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Comparative Study of Physicochemical, Bacteriological and Heavy Metal Quality of Water in Selected Fish Farms in Abuja, Nigeria

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Abstract. Nigeria's growing aquaculture sector faces critical water quality challenges that threaten fish health and food safety, yet limited systematic assessment exists for urban fish farming environments. This study comparatively assessed physicochemical, bacteriological, and heavy metal quality of water in three selected fish farms in Abuja, Nigeria. The study was conducted over five weeks (November 2024–January 2025), with water samples collected in triplicates from inlet and outlet points and analyzed using standard APHA methods for physicochemical parameters, culture and molecular techniques for bacterial identification, and Atomic Absorption Spectroscopy for heavy metals. Data were analyzed using ANOVA and Kruskal-Wallis tests. Results revealed that all farms exhibited suboptimal water quality with dissolved oxygen levels critically below standards (0.18 ± 0.07 mg/L vs. recommended >5 mg/L), acidic pH (6.4 ± 0.41), and elevated chemical oxygen demand (98.72 ± 19.90 mg/L). Nine bacterial species were identified from thirty isolates, with *Escherichia coli* being predominant (60%), followed by *Staphylococcus* sp. and *Shigella* sp. (10% each), while *Campylobacter*, *Proteus*, *Enterococcus*, *Salmonella*, *Enterobacter*, and *Bacillus* species were each detected at 3.3%. Heavy metal concentrations varied significantly across farms with manganese concentrations ranging from 0.18 ± 0.12 mg/L in Farm A to 0.24 ± 0.25 mg/L in Farm C. Zinc (Zn) levels were highest in Farm V at 0.28 ± 0.21 mg/L and lowest in Farm A at 0.20 ± 0.11 mg/L. Nickel (Ni) concentrations remained relatively consistent across all farms. The study concludes that critical water quality deficiencies across all examined fish farms pose significant risks to fish health and public safety, necessitating urgent implementation of water quality management interventions and strengthened regulatory oversight for sustainable urban aquaculture development.

Introduction

Globally, aquaculture has become a key strategy for food security and sustainable protein production, with global output reaching 114.5 million metric tonnes in 2020 (30). As demand for fish increases, aquaculture continues to play a crucial role in providing affordable protein, supporting livelihoods, and boosting economic growth. In Africa, the industry has expanded significantly, and Nigeria remains one of its leading contributors, driven by a growing population, increasing protein needs, and supportive agricultural policies (30, 61).

Nigeria’s aquaculture has evolved from traditional fishing methods to modern, intensive farming practices, adapting to the challenges posed by urbanization and environmental sustainability. In cities like Abuja, urban aquaculture has emerged as an innovative approach to meet food production demands while navigating the environmental constraints of a rapidly growing metropolis. Maintaining high water quality is a fundamental aspect of fish farming, as it directly influences fish health, farm productivity, and the overall sustainability of the sector (1).

Abuja’s urban setting presents a unique case for studying aquaculture challenges, as it combines rapid urbanization, increasing industrial activities, and environmental fluctuations. These factors impact water quality, which in turn affects fish health and consumer safety (12; 51; 49). Water quality is shaped by a variety of physicochemical, bacteriological, and heavy metal parameters, all

of which must be monitored and managed for sustainable fish farming (11; 24). Previous studies, including those by (3) and (62), have underscored the importance of water quality in aquaculture. However, many of these studies focus on specific parameters rather than examining water quality comprehensively. Given the complexities of urbanization, pollution, and agricultural practices, a more holistic approach is needed. This study seeks to fill these gaps by analyzing the physicochemical, bacteriological, and heavy metal quality of water in selected fish farms in Abuja.

Materials and Methods

Study Area

The study was conducted in Ijayapi, Kubwa (9.160294°N, 7.316732°E), Federal Capital Territory, Nigeria. Kubwa is located approximately 26 kilometers northwest of Abuja city center and falls under the Bwari Area Council. The area experiences a tropical climate with distinct wet and dry seasons, receiving an average annual rainfall of 1,200-1,500mm and temperatures ranging from 20°C to 35°C.

Three fish farms were selected within Ijayapi based on proximity and accessibility for weekly sampling over the 5-week study period. The selection of these farms was primarily based on proximity considerations, which facilitated logistical efficiency for consistent weekly sampling and data collection. The close geographical clustering of the farms within the same locality ensured similar environmental conditions across all study sites, reducing confounding variables while enabling comprehensive assessment of physicochemical parameters, bacteriological populations, and heavy metal concentrations in water samples. The farms' location within the same microenvironment provided an ideal setting for conducting comparative analyses of water quality across different fish farming sites, directly supporting the study's research objectives.

The accessibility of the study area also ensured reliable transportation of samples and equipment throughout the research period, while the established nature of aquaculture activities in Ijayapi facilitated cooperation from farm owners and the local community.

