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KMFRI

Editorial

Editorial: Kenya Aquatica Journal Vol 10(1) – *A Showcase of KMFRI's Pioneering Research in Freshwater Ecosystems*

The latest edition of Kenya Aquatica Journal, Vol 10(1) showcases pioneering research by KMFRI scientists on Kenya's freshwater ecosystems. This edition, supported by KMFRI and WIOMSA, covers ecological, socio-economic, and environmental challenges, providing valuable insights into sustainable management practices.

One notable study investigates disease surveillance and antimicrobial resistance in fish from lacustrine caged farms, emphasizing responsible antibiotic use to maintain fish health. Another study explores the impact of organochlorine pesticides on macroinvertebrates in Lake ecosystems, advocating for Rhagovelia spp. as a bioindicator for pesticide monitoring across food webs.

Research on Lake Baringo's small-scale fishery assesses the catch and effort composition, stressing the need for regulatory enforcement to avoid overfishing and advocating for capacity building among stakeholders for sustainable management. Additionally, a study on wild fish kills in Lake Victoria focuses on eutrophication and pollution, recommending integrated watershed management to protect the lake's fisheries and local livelihoods.

A comprehensive study on Lake Elementaita – one of Kanya's flamingos' sanctuaries, combines water quality, fisheries studies, and community surveys, calling for integrated watershed management, conservation, and sustainable agriculture. Research on fisheries co-management in Lake Baringo highlights the importance of local community involvement and sustained achievements in ecosystem management, despite challenges in law enforcement.

An article on the socio-economic dynamics of Lake Victoria proposes establishing a regulatory framework incorporating citizen science to manage the lake's resources for long-term sustainability. Addressing plastic pollution in Lake Turkana, a study recommends waste management solutions, public awareness, and better enforcement of regulations to tackle the issue.

The journal also features research on antimicrobial resistance (AMR), with a review exploring Kenya's aquatic biodiversity for potential novel antimicrobial agents. A genetic research study evaluates freshwater fish populations, identifying gaps and proposing future directions for conservation and management.

Lastly, the journal presents an evaluation of fish market dynamics in Lake Naivasha, recommending infrastructure development like fish markets and hatcheries to support the region's fishery sector.

This edition of Kenya Aquatica Journal provides crucial insights into Kenya's freshwater ecosystems, covering a wide range of research on sustainable management, environmental challenges, and the socio-economic factors influencing aquatic resources. The research highlights KMFRI's ongoing contributions to understanding and addressing these issues, fostering a deeper understanding of Kenya's aquatic biodiversity.

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About Kenya Aquatica

Kenya Aquatica is the Scientific Journal of the Kenya Marine and Fisheries Research Institute (KMFRI). The aim of the Journal is to provide an avenue for KMFRI researchers and partners to disseminate knowledge generated from research conducted in the aquatic environment of Kenya and resources therein and adjacent to it. This is in line with KMFRI's mandate to undertake research in "marine and freshwater fisheries, aquaculture, environmental and ecological studies, and marine research including chemical and physical oceanography", in order to provide sci entific data and information for sustainable development of the Blue Economy.

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Submitting Articles

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Featured front cover picture: Researcher sampling surface plankton in the Kerio River inlet to Lake Turkana. (Photo credit: Mr. John Malala)

Featured back cover picture: Chair of KMFRI Board of Management Amb. Dr. Wenwa Akinyi Odinga Oranga (seated middle), on her right, Ag. KMFRI CEO Dr. James Mwaluma, flanked by KMFRI Heads of Sections: Front (L-R) Dr. Victoria Tarus, Ms Caroline Mukiira, Dr. Jacob Ochiewo, Dr. Irene Githaiga, Mr. Abraham Kagwima. Back (L—R) Mr. Paul Waluba, Ms Jane Kiguta, Dr. Gladys Okemwa, Dr. Eric Okuku, Dr. Joseph Kamau, Mr. Isaac Kojo, Ms Joan Karanja, Mr. Milton Apollo. (Photo credit KMFRI)

Research Vessel MV Mtafiti in the background

A review on the descriptive approach on disease surveillance and antimicrobial susceptibility profile of bacterial isolates from fish samples in lacustrine caged farms

