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Exploring depletion techniques using CRISPR and Nanopore technologies for enhanced eDNA sequencing

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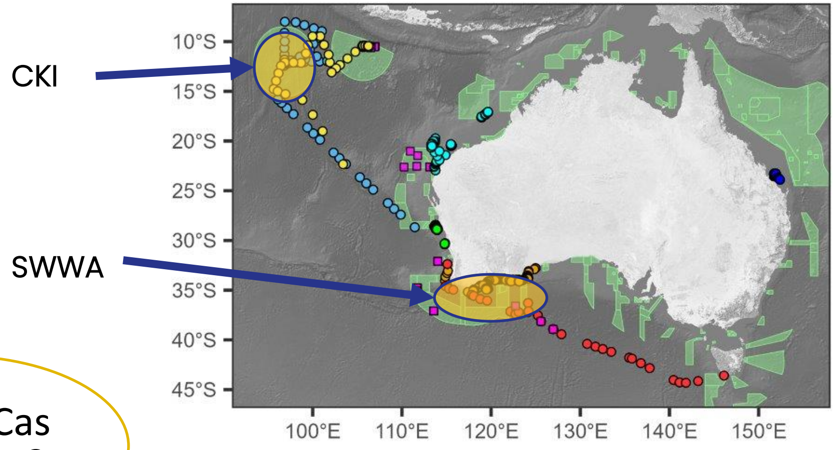
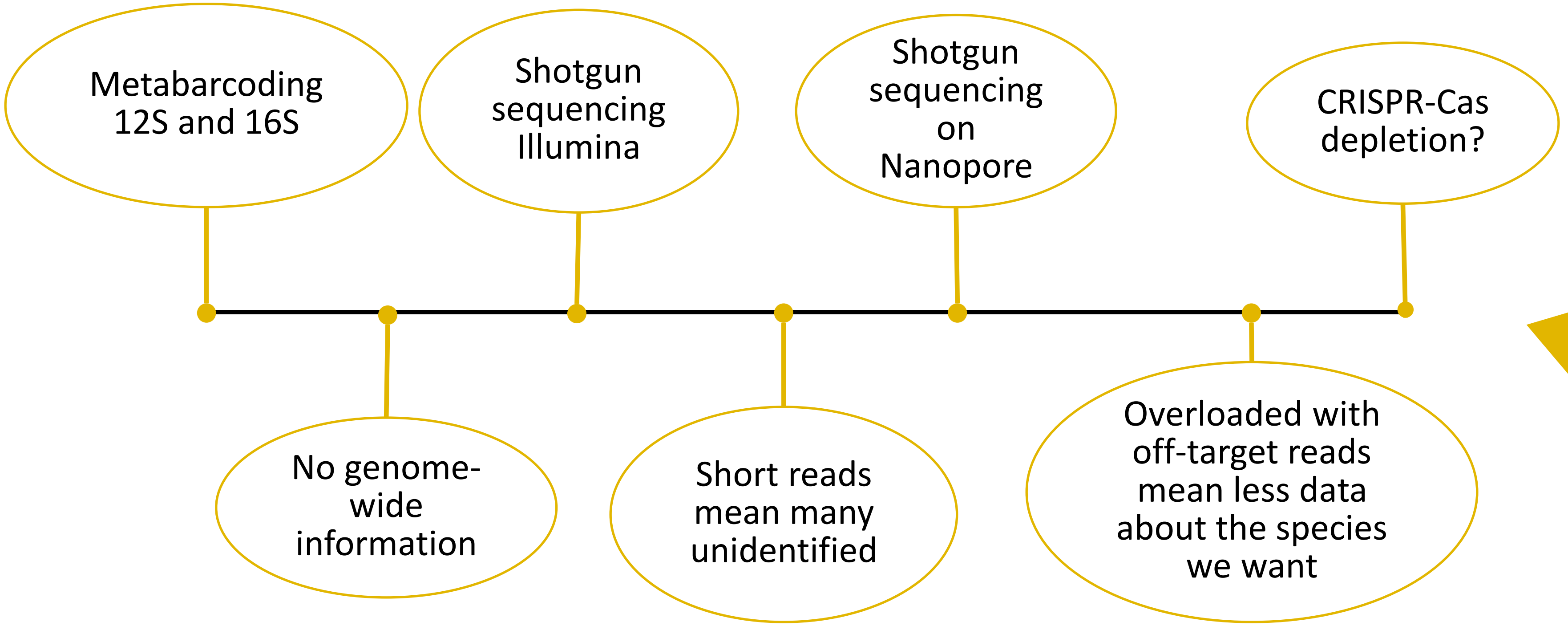
Study outline

Two samples from distinct sites were used for all these studies:





Metabarcoding 12S and 16S

No genome-wide information

Shotgun sequencing Illumina

Short reads mean many unidentified

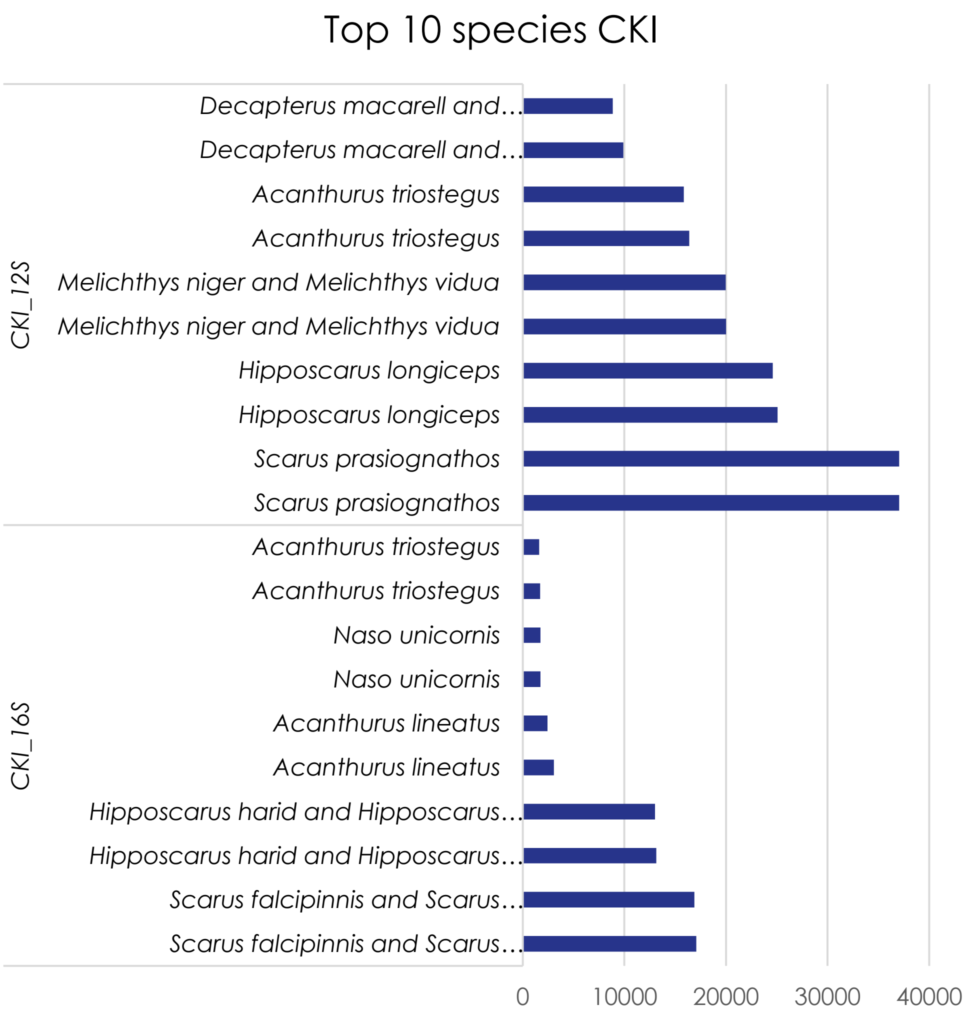
Shotgun sequencing on Nanopore

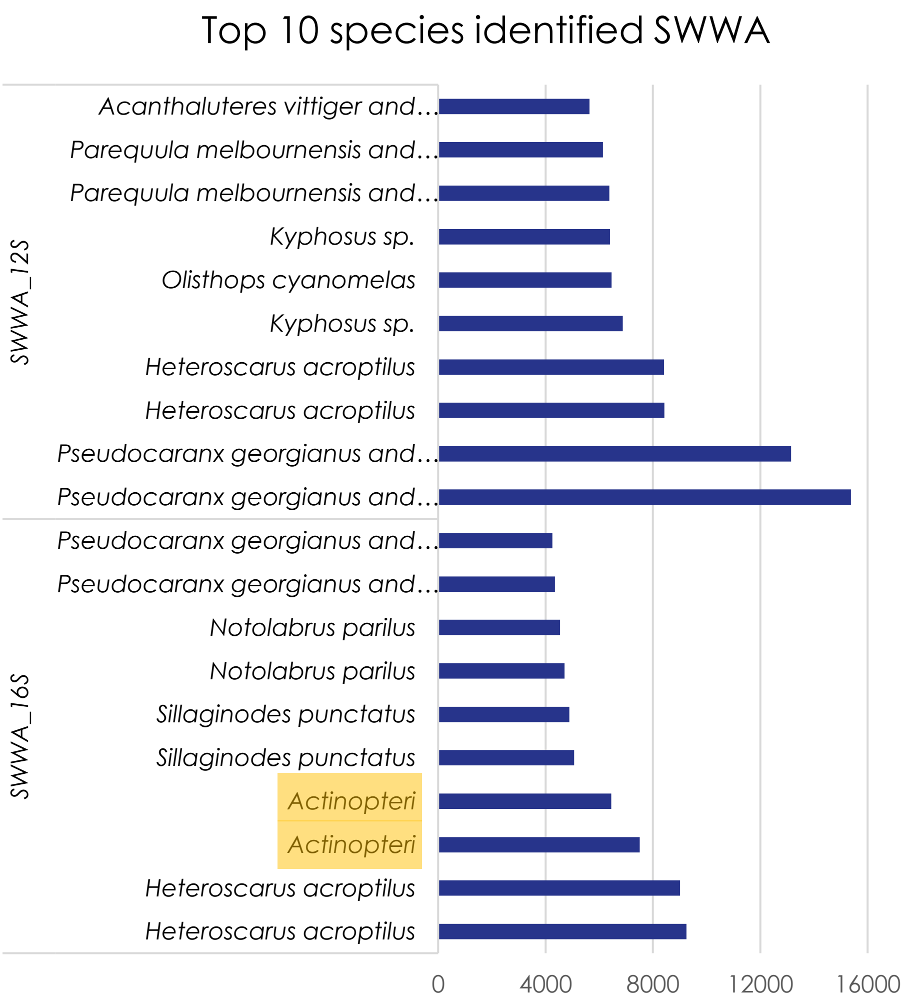
Overloaded with off-target reads mean less data about the species we want

CRISPR-Cas depletion?

