First Draft Genome of *Neodermopthirius harkemai*, an Ectoparasite of Lemon Sharks, and its Detection using Metagenomics

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Introduction

Ectoparasitic flatworms are an important but understudied component of marine disease ecology, particularly in captive shark systems where parasites can persist and spread rapidly. *Neodermopthirius harkemai* is a monogenean ectoparasite that infects Lemon Sharks (*Negaprion brevirostris*), causing hemorrhagic skin lesions and chronic irritation. In aquarium environments, the parasite is capable of transmission through free-swimming larval stages and resistant eggs, making outbreaks difficult to control and monitor.

Despite being morphologically described over 60 years ago, *N. harkemai* lacks any publicly available genomic or ribosomal DNA reference data. This absence of molecular resources limits accurate taxonomic classification, prevents development of genetic detection assays, and hinders studies of parasite ecology and transmission dynamics. Traditional diagnosis currently relies on visual identification of parasites on infected hosts, which may fail to detect low-level or environmental persistence.

Recent advances in metagenomic sequencing provide new opportunities for non-invasive pathogen surveillance in aquatic systems. Environmental DNA (eDNA) and shotgun metagenomics can simultaneously detect parasites, characterize microbial communities, and monitor ecosystem responses to treatment interventions. Saltwater aquaria are particularly valuable model systems because they contain complex microbial assemblages that may reflect broader marine host–parasite–microbe interactions.

Purpose

In this study, we generated the first draft genome of *N. harkemai* using Illumina whole-genome sequencing and evaluated whether parasite DNA could be detected directly from aquarium water samples collected before, during, and after praziquantel treatment. We additionally characterized the microbial composition of the aquarium system to assess broader biodiversity patterns associated with parasite presence and treatment dynamics

Methods

Parasite Collection and Genome Assembly

Adult *N. harkemai* specimens were collected from two infected Lemon Sharks (*N. brevirostris*) housed in a saltwater aquarium system. Individual parasites were morphologically identified prior to DNA extraction. Worms were surface sterilized and Genomic DNA was extracted from adult worms and prepared for whole-genome shotgun sequencing. Libraries were sequenced on an Illumina platform.

Raw sequencing reads were quality filtered and assembled into a draft genome assembly. Putative contaminant contigs originating from bacterial, or host were identified and removed through taxonomic screening and sequence similarity analysis. The resulting decontaminated draft genome was used as the reference for downstream metagenomic detection analyses.

Aquarium Water Sampling

Aquarium water samples were collected at 14 timepoints relative to praziquantel treatment: pre-treatment and 13 post-treatment. For each sampling event, two liters of aquarium water were collected and sequentially filtered using 40 µm and 0.1 µm membrane filters to capture organisms across multiple size fractions.

DNA Extraction and Sequencing

DNA was extracted from filters using standard eDNA extraction protocols optimized for marine samples. Sequencing libraries were sequenced on an Illumina platform to a depth of approximately 20 million paired-end reads per sample.

Metagenomic and Taxonomic Analysis

Shotgun metagenomic datasets were analyzed using Sourmash for taxonomic profiling and microbial community characterization. Reads matching the *N. harkemai* draft genome were used to assess parasite detectability across treatment timepoints. Relative abundances of microbial taxa, including prokaryotic and eukaryotic organisms, were compared between filter sizes and treatment stages to evaluate community composition and environmental dynamics.