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Abstract

In Kenya, cage farming has expanded along the shores of Lake Victoria, primarily focusing on the monoculture of tilapia (*Oreochromis niloticus*). Despite the global growth of aquaculture, infectious diseases, particularly bacterial infections are a significant source of economic losses in this sector, especially for cultured tropical freshwater species. There is limited information regarding bacterial communities, prevalence, and patterns of antimicrobial resistance (AMR) in bacteria isolates from caged tilapia on the Kenyan side. This study aimed to give a descriptive and quantitative approach on an investigation of the bacterial communities in caged fish in Lake Victoria and their antibiotic susceptibility. The approach uses examples of study sites of the five riparian counties along Lake Victoria - Migori, Homa Bay, Kisumu, Siaya, and Busia. Currently, there are over 5,635 cages in these counties, with dimensions varying from 8 m² to circular cages of 20 m in diameter. A total of 100 fish samples were collected across the counties as follows: Busia (30), Homa Bay(20), Kisumu (20), Migori (10), and Siaya (20). In the approach, the fish samples underwent clinical examination for disease signs, while parasitological assays were performed on gill biopsies and skin swabs. A bacteriological assay was conducted using aseptically taken kidney swabs, and internal organs were inspected for infectious lesions. Molecular identification of the isolates was conducted at the International Livestock Research Institute (ILRI) in Nairobi, Kenya, using Matrix-Assisted Laser Desorption Ionization–Time of Flight (MALDI-TOF) mass spectrometry (MS). Antimicrobial susceptibility testing (AST) followed standard disk diffusion method as outlined by the Clinical and Laboratory Standards Institute (CLSI). Statistical analysis was conducted to achieve the aim of the objective.

Keywords: cage farming, Lake Victoria, Kenya, infectious diseases, antimicrobial resistance, antimicrobial susceptibility

Introduction

Aquaculture has the potential to significantly contribute to food security, especially as capture fisheries have stagnated over recent decades (Obirikorang *et al.,* 2024). The consumption of fish remains to be essential in the human diet globally and it is a source of important nutrients that are necessary for good health (Wang *et al.,* 2022). Forecasts suggest that the future growth of fish food production will largely stem from aquaculture. For instance, according to FAO (2020), aquaculture production is expected to increase from 60 million metric tonnes in 2010 to 100 million metric tonnes by 2030, and further increase to 140 million metric tonnes by 2050 (Aura *et al.,* 2024).

Food security is recognized as a major global challenge. Despite progress in ensuring safe and nutritious food for all and addressing malnutrition, FAO (2024) estimated that 713 to 757 million people (8.9-9.4% of the global population) faced undernourishment in 2023. According to FAO (2023), as we confront escalating global challenges such as food shortages, limited access to food, and rising food costs - exacerbated by the climate crisis, biodiversity loss, economic slowdowns, worsening poverty, and other overlapping issues - put us at a critical stage. With global populations projected to increase to over 9.7 billion by 2050, seafood in general and fish in particular will continue to play a vital role in providing nutrition and food security globally, especially in developing countries (Obirikorang *et al.,* 2024).

In Africa, fisheries and aquaculture support around 6 million people, a number that continues to rise. In Sub-Saharan countries like Kenya, aquaculture relies mainly on extensive and semi-intensive practices, which hinder production and do not meet the demands of a growing population (Aura *et al.,* 2024). In Kenya, freshwater fish accounts for close to 95% of reported aquaculture production, of which 90% are from Lake Victoria (Orinda *et al.,* 2021). Lake Victoria is revealed to produce more fish than all five Laurentian Great Lakes combined, triple the production of Lake Tanganyika, and more than quadruple the harvest of Lake Malawi (Nyamweya *et al.,* 2023). Tilapia represents about 75% of the total fish produced from aquaculture, followed by African catfish (18%), common carp (6%), and trout (<1%) (Opiyo *et al.,* 2018).