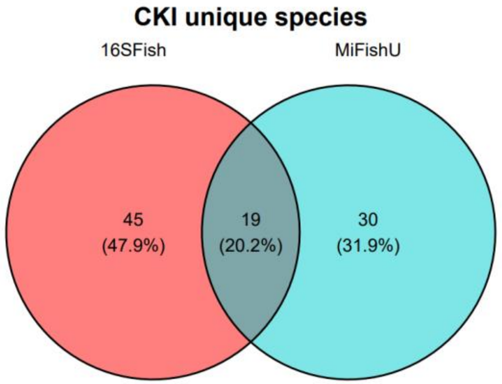
Genome-wide eDNA characterisation

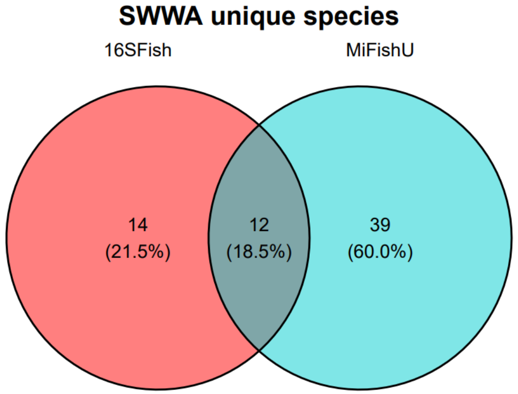
Metabarcoding assay using 12S and 16S as targets