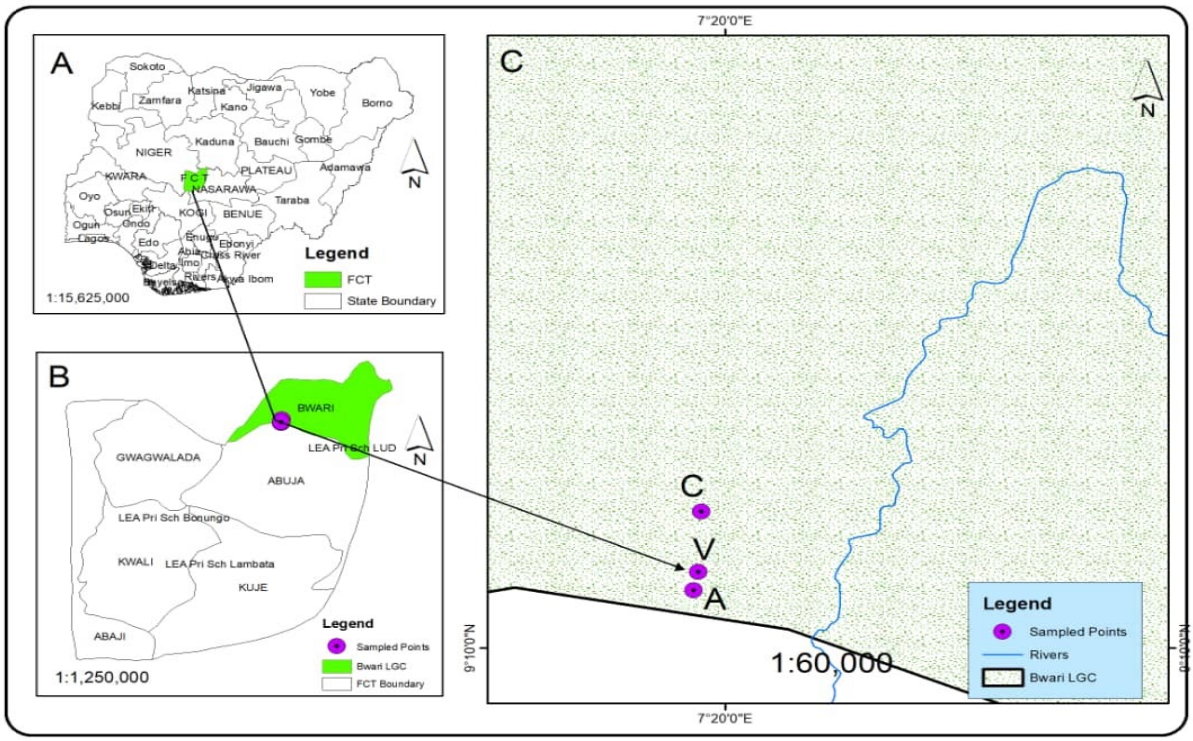

Fig. 1. Map of the study area in Ijayapi, Kubwa, Federal Capital Territory, Nigeria.

Collection of Samples

Samples were collected in triplicate from each farm to enhance data reliability. Water samples were collected from the inlet and outlet sources at each farm to capture variations in water quality. Six water samples were collected in sterile bottles, which were dipped below water surface level and capped in the water before the bottles were brought out. Temperature and pH levels of each sample were taken immediately at the site of collection. The collection period was between 8am-2pm after which they were then transported, in ice packs, to Nile University Microbiology Laboratory and Sheda Science and Technology Complex, Kwali for further analysis. The collection period was between November 2024 and January 2025.

Physicochemical Analysis of Water Samples Collected from the Fish Farms

A comprehensive physicochemical analysis was carried out following standard methods described by (5) to assess parameters including pH, temperature, conductivity, total dissolved solids (TDS), salinity, turbidity, dissolved oxygen (DO), total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), and nitrate. Standard analytical procedures were followed using the following instruments: Thermo Scientific iCE 3000 Atomic Absorption Spectrometer for trace element analysis (Thermo Fisher Scientific, 2019), a Hanna Instruments turbidity meter for turbidity measurements (Hanna Instruments, n.d.), and the Thermo Scientific Orion Versa Star Pro multi-parameter electrochemistry meter for pH, conductivity, and DO analysis (Thermo Fisher Scientific, 2023; Thermo Fisher Scientific, n.d.). All analyses were conducted using analytical-grade reagents, and the water samples were processed under controlled laboratory conditions.

Bacteria Analysis

Media Preparation

Culture media were prepared to facilitate the isolation and enumeration of microorganisms from water samples collected from three selected fish farms. The preparation followed standard microbiological protocols to ensure sterility and accuracy (5).

For the enumeration of total heterotrophic bacteria, Nutrient Agar (NA) was used as a general-purpose medium. MacConkey Agar (MA) was employed for the detection and differentiation of coliform bacteria based on lactose fermentation, while Eosin Methylene Blue (EMB) Agar was used to confirm the presence of bacteria.