Cage farming has sporadically expanded throughout the shores of Lake Victoria, primarily involving the monoculture of Nile tilapia, *Oreochromis niloticus*, and it has been considered as a game-changer (Obiero *et al.,* 2022). Currently, cage farming is being practiced in five riparian counties including Migori, Siaya, Homabay, Busia and Kisumu, with Siaya County leading with the highest number of cages (Opiyo *et al.,* 2018). The stocking density in the cages ranges between 60 and 250 fish m^{-3}, with cage sizes ranging from 8 to 125 m^3. *Oreochromis niloticus* is the only fish cultured in the lake, with a production of 12 million kg of fish every cycle annually (Opiyo *et al.,* 2018).

Despite the global expansion of aquaculture, diseases significantly contribute to economic losses in the aquaculture industry, with bacterial diseases being the most common and primary cause of mass mortality in fish, particularly affecting cultured tropical freshwater species (Siamujompa *et al.,* 2023). Worsenning the situation, antimicrobial resistance (AMR) in cultured fish has emerged as a major challenge in aquaculture (Preena *et al.,* 2020). AMR is known to arise from mutations or the spread of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs), largely due to the widespread use of antibiotics (Wang *et al.,* 2022). The AMR is an unavoidable evolutionary phenomenon where by microorganisms including bacteria, viruses, fungi and parasites have the ability to thrive and grow in the midst of drugs designed to kill them, mainly as a result of genetic mutations (Salam *et al.,* 2023). However, long-term overuse of antibiotics in aquaculture has led to acceleration and acquisition of drug resistance (Wang *et al.,* 2022). This AMR can transfer to important strains in nature, impacting the ecosystem (Preena *et al.,* 2020).

Many cultured fish, including ornamental types, carry diverse pathogens with multiple antibiotic resistance (Preena *et al.,* 2020). Studies have also shown that there is a continuous increase in resistance of bacteria to antibiotics due to their widespread use and misuse in fish farming in the treatment of specific and non-specific infections and as growth promoters, resulting in emergence of resistant bacterial strains (Gousia *et al.,* 2011). This makes farmed animals including fish, which are reared majorly for human consumption to become reservoirs of antibiotic resistant bacterial strains. Interestingly, in human beings, AMR has been listed as number one global threat and a significant concern for public health as by the global health security (Velazquez-Meza *et al.,* 2022). Although bacteria intrinsically develop resistance against particular antimicrobials, the significant key factor in the selection of resistant bacteria is probably the use of antibiotic-type and antimicrobial agents for the treatment of diseases and infections in human and animals (Onjong *et al.,* 2021).

According to Onjong *et al.* (2021), AMR is greatly associated with antimicrobials being incorporated into commercial livestock, poultry, and aquaculture production at sub-therapeutic doses to promote growth, improving the feeding efficiency, metaphylaxis and prophylaxis. However, this is immensely unregulated in developing countries. Consequently, due to unregulated use of antibiotics, human beings are indirectly exposed to AMR bacteria through the food chain. Antibiotic resistance determinants found in food and water can be transferred to bacteria of human clinical significance (Onjong *et al.,* 2021).

Numerous studies have been conducted on cage farming in Lake Victoria, Kenya. For instance, Aura *et al.* (2018) reviewed the integration of mapping and socio-economic indicators of fish cage culture in the lake. Njiru *et al.* (2019) reviewed the establishment of cages in the lake and the need for coming up with a decision-support tool for efficient management of the lake. Mwainge *et al.* (2021) conducted a study in determining the fish disease and parasite occurrence in cage culture system. Mboya and Ouko (2023) reviewed the economic aspects of fish cage farming in the lake and Mawundu *et al.* (2023) conducted a study on the effect of stocking density on growth performance, and survival of Nile tilapia (*O. niloticus*) in cage culture system in the lake. Aura *et al.* (2024) did a case study to evaluate the sustainability features of a community-based cage aquaculture that included socio-economic, physical, chemical, biological, production and risks variables that were aimed at identifying and proposing potential mitigation measures for the challenges the cage culture industry may experience. However, there is paucity of information on the bacterial community, prevalence, approach and pattern of AMR- bacterial isolates from caged tilapia (*O. niloticus)* on the Kenyan side of the Lake Victoria. Therefore, the present study targeted to give a detailed or coherent discussion on the approach that is required in investigating the level of bacterial community in caged fish in Lake Victoria and their antibiotic susceptibility. The information from this study is of significance in addressing proper use of antibiotic-type and antimicrobial agents in cage farming in an aquatic environment.