Top 10 species CKI


Top 10 species identified SWWA

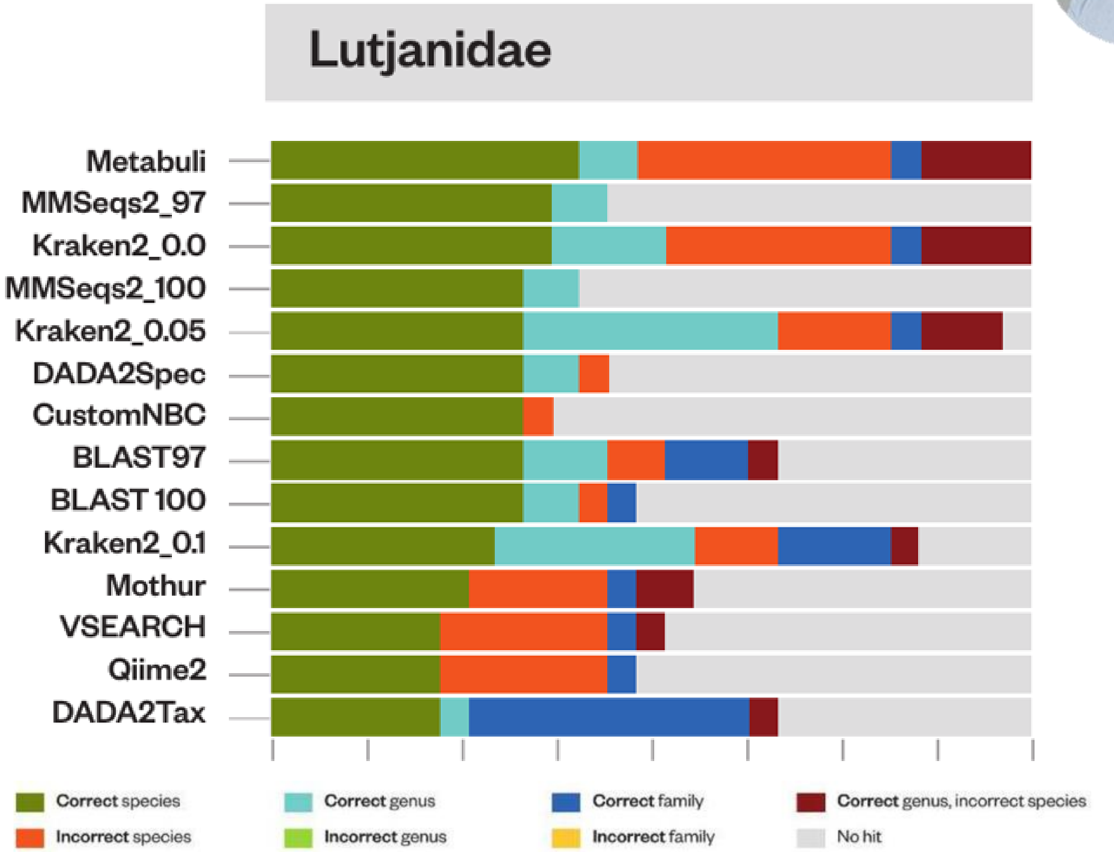

Adam Bennett


CKI unique species

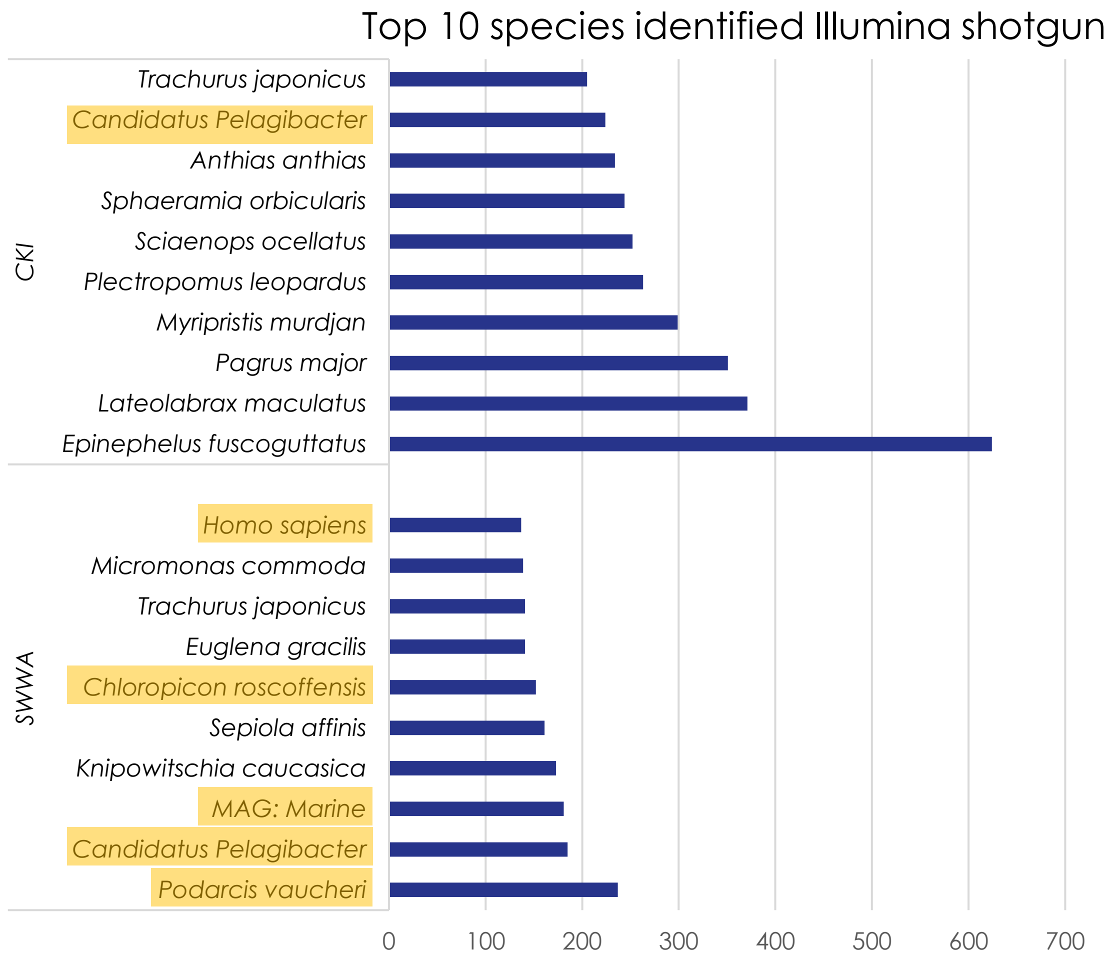

SWWA unique species

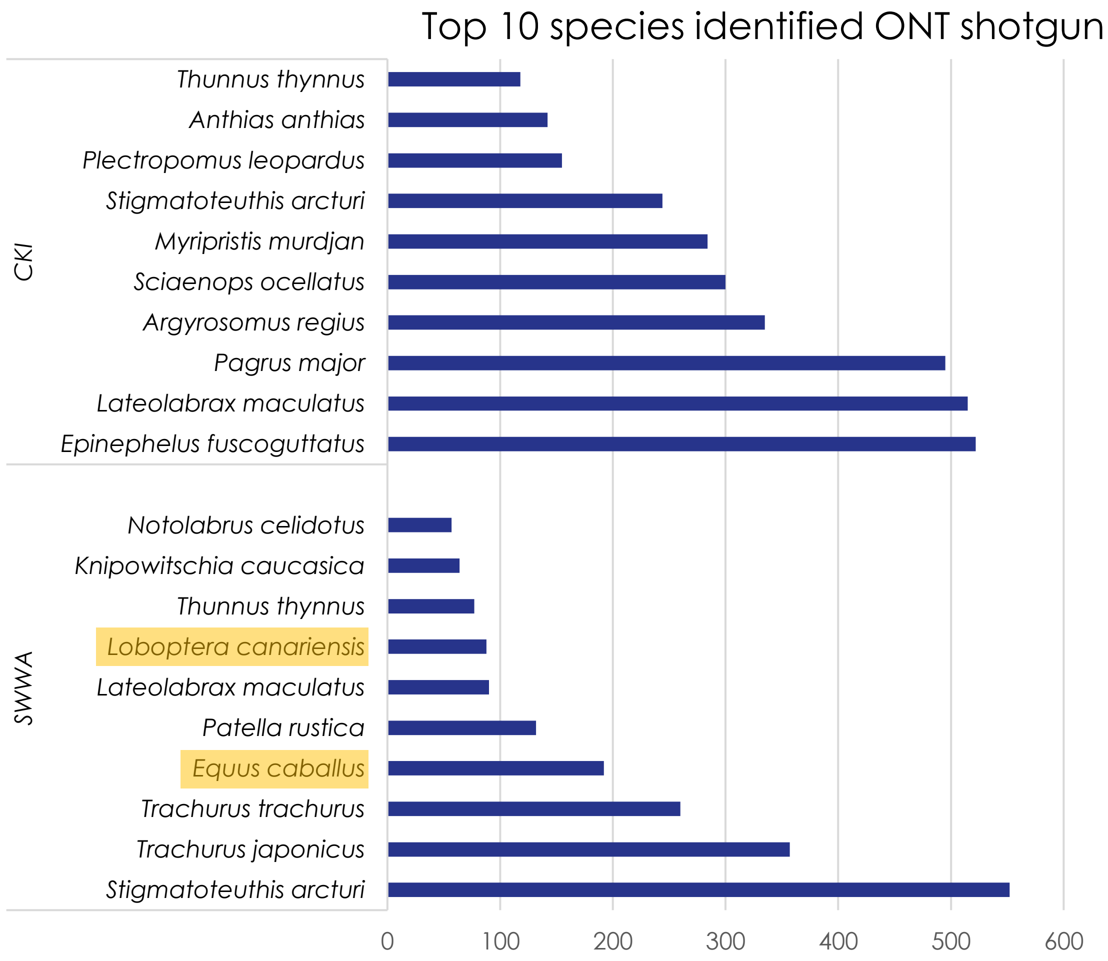
Metabarcoding limitations

Works for some species but not all and misses many species and most of the available data.