The required amounts of dehydrated media were weighed using an analytical balance and suspended in distilled water according to the manufacturer's instructions. The mixtures were heated with continuous stirring until fully dissolved, followed by sterilization in an autoclave at 121°C for 15 minutes at 15 psi. After sterilization, media requiring selective agents were cooled to 45–50°C before the addition of heat-sensitive supplements under aseptic conditions.

Molten agar was dispensed into sterile Petri dishes in a laminar airflow cabinet and allowed to solidify at room temperature. The plates were then stored at 4°C in an inverted position to prevent condensation. Liquid media, such as Lactose Broth for coliform analysis, were dispensed into sterile test tubes before autoclaving.

To verify the sterility of the prepared media, one randomly selected plate from each batch was incubated at 37°C for 24 hours. Plates showing microbial growth were discarded, and the preparation process was repeated as necessary.

Bacterial Colony Count

Water samples were serially diluted to (10^{-5}) using distilled water. The serial dilution was done by 9 ml of distilled water and 1ML of samples 10^{-3} dilution was used because the concentration was neither too high nor low. The pour plate method (20) was used, where 0.1ml of the 10^{-3} dilutions for each sample were inoculated on media in triplicates and the plates were placed on plate count medium (HIMEDIA®), the plates were then placed in an incubator at 37 ^{0} C for 24 hours, after

which the plates were brought out for colony counts. This was done using a colony counter (Stuart") and each distinct colony was counted, below 30 was considered too few to count and above 300 is considered too much to count. The CFU/100 ml was estimated using:
CFU/ML = (Colonies formed) / (Volume plated × Dilution factor) × 100.

Isolation and Identification of Bacteria

Bacterial colonies obtained from the water samples were subcultured on Eosin Methylene Blue (EMB) agar and Plate Count Agar (PCA) to facilitate bacterial isolation. The plates were incubated at 37°C for 24 hours to allow for the growth of distinct bacterial colonies. To confirm bacterial identity, Gram staining and various biochemical tests, including the indole test, catalase test, coagulase test, methyl red, voges-proskauer, and citrate test were performed. These assays helped differentiate bacterial species based on their physiological and metabolic characteristics. Each sampling point was analyzed in triplicate to enhance the reliability of the results.

Molecular Characterization

Genomic DNA was extracted from the bacteria isolates using ZymoBIOMICS® DNA Miniprep kit (Zymo Research) according to the manufacturer's protocol. 16s rRNA was targeted using universal bacteria primers and Polymerase chain reaction (PCR) amplification of the targeted gene was performed. The PCR products were then sent to a commercial lab for Sanger sequencing and the sequences were queried against GenBank at the National Centre for Biotechnology Information (NCBI).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) was conducted using the disk diffusion method. Isolates were cultured in Mueller-Hinton medium and incubated at 37 °C for 24 hours. The bacterial suspension was evenly spread on Mueller-Hinton agar, and susceptibility discs with fixed antibiotic concentrations were placed on the inoculated plates, ensuring a minimum gap of 24 mm between discs. The plates were then incubated at 37 °C for 24 hours.
The antibiotics tested included Rifampicin (RD) 20mcg, Ceftazidime (CTZ) 30mcg, Streptomycin (S) 30mcg, Azithromycin (AZM) 10mcg, Amoxil (AMX) 20mcg, Ciprofloxacin (CPX) 10mcg, Erythromycin (AND) 30mcg, Levofloxacin (LEV) 20mcg, Gentamicin (CN) 10mcg, and Cefuroxime (CEF) 30mcg. The results were interpreted according to (20) guidelines.

Heavy Metal Analysis

Heavy metal concentrations in water samples were determined using Atomic Absorption Spectroscopy (AAS). The metals analyzed included lead (Pb), manganese (Mn), copper (Cu), magnesium (Mg), cadmium (Cd), chromium (Cr), and zinc (Zn). The results were compared with established water quality standards (31; 30; 15) to assess contamination levels and potential risks to fish health and human consumption.

Data Analysis

Data analysis in this study was conducted using SPSS Version 27 for statistical tests like ANOVA, Python for advanced analysis and visualizations, Excel for data organization and descriptive statistics. These tools are collectively enabled through statistical evaluation and effective presentation of results.

Results

Physicochemical Properties of Water in Selected Fish Farms in Abuja

Table 1 shows the physicochemical water quality parameters measured across three fish farms in Abuja, comparing observed values against established regulatory standards for aquaculture operations. The table displays mean values with standard deviations for each parameter, along with the measured range, applicable regulatory thresholds, and compliance status for each farm. The data

represents comprehensive water quality assessment covering nine key physicochemical parameters essential for evaluating aquaculture water suitability, including pH, dissolved oxygen, conductivity, total dissolved solids, salinity, temperature, turbidity, chemical oxygen demand, and biochemical oxygen demand.

Table 1. Result of Water Quality tests conducted on Water Samples from V, A, and C Fish Farms.