Discussion

The methodology followed in this study is explained in the schematic diagram indicating the flow of processes from sample collection in the field, to the final processes in the laboratory.

Study identification

The review of the approach was based on the five riparian counties along Lake Victoria, Kenya i.e., Migori, Homa Bay, Kisumu, Siaya and Busia counties where cage farming is being practiced (Obiero *et al.,* 2022). Lake Victoria is ranked to be the second largest freshwater lake globally and Africa's largest by surface area and it is being shared by Kenya (6%), Uganda (43%), and Tanzania (51%) (Nyamweya *et al.,* 2023). Currently, the lake is revealed to hold a little over 5,635 cages in the five riparian counties of the Kenyan side with dimensions ranging from 8 m^2 to circular cages of 20 m diameter (Mwainge *et al.,* 2021).

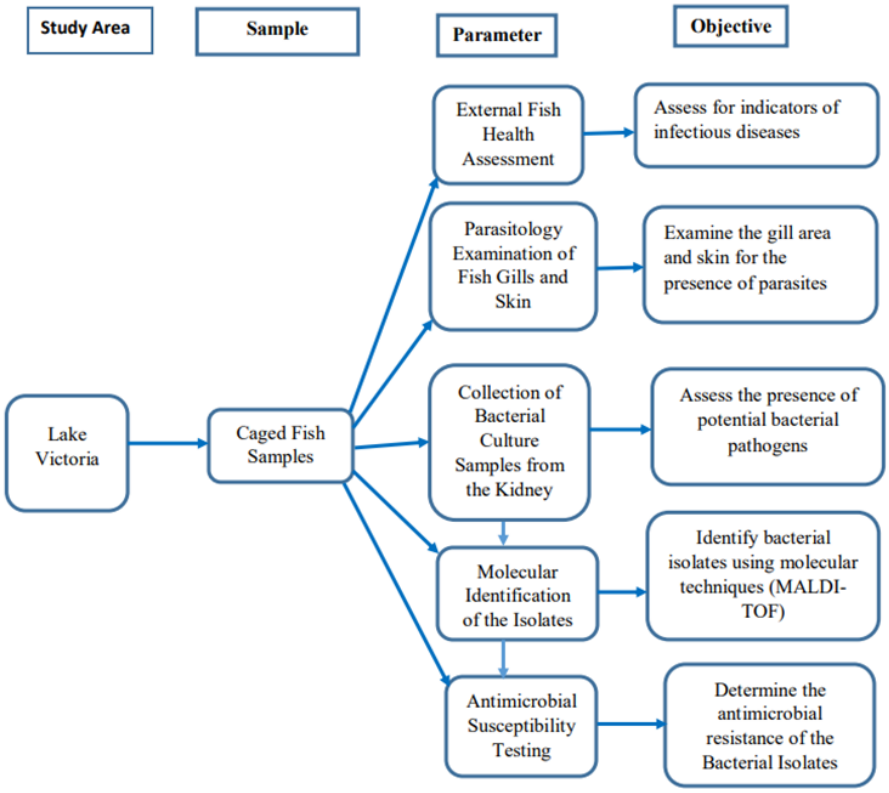

Figure 1. A schematic descriptive approach of the entire process showing the various processes from the sample collection to final laboratory analyses.

Sample collection and sizing

A total of five healthy fish samples, each weighing approximately 200 g or more, were randomly collected from selected cages using a sweep net. The cages from which the samples were taken were also chosen randomly, with consideration given to the distance between the selected cages to ensure diversity. In total, 100 fish samples were collected for analysis from five riparian counties. The distribution of samples included 30 from Busia, 20 from Homa Bay, 20 from Kisumu, 10 from Migori, and 20 from Siaya. Additionally, water samples for metagenomic analysis were collected from the same cages where the fish samples were obtained (Nogueira and Botelho, 2021).