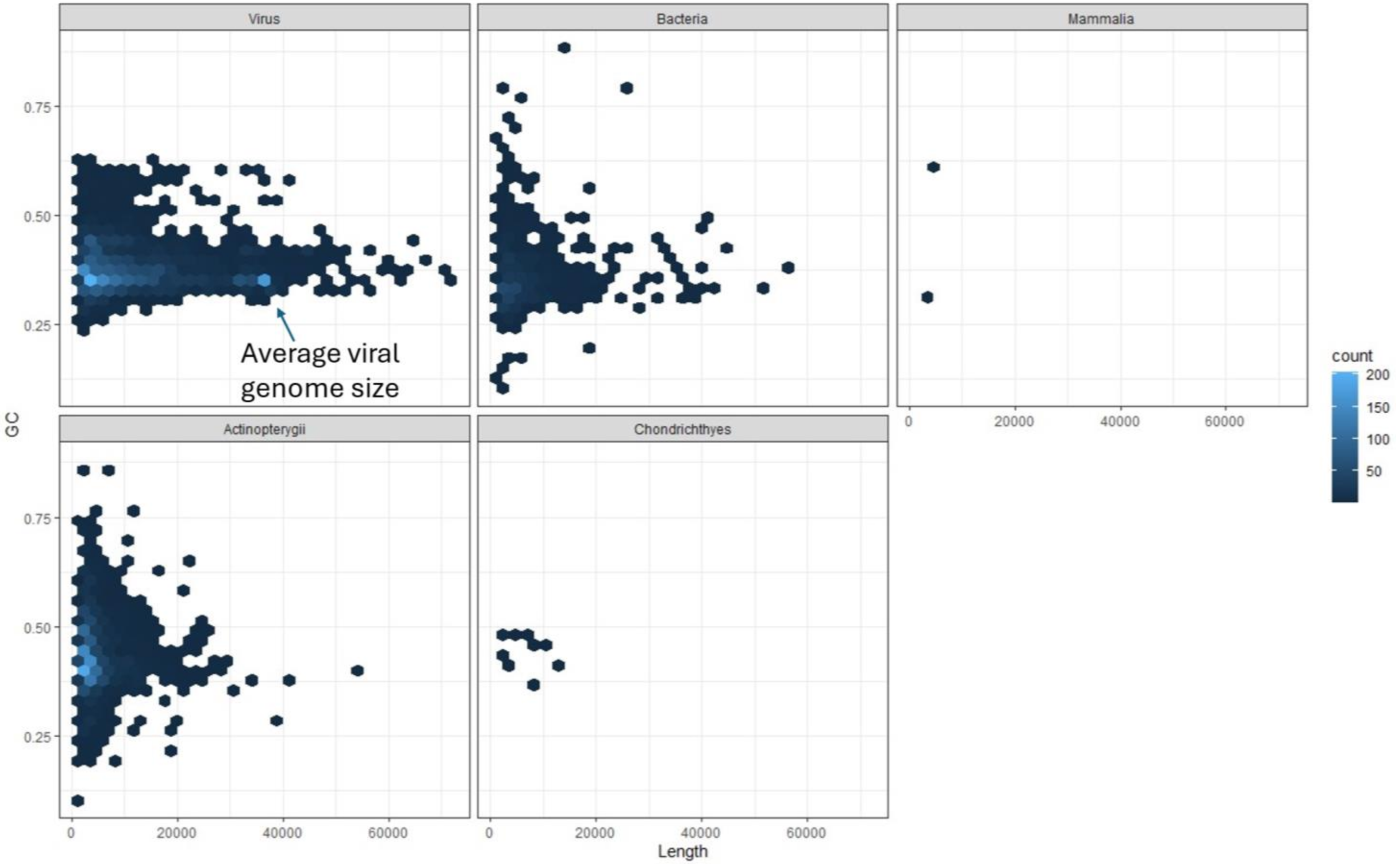
Shotgun sequencing top 10 species





Long read exploration

A range of fragment sizes are present in eDNA, much longer than what we normally target in metabarcoding.