Parameter	V Farm (Mean ± Std)	A Farm (Mean ± Std)	C Farm (Mean ± Std)	Range	Regulatory Standard	Compliance Status
Ph	6.4 ± 0.41	6.4 ± 0.41	6.4 ± 0.41	0.98	6.6–8.5 (FAO, 2006)	**Non-compliant**
DO (mg/L)	0.18 ± 0.07	0.18 ± 0.07	0.18 ± 0.07	0.17	>5.0 (WHO, 2023)	**Non-compliant**
Conductivity (μS/cm)	189.67 ± 69.21	189.67 ± 69.21	189.67 ± 69.21	175.00	100-2000 (Boyd, 2003)	Compliant
TDS (mg/L)	0.09 ± 0.03	0.09 ± 0.03	0.09 ± 0.03	0.09	≤0.13 (Davis, 1993)	Compliant
Salinity (ppt)	0.10 ± 0.02	0.10 ± 0.02	0.10 ± 0.02	0.05	0-5 (FAO, 2006)	Compliant
Temperature (°C)	28.14 ± 1.58	28.14 ± 1.58	28.14 ± 1.58	4.63	25–30 (FAO, 2006)	Compliant
Turbidity (NTU)	9.03 ± 12.02	9.03 ± 12.02	9.03 ± 12.02	24.44	<25 (Boyd, 2003)	Compliant
COD (mg/L)	98.72 ± 19.90	98.72 ± 19.90	98.72 ± 19.90	53.00	<50 (WHO, 2023)	**Non-compliant**
BOD (mg/L)	6.65 ± 4.08	6.65 ± 4.08	6.65 ± 4.08	9.20	3–20 (FAO, 2006)	Compliant

Note: Values represent mean ± standard deviation; Range represents the maximum difference observed within each parameter across all farms.

Temporal Variation in Water Quality Parameters

Repeated Measures ANOVA showed no significant temporal variation in pH, DO, conductivity, TDS, salinity, temperature, turbidity, COD, and BOD across the five-week study period (Table 2). This indicates that these water quality parameters remained relatively stable over time for all three farms.

Table 2. Repeated Measures ANOVA Results for Water Quality Parameters (5-weeks).

Parameter	F-value	p-value	Interpretation
pH	0.2500	0.9018	No significant variation across weeks
DO	-2.0000	1.0000	No significant variation across weeks
Conductivity	1.1246	0.4218	No significant variation across weeks
TDS	-2.0000	1.0000	No significant variation across weeks
Salinity	0.9540	0.4762	No significant variation across weeks
Temperature	2.1278	0.1687	No significant variation across weeks
Turbidity	1.5642	0.2835	No significant variation across weeks
COD	1.2475	0.3752	No significant variation across weeks
BOD	0.1651	0.9502	No significant variation across weeks

Bacterial Populations and Diversity across Fish Farms

Bacteriological Identification

Table 3 shows the morphological and biochemical characteristics of thirty bacterial isolates recovered from fish farm water samples during a five-week sampling period. The isolates were designated A1-A6, B1-B6, C1-C6, D1-D6, and E1-E6 corresponding to weeks 1, 2, 3, 4, and 5 respectively. Each isolate was characterized using Gram staining for cell morphology and gram reaction, followed by standard biochemical tests comprising coagulase, catalase, indole, methyl red, Voges-Proskauer, citrate utilization and sugar fermentation tests for lactose, glucose and sucrose. The results obtained were used to identify the probable bacterial species present in the water samples. The identified organisms included *Escherichia coli, Staphylococcus sp., Shigella sp., Campylobacter sp., Proteus sp., Enterococcus sp., Salmonella sp., Enterobacter sp., and Bacillus sp.*

Table 3. Morphological and Biochemical Characteristics of Bacterial Isolates from Fish Farm Water Samples (Weeks 1–5).