Results

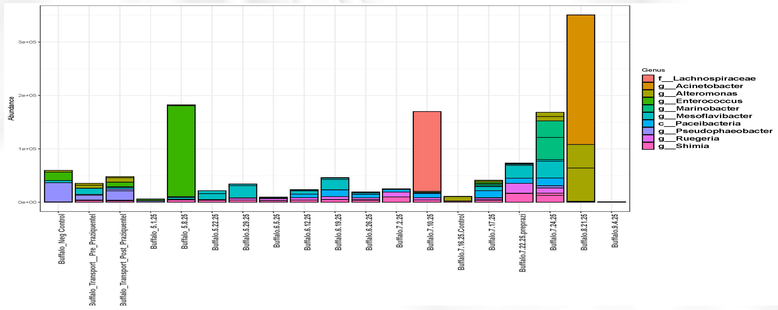


Figure 1) : The bacterial composition of the filter caught samples over time throughout the course of the experiment. The abundances of the top 10 bacterial genera are shown. The most conserved bacteria included the genera *Mesoflavibacter* and *Marinobacter*. The pathogenic bacterium *Enterococcus* was observed before Praziquentel treatment and ~1 week following treatment but was not observed in later sampling events

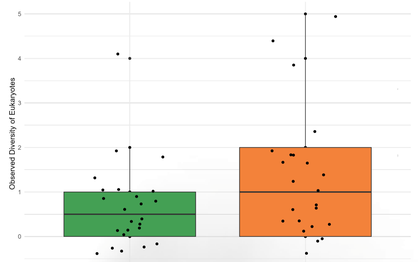
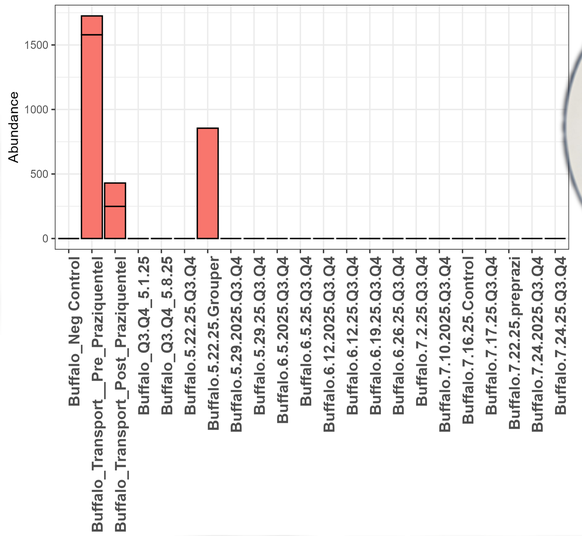


Figure 2) : The detection of marine Eukaryote species observed with the separate filters. In this study, filters with the larger 40uM pore size (orange) captured a greater diversity of marine Eukaryotic organisms (including parasite presence) compared to 0.1uM filters, though the results fell below significance thresholds.





Neodermopthirius harkemai

Figure 3): Detection of the parasite *N. harkemai* throughout the course of the experiment. Each timepoint includes two filter samples; 0.1uM filters (top) and 40uM filters (bottom).

Conclusion

This study presents the first draft genome of *Neodermopthirius harkemai* and demonstrates the utility of metagenomic sequencing for non-invasive parasite detection in marine aquarium systems. Using environmental DNA collected from aquarium water, *N. harkemai* was consistently detected in pre-praziquantel treatment samples and was detected only once approximately one month following treatment, suggesting a substantial reduction in parasite abundance after therapeutic intervention.

Filter size influenced observed community composition, with 40 µm filters capturing greater diversity of marine micro-eukaryotes compared to 0.1 µm filters, although these differences were not statistically significant. These findings suggest that larger pore-size filtration may improve recovery of eukaryotic environmental DNA in aquarium metagenomic workflows.

Metagenomic profiling also identified diverse bacterial taxa within the aquarium system. Notably, *Enterococcus* spp. were detected prior to praziquantel treatment and at one sampling point approximately eight days post-treatment but were absent from other timepoints. The biological or ecological mechanism underlying this transient pattern remains unclear and warrants further investigation.

Overall, this work establishes foundational genomic resources for *N. harkemai* and highlights the broader potential of aquarium metagenomics for monitoring parasite dynamics, microbial community structure, and host-associated ecosystem health in controlled marine environments.

References



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