I. Title of Research Project

Metagenomic analysis of antimicrobial-resistant bacteria of endangered *Sardinella tawilis* and farmed *Oreochromis niloticus* in Taal lake, Philippines

II. Names of Project Members (including affiliation)

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III. Purposes / Objectives

Taal Lake, a caldera lake formed by volcanic activity, supports a rich biodiversity, including the endemic *Sardinella tawilis*, the world's only freshwater sardine. However, intensive aquaculture of *Oreochromis niloticus* (tilapia) has introduced potential environmental challenges, including antibiotic resistance. The increased reliance on antibiotics to mitigate disease outbreaks in aquaculture has raised concerns about ARGs in aquatic environments. Despite the significance of Taal Lake as a water resource, no prior studies have documented antibiotic use or resistance in the system. This study aims to determine the presence of ARGs and antibiotic residues in Taal Lake, Philippines. The research will analyze microbial diversity and functional genes through metagenomics, as well as assess the potential risks posed by antibiotic resistance to aquaculture and endemic species. This study seeks to fill this gap and provide a foundation for future monitoring and management strategies. The following are the objectives of the research:

1. To determine antibiotic residues in Taal Lake, Philippines

2. To perform metagenomics analysis in water samples from Taal Lake and its fish inhabitants, endangered *Sardinella tawilis* and aquaculture fish *Oreochromis niloticus*

IV. Methods

Study site and sample collection: Taal Lake has a surface area of 234.2 km^2, shore length of 82.5 km and a mean depth of 100m. Normal range of annual water level fluctuation is around 2m. Ten (10) sampling sites will be identified to cover the entire lake surface. A total of ten sampling sites were selected to cover the lake's ecosystem. The sites included: seven (7) locations covering the surface of Taal Lake; one (1) sampling point from the outflow river, Pansipit River; and two (2) fish gut samples: one from *O. niloticus* (tilapia) and one from *S. tawilis*.

Detection of antibiotic residues: 100 ml surface water samples were collected from 8 collection points (7 samples across the lake and 1 from the Pansipit River) locations across the lake. All samples were put in amber sterile containers and immediately

placed on ice after collection. Upon reaching the laboratory, these were transferred to -80°C freezer until pretreatment. HPLC-MS/MS analysis of antibiotics were done.

Metagenomic Analysis

Water sample collection for DNA Extraction. 1-liter water samples were collected from the top 0.5 m of surface water. For each sampling point. All samples were stored in sterile bottles and then transported immediately to the laboratory. These were filtered through 0.22 µm pore size polycarbonate membranes (GTTP, Millipore, Ireland) using a vacuum filter.

DNA extraction in water samples. Filter membranes were cut into small pieces and placed in extraction tubes provided in a Power Water Kit (Qiagen). Total DNA were extracted from the filter membranes according to the kit manufacturer's protocol instructions. Quality of the DNA were checked on 1.0% agarose gel ran at 110 V for 30 mins. DNA Concentration were determined using Qubit® 2.0 Fluorometer through 1 µl of sample.

Fish samples from *S. tawilis* and *O. niloticus*

10 *O. niloticus* samples were randomly collected from fishermen with fish cages in the lake. 10 S. tawilis samples were obtained from fishermen after being caught in the lake. The fish were dissected and the contents were subjected DNA extraction following the manufacturer's protocol (DNeasy, Qiagen).

Sequencing

DNA samples will be sent to Novogene for the library preparation. The sequencing will be done on the Illumina platform NovaSeq. For metagenomic analysis in the study all the reads will be taken to study the taxonomic and functional gene characterization.

V. Results (Preliminary Findings)

A total of ten sampling sites were selected to represent the lake's total area (Figure 1). Sampling was conducted on January 4, 2025, with immediate processing in the laboratory. The sites included:

- Seven (7) locations covering the surface of Taal Lake.
- One (1) sampling point from the outflow river, Pansipit River.
- Two (2) fish gut samples: one from *O. niloticus* (tilapia) and one from *S. tawilis*.