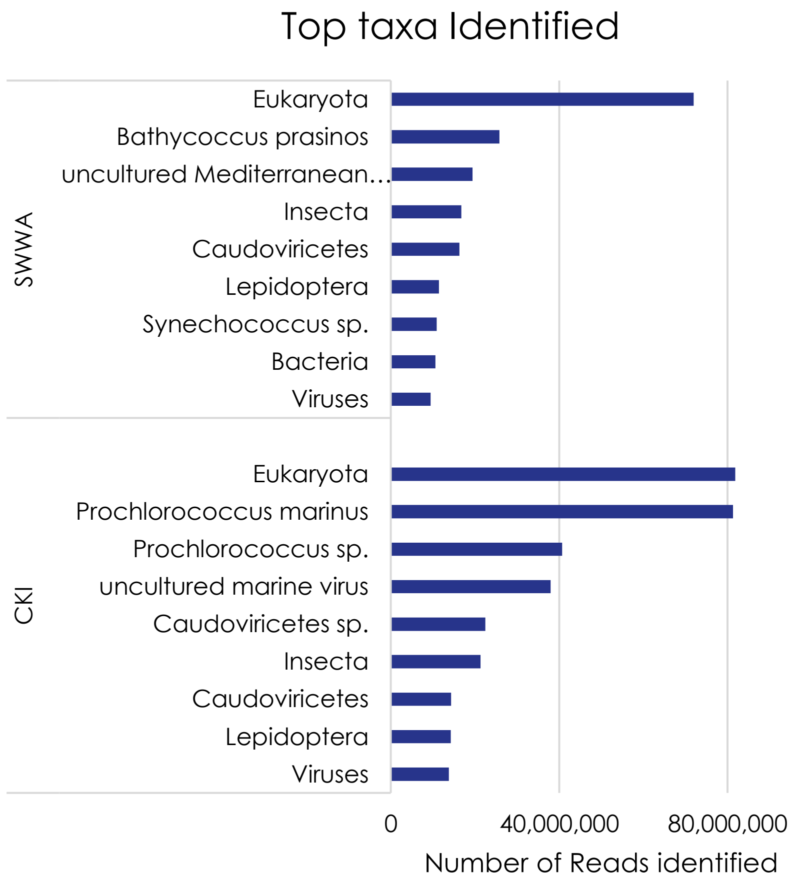


Average viral genome size

Top taxa across all samples

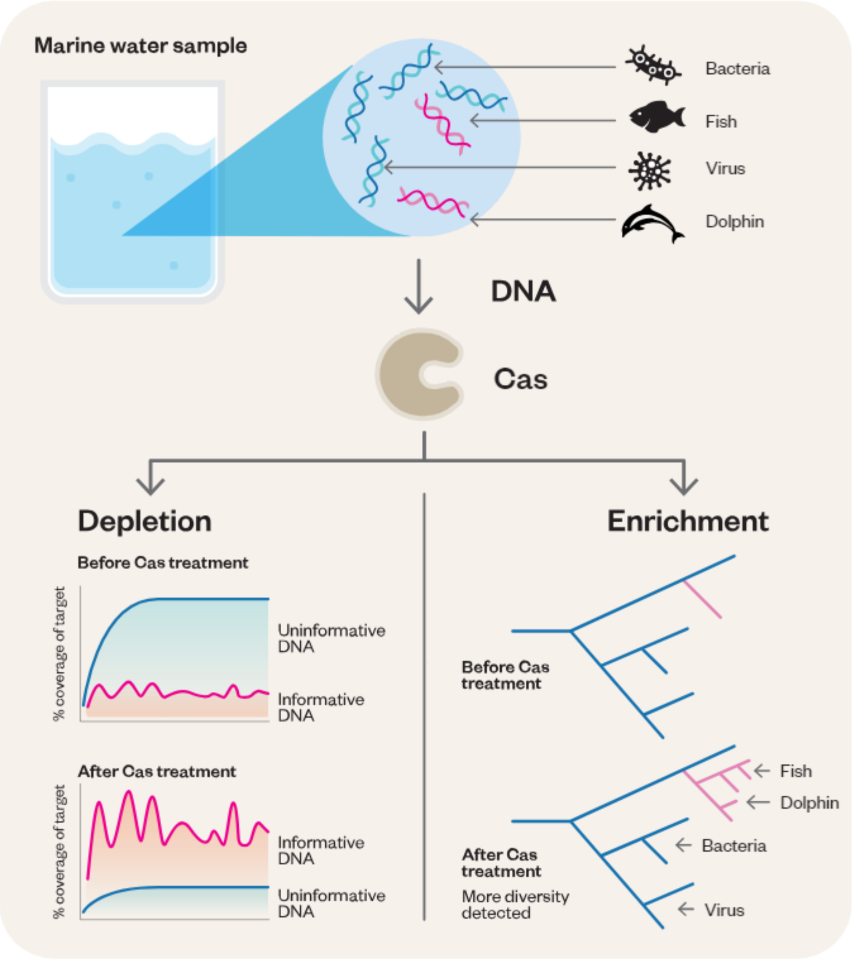
Most of what we sequence is not the vertebrate DNA that we are looking for or can only be identified to domain level.

- –For ONT around 16% of reads could be classified.
- –For Illumina around 23% of reads could be classified.
- –Most of the unclassified reads are very repeat rich.

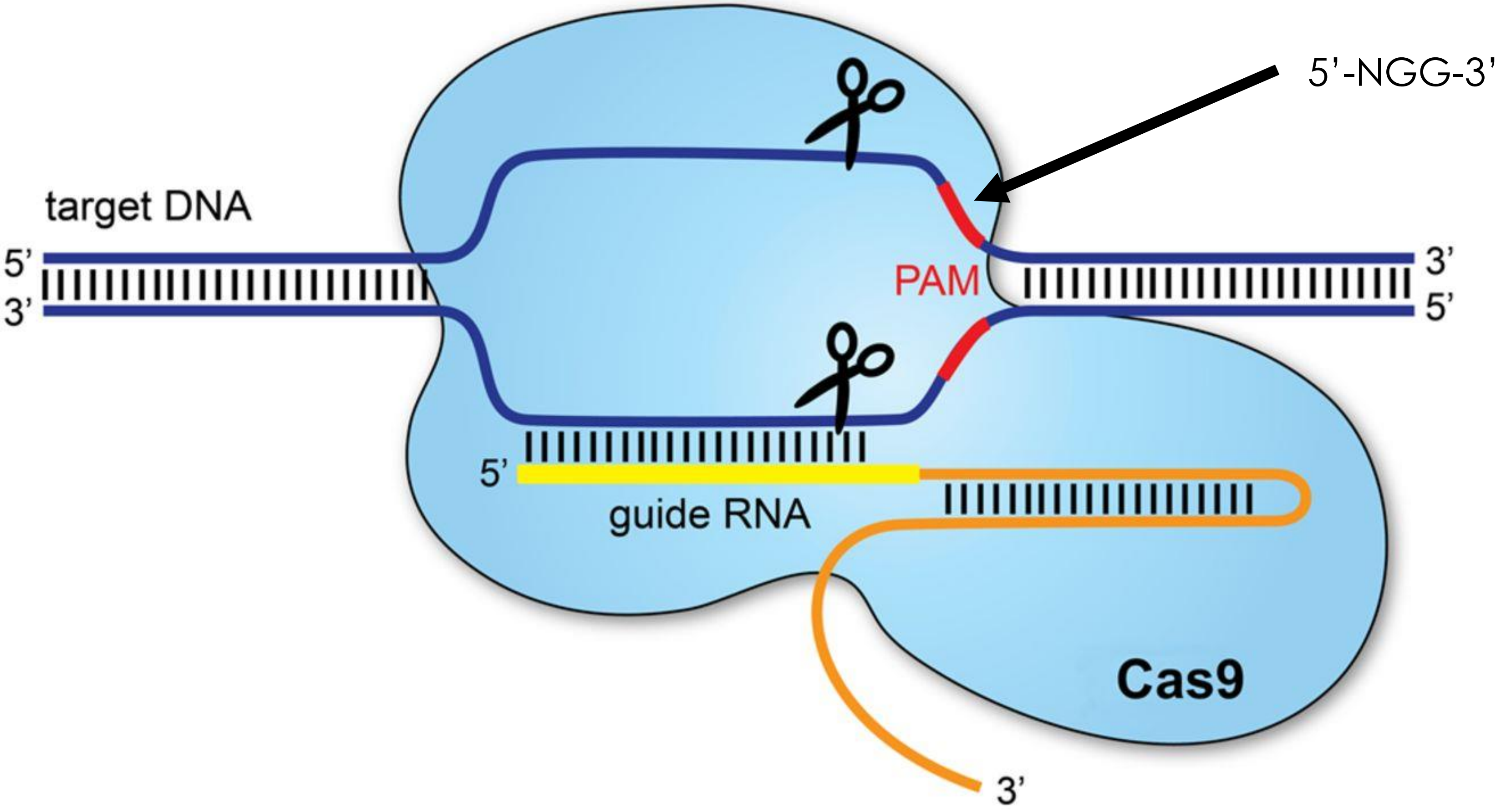


Goals with using CRISPR-Cas

Simplify the results from shotgun sequencing of marine eDNA by depleting uninformative DNA (repeats, bacteria, viruses) or enriching for target DNA (fish, dolphins, etc.).



