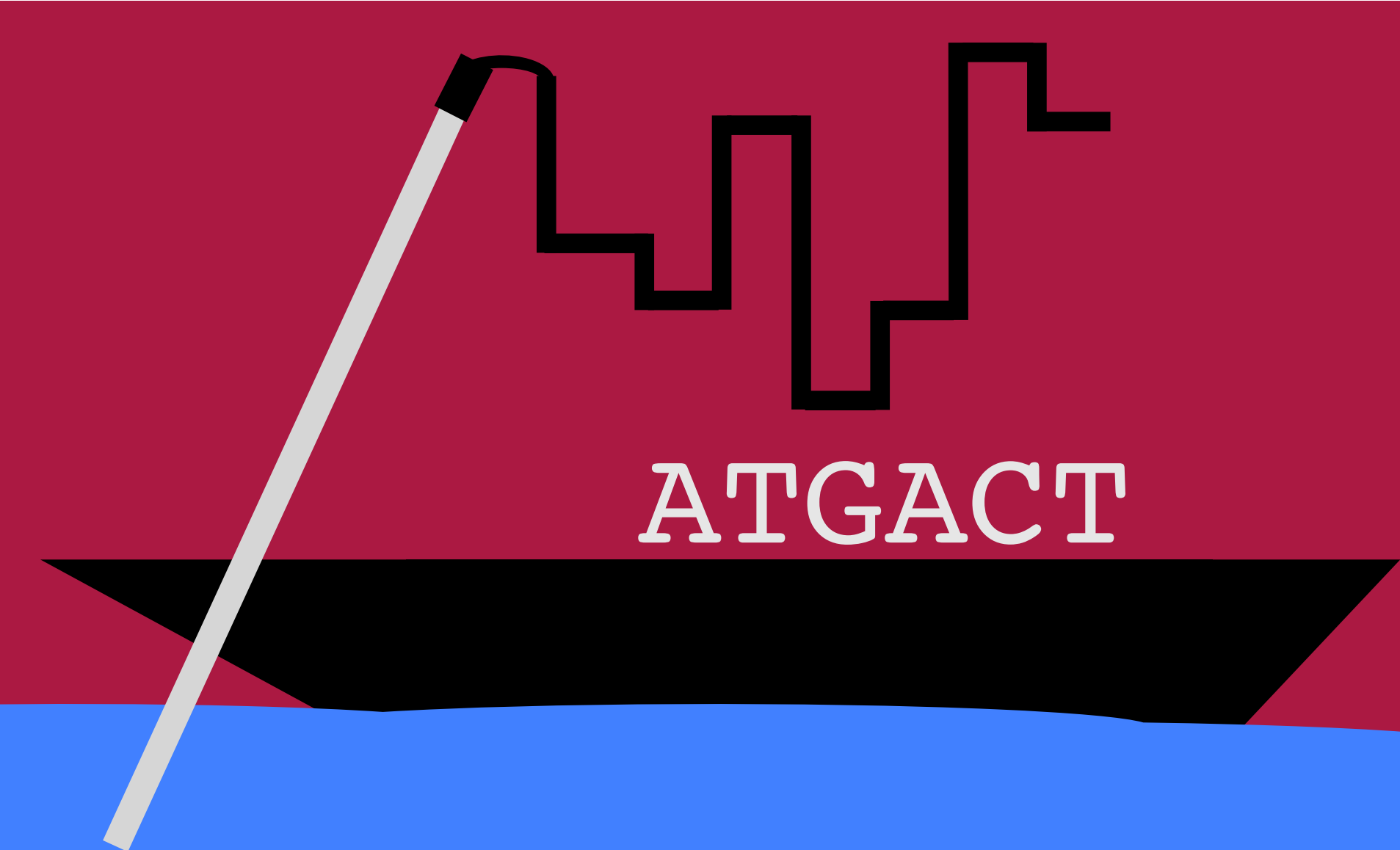


PuntSeq

River water DNA and microbial analyses
for everyone



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References

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[3] <https://github.com/rwwick/Basecalling-comparison> [12/03/18]

[4] Wagstaff *et al.* (2017) Isolation and Characterization of a Double Stranded DNA Megavirus Infecting the Toxin-Producing Haptophyte *Prymnesium parvum*. *Viruses*, 9 (3), 40.