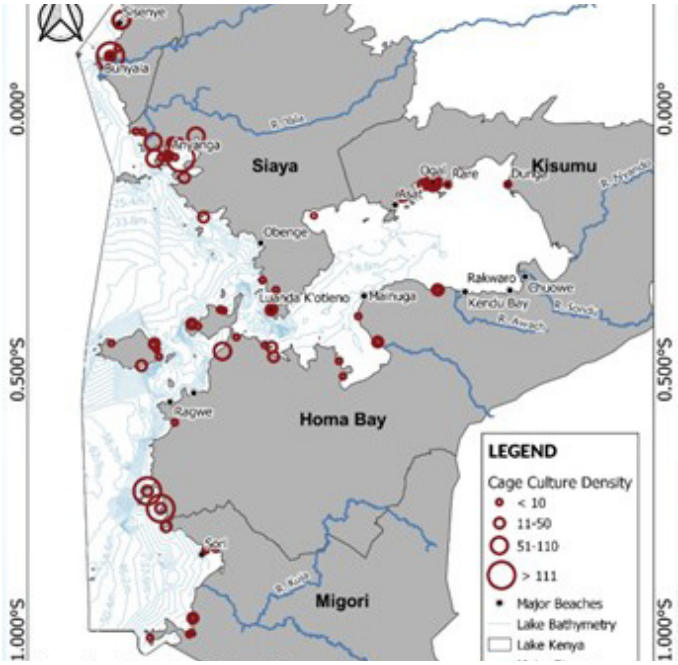

Figure 2. A map illustrating the five riparian counties around Lake Victoria in Kenya where cage farming is practiced and their respective density (Source: KMFRI-ABDP Unpublished Report, 2022).

External fish health assessment

The collected fish samples were first examined clinically for signs of infectious diseases (Mwainge *et al.,* 2021). This assessment involved checking for lesions, ulcerations, deformities such as eye opacity, and skin discolorations on the external parts of the fish. In case of any observations, they were recorded.

Parasitology examination of fish gills and skin

Precise fish gill samples were taken using dissecting scissors and placed on a microscope slide to check for the presence of parasites in the gill area. Two to three drops of distilled water were added to the gill samples to keep the contents fresh and improve visibility during microscopy. Additionally, skin swabs were obtained using coverslips, which were positioned parallel to the gill samples. Both samples were then observed under a compound microscope, and any observable parasites were recorded, noting their number and species (Mwainge *et al.,* 2021).

Bacterial culture sample collection

A bacteriological assay was performed by taking kidney swabs aseptically (Jia *et al.,* 2023). The fish samples were dissected on the dorsal side to expose the internal organs. They were then examined for internal lesions except the kidney to avoid contamination of the site for possible cultures. The swim bladder membrane was carefully dissected to access the kidney, located along the backbone. A sterile hot wire loop was used to puncture the kidney. To avoid contamination, special care was taken to prevent the wire loop from contacting any part of the fish or external objects other than the internal part of the kidney. The swabs were then streaked aseptically onto sterile tryptose agar plates. The inoculated plates were sealed with parafilm and transported to the KMFRI microbiology laboratory. In the laboratory, the plates were incubated at 30°C for 24 to 48 hours, after which bacterial growth was observed. A negative control, consisting of a medium without an inoculum, was also incubated to confirm the sterility of the medium. Only bacterial growth along the streaking pattern was considered. The cultures of interest were sealed with parafilm and preserved in the refrigerator at 2°C as a backup until the process was completed, after which they were discarded aseptically.

Broth culture and glycerol stocks preparation

Colonies of interest were picked from the culture medium and inoculated into sterile broth in sterile test tubes using sterile toothpicks, held with flamed forceps to maintain aseptic conditions. The inoculated broth was then incubated at 30°C for 24 to 48 hours to allow for sufficient growth, which was determined by the turbidity intensity. A negative control, consisting of broth containing only a sterile toothpick, was also incubated to ensure the sterility of the broth. To prepare glycerol stocks, 600 µl of 50% glycerol was pipetted into sterile cryovials, followed by an equal amount of broth culture, and the mixture was homogenized. The vials were labeled according to the various bacterial isolates, with one as a backup. The stock solutions were then preserved in a freezer at -35°C for further analysis.