The collected water samples from the 8 sampling sites were analyzed for the following parameters:

- pH : 6.0 to 6.60
- Temperature: 26.8 °C to 27.3 °C
- Total Dissolved Solids (TDS) (ppm): 0.94
- Electrical Conductivity (EC) (µS/cm): 852
- Total Particulate Matter (TPM): 557

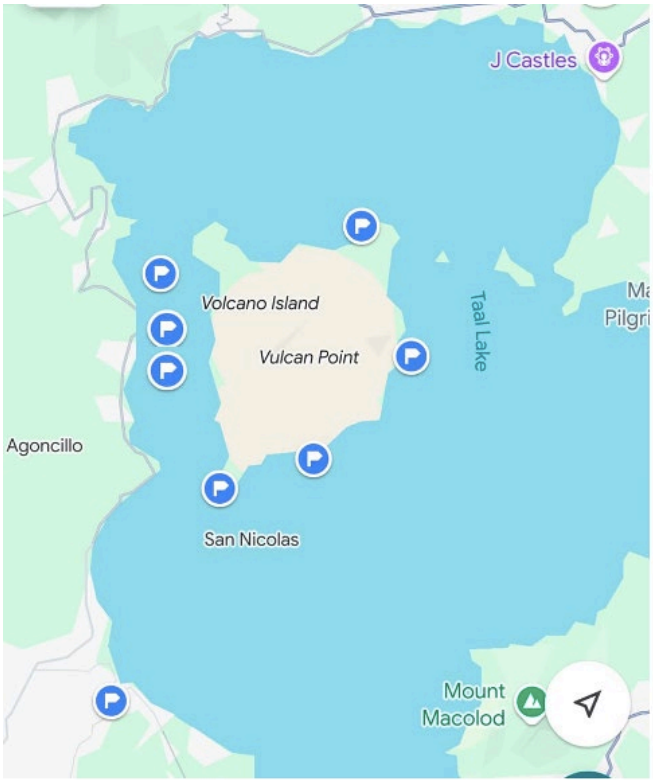

Figure 1. Collection points at Taal Lake, Philippines

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DNA Extraction

DNA extraction from water and fish samples yielded high-quality DNA with sufficient concentration for sequencing (Table 1.).

Table 1. DNA concentration of samples for sequencing

Samples	DNA Concentration (ng/µl)
1 (Taal Lake)	56.3
2 (Taal Lake)	34.6
3 (Taal Lake)	18.0
4 (Taal Lake)	30.0
5 (Taal Lake)	16.2
6 (Taal Lake)	30.6
7 (Taal Lake)	23.8
8 (Pansipit River)	19.6
S. tawilis	46.4
O. niloticus	44.3

Summary and Future Work

Sampling and DNA extraction have been successfully completed, covering seven lake sites, one outflow river site (Pansipit River), and two fish gut samples (*S. tawilis* and *O. niloticus*). The DNA is of high quality and is now ready for sequencing. The results from

the upcoming metagenomic analysis and antibiotic residue detection will provide critical insights into the extent of antimicrobial resistance in Taal Lake, contributing to sustainable aquaculture and conservation efforts for endemic species such as *Sardinella tawilis*.

Future work will focus on:

- Completing the antibiotic residue analysis.
- Conducting bioinformatics analysis of sequencing data to identify ARGs and microbial diversity.
- Assessing correlations between antibiotic residues, ARG prevalence, and environmental parameters.

VI. Future challenges

One of the challenges in this research is the lack of baseline data on antibiotic usage in Taal Lake, making it difficult to assess the extent of contamination and resistance development over time. Additionally, no previous metagenomic study has been conducted on the microbial communities and ARGs in the lake, presenting a challenge in establishing reference points for comparison. Metagenomic data analysis requires advanced bioinformatics approaches to accurately identify ARGs and their potential risks. Future research must address these gaps by conducting long-term monitoring of antibiotic residues and resistance genes to better understand their environmental and ecological impacts.

Further studies are also needed to assess the direct effects of antibiotic resistance on endemic species such as *Sardinella tawilis* and the potential transmission of resistant bacteria to human populations. This will require interdisciplinary approach and sustainable aquaculture practices to mitigate the spread of antimicrobial resistance while preserving the ecological balance of Taal Lake.