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Overview

Year by year, Cambridge rowers, swimmers and punters obtain serious infections associated with pathogens obtained from the river's water. An information and research framework that targets the involved microbial culprits is still lacking. Here we present PuntSeq, a citizen science effort that will provide an in-depth resolution of the **Cam river microbial and pathogenic landscape** with minimum expense. Led by a small group of graduate students at different Life Science Departments of the University of Cambridge, we have designed a workflow for the Oxford Nanopore MinION sequencing device, in addition to processing large volumes of DNA sequencing and surface water metadata.

Methods

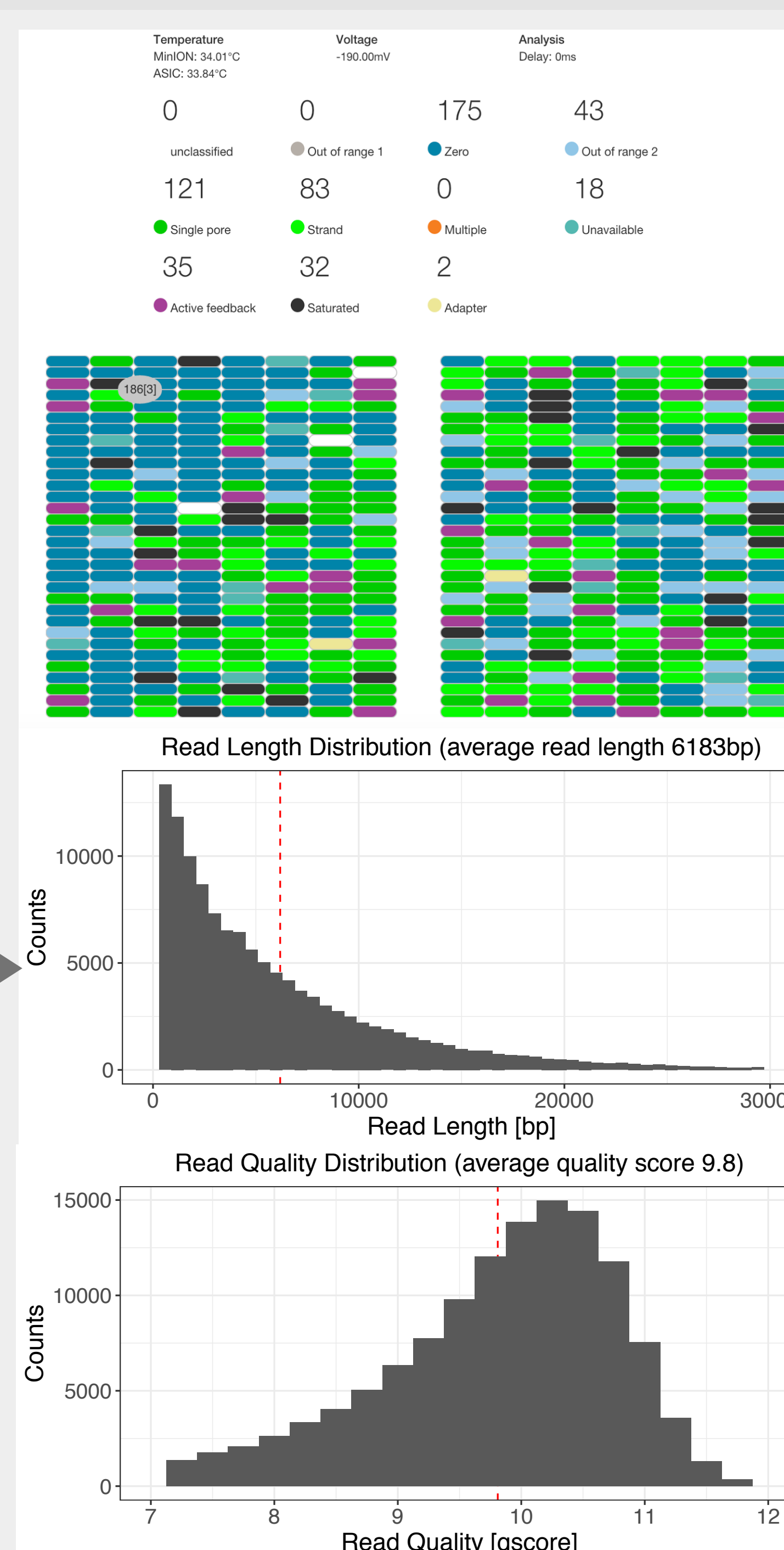
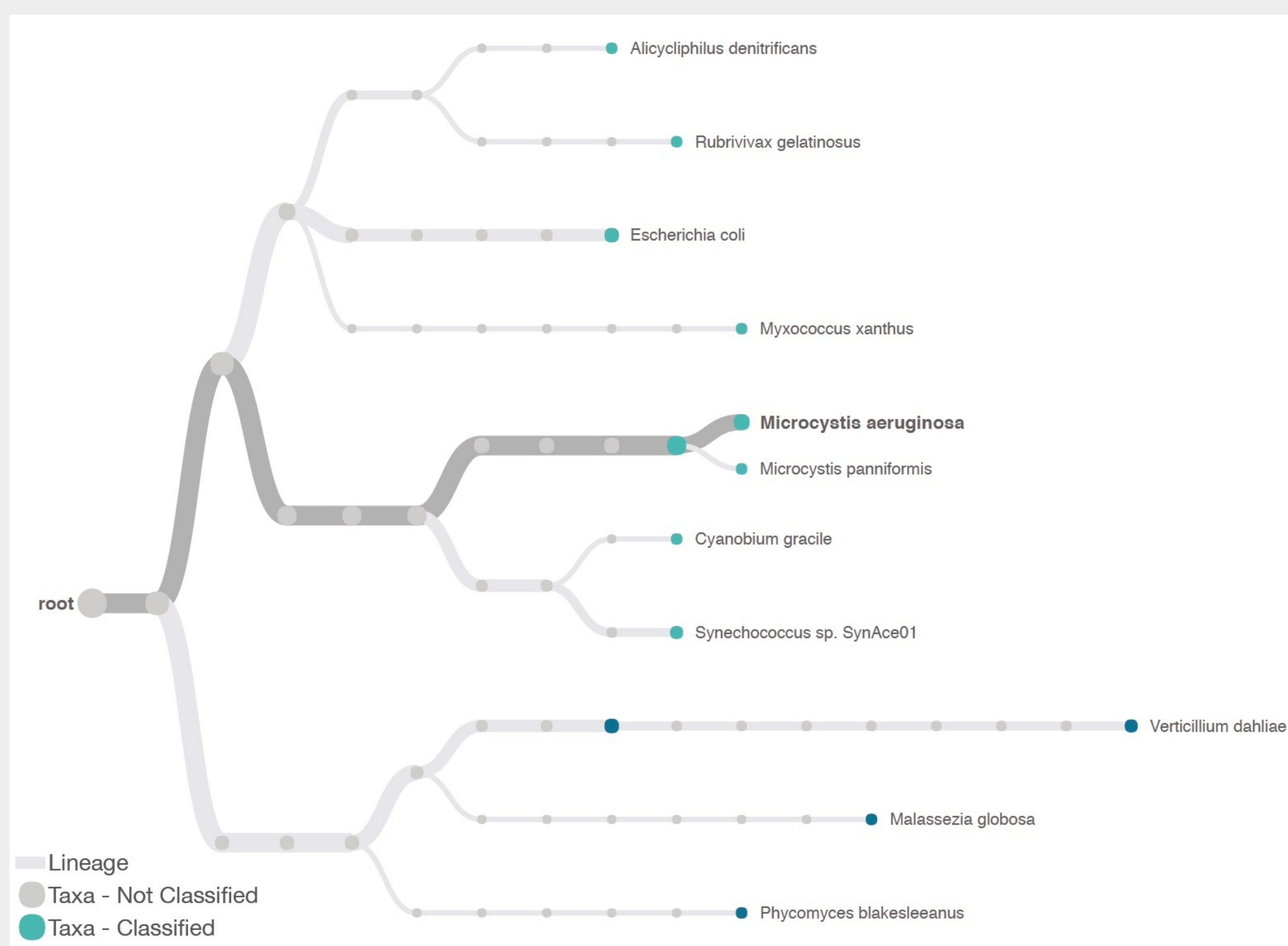
Summary. The analysis of the bacterial community of the Cam will involve (i) aquatic fieldwork sampling, (ii) DNA extraction, (iii) MinION usage, (iv) MinION raw data processing, and (v) metagenomics data interpretation. PuntSeq serves as a real world metagenomics pipeline for field work in the context of freshwater analysis, and will be applicable to a wide range of monitoring studies in Cambridge and beyond.