What is CRISPR-Cas?

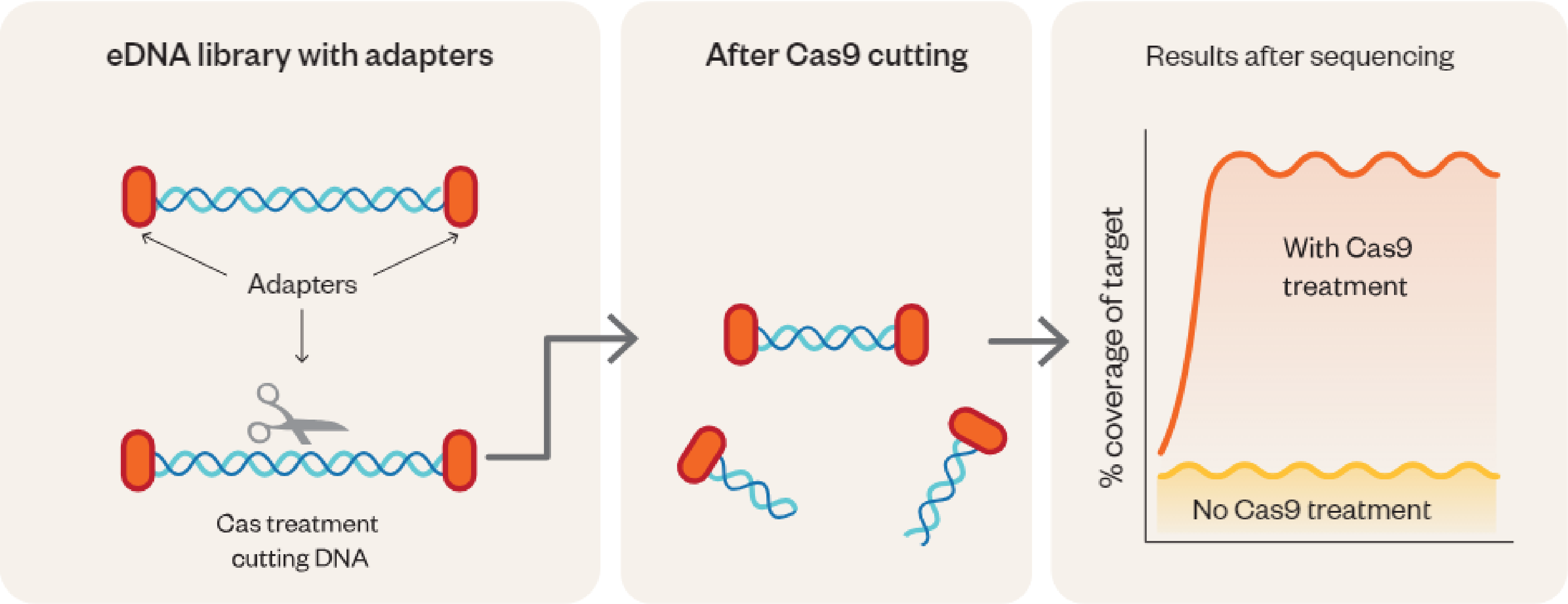


Redman, M., King, A., Watson, C., & King, D. (2016). What is CRISPR/Cas9? *Archives of Disease in Childhood: Education and Practice Edition*, *101*(4), 213–215. https://doi.org/10.1136/archdischild-2016-310459

CRISPR-Cas based depletion

Remove uninformative sequences

DASH (Depletion of Abundant Sequences by Hybridization)



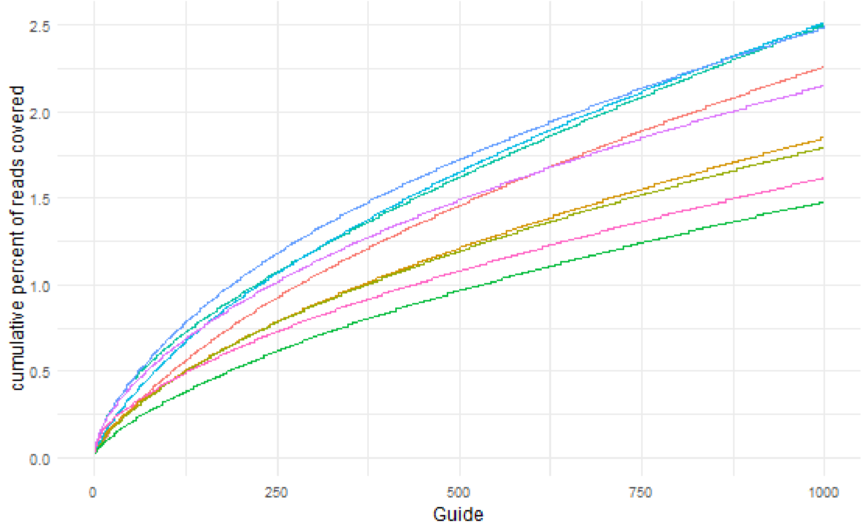
Initial results of CRISPR-Cas depletion in silico testing

Made 15,000 guide sequences which can theoretically cover 21% of the shotgun sequences.

Tested these guides in silico.

Most of these guides are repetitive sequences which is useful to design fewer guides.