Sample	Gram Reaction	Coagulas	Catalase	Indole	Methyl Red	VP	Citrate	Lactose	Glucose	Sucrose	Probable Organism
A1	- Rod	-	-	-	-	-	-	-	-	-	*Escherichia coli*
A2	- Cocci/Rod	-	-	-	-	-	-	-	-	-	*Campylobacter spp.*
A3	- Rod	-	-	-	-	-	-	-	-	-	*Shigella spp.*
A4	+ Rod	-	-	+	+	-	-	+	+	+	*Shigella spp.*
A5	+Cocci/Rod	-	-	+	+	-	-	+	+	+	*Escherichia coli*
A6	- Cocci/Rod	-	-	+	+	-	-	+	+	+	*Escherichia coli*
B1	- Cocci/Rod	-	-	+	+	-	-	+	+	+	*Escherichia coli*
B2	+Rod/Cocci	+	+	+	+	+	+	+	+	+	*Staphylococcus spp.*
B3	- Rod	-	-	+	+	-	-	+	+	+	*Escherichia coli*
B4	- Spiral/Rod	-	-	+	+	-	-	+	+	+	*Proleus spp.*
B5	- Cocci/Rod	-	-	+	+	-	-	+	+	+	*Escherichia coli*
B6	- Rod	-	-	+	+	-	-	+	+	+	*Escherichia coli*
C1	- Cocci/Rod	+	+	+	+	+	+	+	+	+	*Staphylococcus spp.*
C2	+Rod/Cocci	-	-	+	+	-	-	+	+	+	*Escherichia coli*
C3	- Rod	-	+	-	-	+	-	+	+	+	*Enterococcus spp.*
C4	- Spiral/Rod	-	+	-	+	-	+	+	+	+	*Salmonella spp.*
C5	- Cocci/Rod	-	-	+	+	-	-	+	+	+	*Escherichia coli*
C6	- Rod	-	-	+	+	-	-	+	+	+	*Escherichia coli*
D1	+ Cocci	+	+	+	+	+	+	+	+	+	*Staphylococcus spp.*
D2	- Rod	-	-	+	+	-	-	+	+	+	*Escherichia coli*
D3	- Rod	-	-	+	+	-	-	+	+	+	*Escherichia coli*
D4	- Rod	-	-	+	+	-	-	+	+	+	*Escherichia coli*
D5	- Rod	-	-	+	+	-	-	+	+	+	*Shigella spp.*
D6	- Rod	-	-	+	+	-	-	+	+	+	*Enterobacter spp.*
E1	+ Rod	-	-	-	-	-	-	-	-	-	*Bacillus spp.*
E2	- Cocci/Rod	-	-	-	-	-	-	-	-	-	*Escherichia coli*
E3	- Cocci/Rod	-	-	-	-	-	-	-	-	-	*Escherichia coli*
E4	- Cocci/Rod	-	-	-	-	-	-	-	-	-	*Escherichia coli*
E5	- Rod	-	-	-	-	-	-	-	-	-	*Escherichia coli*
E6	- Cocci/Rod	-	-	-	-	-	-	-	-	-	*Escherichia coli*

Distribution and Prevalence of Bacterial Species

Table 4 shows the prevalence of bacterial species recovered from fish farm water samples during the five-week study period. Nine different bacterial species were identified from the thirty isolates examined. *Escherichia coli* was identified as the predominant organism, comprising 18 isolates (60.0% of total isolates). *Staphylococcus sp.* and *Shigella sp*. were the second most prevalent organisms, with 3 isolates each (10.0% respectively). The remaining bacterial species—*Campylobacter sp., Proteus sp., Enterococcus sp., Salmonella sp., Enterobacter sp.,* and *Bacillus sp*. were each isolated once, representing 3.3% each of the total isolates recovered from the study locations.

Table 4. Prevalence of Bacterial Species Isolated from Fish Farm Water Samples (Weeks 1-5).

Bacterial Species	Frequency of Isolates (n)	Prevalence (%)
Escherichia coli	18	60.0
Staphylococcus sp.	3	10.0
Shigella sp.	3	10.0
Campylobacter sp.	1	3.3
Proteus sp.	1	3.3
Enterococcus sp.	1	3.3
Salmonella sp.	1	3.3
Enterobacter sp.	1	3.3
Bacillus sp.	1	3.3
Total	**30**	**100.0**

Additionally, molecular identification using 16S rRNA gene sequencing revealed distinct bacterial species across different water samples (Figure 1). Sample A4 from fish farm outlet water contained *Shigella sp*. with a 98.45% identity (NCBI: MH123456.1), sample B4 from fish farm inlet water identified Proteus sp. with a 97.83% identity (NCBI: KX987654.1), and sample C4 from fish farm outlet water showed *Salmonella sp*. with a 96.72% identity (NCBI: JQ456789.1). The molecular identification results confirmed the bacterial species previously identified through conventional biochemical methods. The corresponding BLAST results are presented in Table 5.

Table 5. BLAST Results for Organisms Identified in Water Samples from Fish Farms.

Sample Code	Fish Farm Source	Accession Number (NCBI)	Scientific Name of Organism	Percentage Identity (%)
A4	Fish farm (outlet wastewater)	MH123456.1	*Shigella sp.*	98.45
B4	Fish farm (inlet water)	KX987654.1	*Proteus sp.*	97.83
C4	Fish farm (outlet wastewater)	JQ456789.1	*Salmonella sp*.	96.72