Aquatic fieldwork sampling. We take sample volumes of 1l from the water surface from multiple locations along the Cam. The metadata parameters for each sample are collected by an Arduino-based system and include (i) date and time, (ii) air and water temperature, (iii) date/time of last rainfall, (iv) GPS location, (vi) pH, (vii) water oxygen content, (viii) flow current of the river, and (ix) turbidity.

DNA extraction. Water samples are filtered through a nitrocellulose membrane by applying vacuum pressure. We make use of a pump, sewage pipes, two glass bottles and a tube. DNA is isolated directly from the membrane with the *Qiagen DNeasy PowerWater* kit.

MinION usage. We sequence the extracted DNA of all Cam river surface water samples, using the pocket-sized MinION from Oxford Nanopore Technologies [1-2]. To this end, we employ a multiplex barcoding strategy and analyse several samples in parallel. A purified Lambda-phage DNA sequencing trial run with the MinION has confirmed the technical functionality of the instrument.

Data processing and interpretation. To assess read accuracy and consensus sequence accuracy of different basecallers, we compare the performance of Albacore, Scrappie raw and Chiron [3]. The aim will be to assess and compare the bacterial community and diversity in our samples, and to potentially identify pathogenic species.



Results In a collaboration with Rob Field's laboratory at the John Innes Centre Norwich, we performed our first metagenomics analysis on a sample of Norfolk's Hickling broad. Here, fast metagenomics surveillance is of particular importance since the Norfolk Broads regularly suffer from toxic algal (*Prymnesium parvum*) blooms likely triggered by the dsDNA virus PpDNAV [4]. With Albacore, we were able to draw a preliminary map of the bacterial and fungal community of the broad (average qscore=15), and we also found evidence of PpDNAV. Our Arduino system showed robust results. *E.g.*, a measurement of the Mill Pond provided us with information about the pond's pH (7.26 +/-0.02), turbidity (900 +/-18 dtu) and dissolved oxygen level (9.87 +/-0.39 dtu).

Outreach

Our data and results will be made openly accessible through a wide range of media, including a website and YouTube channel, radio features, newspaper and journal articles. To make use of the portability of our devices, we ultimately aim to design a sequencing lab with which we can analyze the metagenomics of a river *in situ*, *e.g.* while punting on the Cam.

Follow our regular team updates on Twitter:
<https://twitter.com/puntseq>

