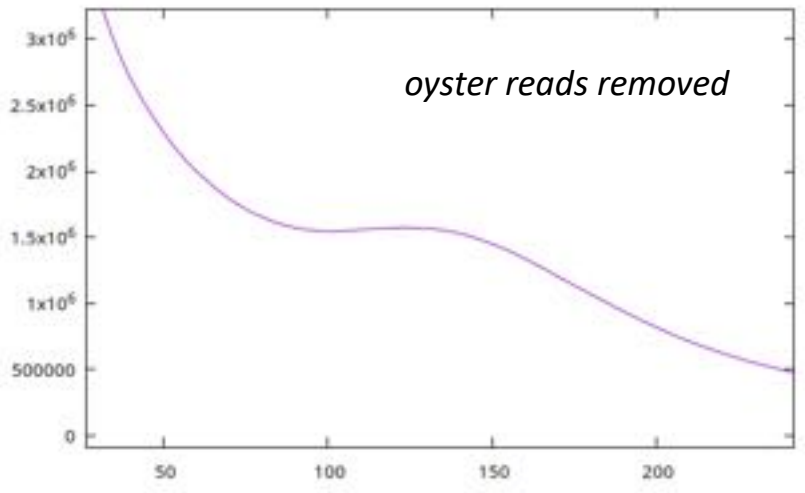
H. nelsoni DNA sequencing

AVITI short-read sequencing

H. nelsoni DNA sequencing

AVITI short-read sequencing





982.8M reads

Remove contamination

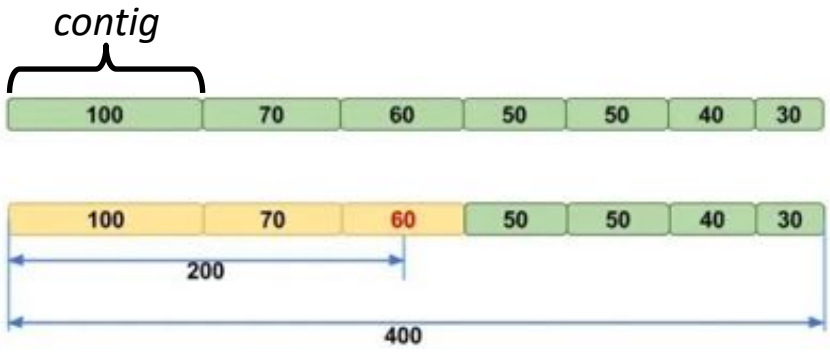
- Reads mapped to *C. virginica* reference genome
- Removed ~50% of the sequences
- Bump ~125X (potentially *H. nelsoni*)

H. nelsoni preliminary assembly

- Kraken2 annotation: 2M trimmed and filtered reads mapped to Haplosporidia/Haplosporidium

Draft assembly with MaSuRCA short read assembler:

- Total length of assembly: 756 Mbp - bigger than we expect
- N50 = 1,623 bp
- Kraken2: 550 Mbp (73% of assembled genome) maps to contaminants (mostly molluscs); 63 Mbp is “unclassified”
- ~3000 contigs (~288,000 bp) map to different Haplosporidia (< 1% of assembled genome)



Conclusion & Next Steps

- Difficult to isolate *H. nelsoni* DNA from contaminants – we knew this would be a challenge!
- Relative Cq values give an idea of the proportion of parasite DNA, but better understanding the relationship between detection estimates and sequencing output is needed
- Currently have a very partial assembly, with a small proportion of contigs that are potentially *H. nelsoni*
- Methods for enrichment of *H. nelsoni* cells prior to DNA extraction
- HiFi sequencing ideal for genome assemblies; depends on getting high molecular weight DNA

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Thank you for your attention!

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IBIS Du fondamental à l'appliqué

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