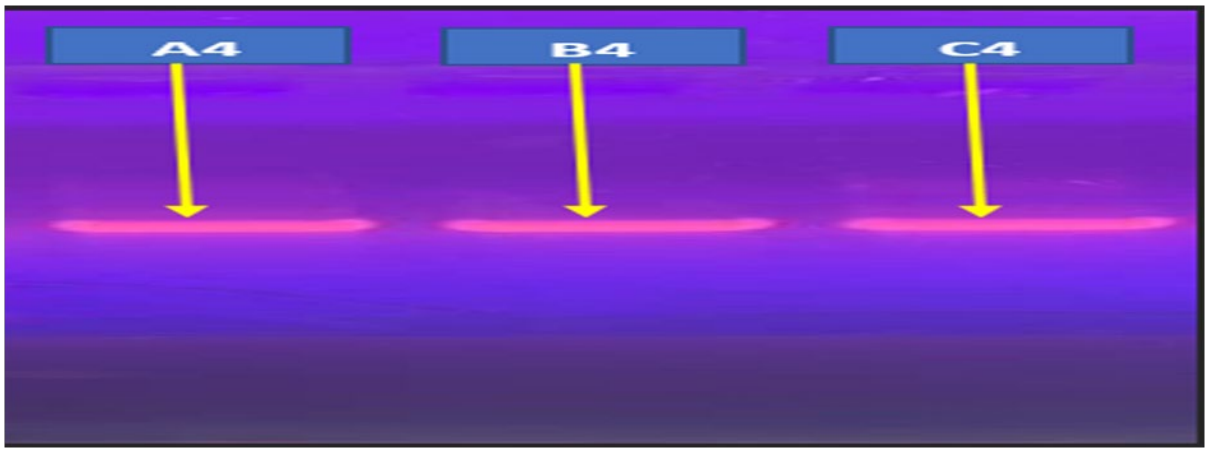

Fig. 2. Gel Electrophoresis of 16S rRNA Sequences from Water Samples.

Note; where A4 = A fish farm outlet wastewater, B4 = C fish farm inlet water, C4 = C fish farm outlet wastewater

Table 6. Comparison of Bacterial Species Prevalence in Water Samples from Three Fish Farms (Weeks 1-5).

Bacterial Species	Fish Farm 1		Fish Farm 2		Fish Farm 3		Total	
	Number of Isolates	Percentage (%)	Number of Isolates	Percentage (%)	Number of Isolates	Percentage (%)	Number of Isolates	Percentage (%)
Escherichia coli	5	50.0	7	70.0	6	60.0	18	60.0
Staphylococcus sp.	2	20.0	1	10.0	0	0.0	3	10.0
Shigella sp.	1	10.0	0	0.0	2	20.0	3	10.0
Campylobacter sp.	0	0.0	1	10.0	0	0.0	1	3.3
Proteus sp.	0	0.0	1	10.0	0	0.0	1	3.3
Enterococcus sp.	0	0.0	1	10.0	0	0.0	1	3.3
Salmonella sp.	0	0.0	1	10.0	0	0.0	1	3.3
Enterobacter sp.	0	0.0	0	0.0	1	10.0	1	3.3
Bacillus sp.	1	10.0	0	0.0	0	0.0	1	3.3

Antibiotic Susceptibility Profiles

Antibiotic susceptibility testing revealed that all identified bacterial species were sensitive to all ten antibiotics tested: Gentamicin, Amoxil, Cefuroxime, Rifampicin, Erythromycin, Ceftazidime, Streptomycin, Levofloxacin, Ciproflaxicin, and Azithromycin (Table 7).

Table 7. Antibiotic Susceptibility of Pathogens across V, A, and C Fish Farms.

Antibiotic	*E. coli* (Susceptible)	*Salmonella sp*. (Susceptible)	*Shigella sp* (Susceptible)
Gentamicin	+	+	+
Amoxil	+	+	+
Cefuroxime	+	+	+
Rifampicin	+	+	+
Erythromycin	+	+	+
Ceftazidime	+	+	+
Streptomycin	+	+	+
Levofloxacin	+	+	+
Ciproflaxicin	+	+	+
Azithromycin	+	+	+

key: + = positive to susceptibility test

Levels and Distributions of Heavy Metal Contamination

Table 8 shows that heavy metal concentrations varied significantly across the three fish farms (Table 7). The three highest recorded concentrations of heavy metals across the fish farms were magnesium (Mg) in Farm C at 5.33 ± 2.43 mg/L, lead (Pb) in Farm V at 0.32 ± 3.98 mg/L, and chromium (Cr) in Farm V at 0.64 ± 1.15 mg/L (Table 7). Magnesium concentrations across Farms A and V were 3.83 ± 1.41 mg/L and 3.01 ± 0.61 mg/L, respectively. Lead concentrations were 0.22 ± 2.57 mg/L in Farm A and 0.19 ± 2.89 mg/L in Farm C. Chromium values in Farms A and C were 0.51 ± 0.63 mg/L and 0.23 ± 0.81 mg/L, respectively.

Manganese (Mn) concentrations ranged from 0.18 ± 0.12 mg/L in Farm A to 0.24 ± 0.25 mg/L in Farm C. Zinc (Zn) levels were highest in Farm V at 0.28 ± 0.21 mg/L and lowest in Farm A at 0.20 ± 0.11 mg/L. Nickel (Ni) concentrations remained relatively consistent across all farms, ranging from 0.13 ± 0.11 mg/L to 0.16 ± 0.14 mg/L. Cadmium (Cd) was 0.10 ± 0.05 mg/L in Farm V and 0.12 ± 0.12 mg/L in both Farms A and C.

Table 8. Heavy Metal Concentrations across V, A, and C Fish Farms.