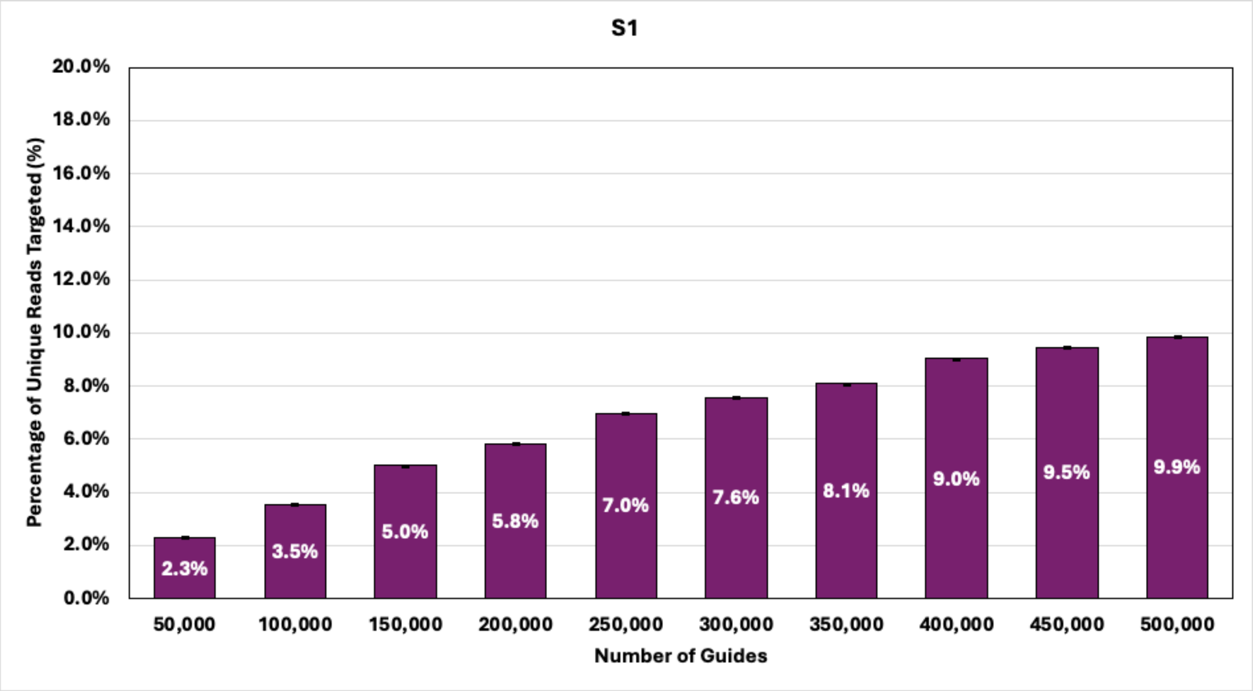
If we can scale it up using Jumpcode. They can make up to 500,000 guides.



Depletion in silico tests by JumpCode Genomics

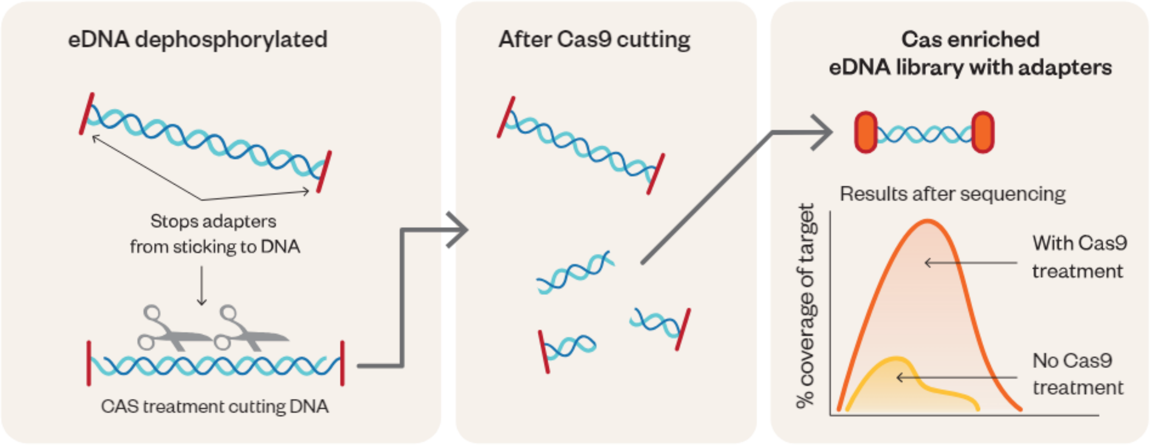
Based on Jumpcode's analysis we need much more than 500,000 guides to get more than 10% depletion.

eDNA is too complex to target unwanted sequences specifically with the technology we are using now.



Future directions

- Many CRISPR-Cas studies that have focused on targeting individual species.
- Could be used to target SNPs and UCEs for population structure information.
- Looking beyond fish species into whole ecosystem interactions across domains?



Acknowledgements

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Presenters


Shannon Corrigan

Ocean Genomes- Reference Quality Genome Resources for Marine Vertebrates

Thursday- 16:15 Session 17


Georgia Nester

Innovative eDNA strategies for advancing deep-sea biodiversity research and conservation

Friday- 10:35 Session 20


Philip Bayer

The OceanOmics dashboard: A visualisation tool for the democratisation of eDNA data

Thursday- 15:00 Session 14


Laura Missen

Scaling-up marine edna biodiversity monitoring: automation of workflows from sampling to sequencing

Thursday – 11:50 Session 10

Whole data results

	Sample site	Reads sequenced	Data sequenced	% classified	% fish reads out of all classified
Metabarcoding	CKI	168,595 -16S 589,591 -12S	58 Mb -16S 200 Mb -12S	80% -16S 79% -12S	29% -16S 54% -12S
	SWWA	216,828 -16S 308,176 -12S	50 Mb -16S 187 Mb -12S	70% -16S 70% -12S	43% -16S 40% -12S
Illumina shotgun	CKI	1,616,159,983	281 Gb	33%	2%
	SWWA	1,890,542,844	329 Gb	23%	2%
ONT shotgun (simplex)	CKI	6,073,841	20 Gb	21%	2%
	SWWA	19,340,305	40 Gb	16%	2%