Molecular identification of the isolates

Molecular identification was performed at the International Livestock Research Institute (ILRI) in Nairobi, Kenya, using Matrix-Assisted Laser Desorption Ionization–Time of Flight (MALDI-TOF) mass spectrometry (MS) (Jia *et al.,* 2023). Spectra acquired from the test isolate were compared with spectra in the reference library and an identification match score was provided based on the similarity to reference library entries. Identification match scores for MALDI-TOF were categorized based on the manufacturer's guidelines

Antimicrobial susceptibility testing (AST)

Antimicrobial susceptibility testing (AST) against various antibiotics was conducted using the disk diffusion assay in accordance with the Clinical and Laboratory Standards Institute (CLSI) standard methodology for bacterial isolates from the fish samples (Siamujompa *et al.,* 2023).

Data processing

The data analysis for this study was conducted using a combination of statistical methods to assess the bacterial communities and their antimicrobial susceptibility profiles in caged tilapia from Lake Victoria. The analysis aimed to identify patterns of parasite and bacterial prevalence, diversity, and bacterial resistance, providing a comprehensive overview of the health status of the fish populations. Statistical analyses were performed using both Excel and R v.4.3.1 (R Core Team, 2023). Descriptive statistics, including means, standard deviations, and frequencies, were calculated to summarize the data collected from the fish samples.

Prevalence and intensities of infection

This section focused on examining the prevalence and intensity of parasites and the prevalence of bacterial infections affecting caged tilapia (*O. niloticus*) in Lake Victoria.

Prevalence and intensity of parasites

- **Prevalence**: The prevalence of parasitic infections was determined as the proportion of infected fish relative to the total number of fish examined. The formula used was:

  Prevalence = (Total number of fish examined ÷ Number of infected fish) ×100.

- **Intensity assessment**: The intensity of infection was calculated as the average number of parasites per infected fish. This provided insight into the severity of parasitic infestations.

Prevalence of bacteria

- **Bacterial prevalence**: The prevalence of each bacterial species was calculated as a percentage of the total number of samples tested. This was done by dividing the number of positive samples for each species by the total number of samples collected and multiplying by 100.

Antimicrobial susceptibility testing (AST): The results from the disk diffusion method were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The diameters of inhibition zones were measured in millimeters, and bacteria were classified as susceptible, intermediate, or resistant based on these measurements.

Diversity analyses

The diversity of both bacterial and parasite communities was assessed using the Shannon-Wiener index (H′) and Simpson's index (D). These indices provide insights into both species richness and evenness within the samples:

- **Shannon-Wiener Index (H′)**: This was calculated using the formula:

  $$H' = -\Sigma(pi \cdot \ln(pi))$$

  where *pi* is the proportion of each species of bacteria or parasite in relation to the total number of species.

- **Simpson's Index (D)**: This index was computed using the formula:

  $$D = \Sigma(pi2)$$

The results from these indices help in understanding the ecological balance within the caged fish populations and can indicate potential impacts on fish health.

Multivariate analyses

A principal coordinate analysis (PCA) was employed to visualize differences in bacterial and parasite communities among samples from different counties. This multivariate approach allows for an assessment of how geographical variations may influence the parasite and microbial diversity and antibiotic resistance patterns.

Conclusions and recommendations

This study provides insights on the approach required during the investigation of the parasitic and bacterial communities in caged tilapia fish (*O. niloticus*) and their antibiotic susceptibility in a lacustrine environment. This is because there is limited information about the bacterial communities and the prevalence and patterns of antibiotic-resistant bacteria (AMR) isolated from caged tilapia in the lake. Despite the global growth of aquaculture, infectious diseases, especially bacterial infections pose a significant economic challenge in this sector, particularly for cultured tropical freshwater species. Antimicrobial resistance in cultured fish presents a major obstacle, with antibiotic use largely unregulated in developing countries. The approach of this study will provide crucial insights into the responsible use of antibiotics and antimicrobial agents in cage farming practices in the region, in accordance with legal and environmental standards.

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