Heavy Metal	**V Fish Farm (Mean ± SD)**	**A Fish Farm (Mean ± SD)**	**C Fish Farm (Mean ± SD)**
Mn	0.19 ± 0.15 mg/L	0.18 ± 0.12 mg/L	0.24 ± 0.25 mg/L
Zn	0.28 ± 0.21 mg/L	0.20 ± 0.11 mg/L	0.27 ± 0.18 mg/L
Mg	3.01 ± 0.61 mg/L	3.83 ± 1.41 mg/L	5.33 ± 2.43 mg/L
Ni	0.16 ± 0.14 mg/L	0.13 ± 0.11 mg/L	0.15 ± 0.16 mg/L
Cd	0.10 ± 0.05 mg/L	0.12 ± 0.12 mg/L	0.12 ± 0.12 mg/L
Cr	0.64 ± 1.15 mg/L	0.51 ± 0.63 mg/L	0.23 ± 0.81 mg/L
Pb	0.32 ± 3.98 mg/L	0.22 ± 2.57 mg/L	0.19 ± 2.89 mg/L

*Values are in the mean ± standard deviation (SD) of heavy metal concentrations.

Compliance with Regulatory Standards of Water Quality

The comparative analysis of water quality parameters revealed consistent patterns across all three farms (Table 9). Critical water quality issues included severely deficient dissolved oxygen (DO) levels (0.18 ± 0.07 mg/L), consistently acidic pH values (6.4 ± 0.41), and elevated Chemical Oxygen Demand (COD) measurements (98.72 ± 19.90 mg/L). The pH values observed were slightly below the recommended range for aquaculture, while DO levels were significantly below the minimum requirement for optimal fish health. In contrast, parameters such as Biochemical Oxygen Demand (BOD) (6.65 ± 4.08 mg/L), water temperature (28.14 ± 1.58°C), and Total Dissolved Solids (TDS) (0.09 ± 0.03 mg/L) remained within acceptable ranges for aquaculture operations.

While the three farms showed similarities in several water quality parameters, site-specific characteristics were evident. Farm C exhibited the highest concentrations of Mn (0.24 ± 0.25 mg/L) and Mg (5.33 ± 2.43 mg/L), as well as the most significant bacterial growth fluctuations. Farm V showed higher levels of Cr (0.64 ± 1.15 mg/L) and Pb (0.32 ± 3.98 mg/L) compared to the other farms. Farm A maintained intermediate levels of most heavy metals and exhibited the most stable bacterial growth pattern over the study period.

Table 9. Merged Paired t-test Results for Water Quality Compliance across V, A, and C Fish Farms.

Parameter	V Farm	A Farm	C Farm	Range	Regulatory Standard	Compliance
Ph	6.4 ± 0.41	6.4 ± 0.41	6.4 ± 0.41	0.98	6.6–8.5 (FAO, 2006)	Non-compliant
DO (mg/L)	0.18 ± 0.07	0.18 ± 0.07	0.18 ± 0.07	0.17	>5.0 (WHO, 2023)	Non-compliant
Conductivity (μS/cm)	189.67 ± 69.21	189.67 ± 69.21	189.67 ± 69.21	175.00	100-2000 (Boyd, 2003)	Compliant
TDS (mg/L)	0.09 ± 0.03	0.09 ± 0.03	0.09 ± 0.03	0.09	≤0.13 (Davis, 1993)	Compliant
Salinity (ppt)	0.10 ± 0.02	0.10 ± 0.02	0.10 ± 0.02	0.05	0-5 (FAO, 2006)	Compliant
Temperature (°C)	28.14 ± 1.58	28.14 ± 1.58	28.14 ± 1.58	4.63	25–30 (FAO, 2006)	Compliant
Turbidity (NTU)	9.03 ± 12.02	9.03 ± 12.02	9.03 ± 12.02	24.44	<25 (Boyd, 2003)	Compliant
COD (mg/L)	98.72 ± 19.90	98.72 ± 19.90	98.72 ± 19.90	53.00	<50 (WHO, 2023)	Non-compliant
BOD (mg/L)	6.65 ± 4.08	6.65 ± 4.08	6.65 ± 4.08	9.20	3–20 (FAO, 2006)	Compliant

Note: Values represent mean ± standard deviation; Range represents the maximum difference observed within each parameter.

Discussion

Physicochemical Properties of Water in Selected Fish Farms in Abuja

The study revealed consistent physicochemical parameters across the three fish farms investigated, with critical compliance issues identified when compared against established regulatory standards. The water pH averaged 6.4 ± 0.41, indicating slightly acidic conditions that fall below the recommended range (6.6-8.5) for optimal aquaculture as established by (31). According to (34), water quality parameters directly influence key physiological functions in fish including feeding, swimming, metabolism, and excretion. The slightly acidic conditions observed in this study could potentially stress fish populations and affect their growth and survival rates, as noted by (19) who found that deviations from optimal pH ranges impose physiological stress on fish.

A major point of concern was the critically low concentration of dissolved oxygen (DO), recorded at 0.18 ± 0.07 mg/L, which falls far below the (30) recommended threshold of >5.0 mg/L. Such low DO levels represent a critical limitation for aquatic life and could severely impact fish health, metabolism, and survival. This deficiency likely poses the most immediate threat to the sustainability of these farming operations. (25) emphasized that maintaining optimal dissolved oxygen levels is vital for sustaining healthy aquatic environments and preventing stress-induced diseases.

While some parameters were within acceptable ranges, including conductivity (189.67 ± 69.21 µS/cm), TDS (0.09 ± 0.03 mg/L), salinity (0.10 ± 0.02 mg/L), temperature (28.14 ± 1.58°C), turbidity (9.03 ± 12.02 NTU), and BOD (6.65 ± 4.08 mg/L), the Chemical Oxygen Demand (COD) values (98.72 ± 19.90 mg/L) were nearly double the recommended limit (<50 mg/L) established by (30). This elevated COD indicates high organic pollution levels that could further deplete the already critical oxygen levels. Similar findings were reported by (29) who observed significant variations between inlet and outlet water quality in rainbow trout farms, largely due to farm effluents.

When compared against established regulatory standards, all three farms failed to comply with requirements for pH (6.6–8.5, (31), DO (>5.0 mg/L, (30), and COD (<50 mg/L). In contrast, parameters such as conductivity (100-2000 µS/cm), TDS (≤0.13 mg/L), salinity (0-5 ppt), temperature (25–30°C), turbidity (<25 NTU), and BOD (3–20 mg/L) remained within acceptable ranges for aquaculture operations, indicating compliance with relevant standards.

These non-compliant parameters represent significant limiting factors for sustainable aquaculture operations and highlight critical areas for improvement in water quality management. (28) emphasized the importance of maintaining pH levels within 6.6 - 8.5 for saline water and 6.0 - 9.0 for freshwater to ensure a stable environment for fish health. (15) emphasized that monitoring water quality parameters like temperature and turbidity is crucial because even small fluctuations can stress aquatic organisms, making them more vulnerable to diseases.

The absence of significant temporal variation in these parameters over the five-week study period, as confirmed by Repeated Measures ANOVA, suggests stable but persistently suboptimal water quality conditions that require immediate management intervention. This aligns with observations by (72) who noted that many farmers, particularly those using pond systems, lack adequate knowledge of required water quality standards, resulting in suboptimal yields and increased fish mortality.

Bacterial Populations and Diversity across Fish Farms

The bacteriological analysis revealed nine different bacterial species from thirty isolates examined across the three fish farms. *Escherichia coli* was identified as the predominant organism, comprising 18 isolates (60.0% of total isolates). *Staphylococcus sp.* and *Shigella sp*. were the second most prevalent organisms, with 3 isolates each (10.0% respectively). The remaining bacterial species-*Campylobacter sp., Proteus sp., Enterococcus sp., Salmonella sp., Enterobacter sp.,* and *Bacillus sp*. were each isolated once, representing 3.3% each of the total isolates recovered from the study locations.

The presence of *E. coli* indicates potential fecal contamination that presents both environmental and public health concerns. This finding aligns with research by (23) who identified aquaculture systems as potential reservoirs for pathogenic bacteria. The distribution across farms showed that Farm 2 had the highest *E. coli* prevalence at 70.0%, followed by Farm 3 at 60.0% and Farm 1 at 50.0%.

Molecular identification through 16S rRNA gene sequencing confirmed the presence of three key pathogenic species: *Shigella* sp. (98.45% identity) in fish farm outlet wastewater, *Proteus* sp. (97.83% identity) in fish farm inlet water, and *Salmonella* sp. (96.72% identity) in fish farm outlet wastewater. The molecular identification results confirmed the bacterial species previously identified through conventional biochemical methods.

Notably, all identified bacterial species demonstrated sensitivity to all ten antibiotics tested, including Gentamicin, Amoxil, Cefuroxime, Rifampicin, Erythromycin, Ceftazidime, Streptomycin, Levofloxacin, Ciproflaxicin, and Azithromycin. This antibiotic susceptibility represents a positive finding amid concerns about antimicrobial resistance in aquaculture settings. This contrasts with findings by (43) who found significant antimicrobial resistance in bacteria isolated from Thailand's coastal aquaculture regions, including *E. coli* and *Salmonella enterica*. Similarly, (9) detected bacteria with plasmid-mediated quinolone resistance genes in 21% of samples from rainbow trout farms in Portugal.

The presence of *Salmonella* species across the farms (3.3% prevalence) aligns with findings by (65) who reported frequent detection of *Salmonella* in culture water, muscle, and intestinal samples in their systematic review of global *Salmonella* occurrence in aquaculture. As highlighted by (59), the consumption of contaminated fish poses serious public health risks, particularly when raw or undercooked seafood can transmit *E. coli*, *Salmonella*, and other infections to humans.

Levels and Distributions of Heavy Metal Contamination

Heavy metal analysis revealed varying concentrations across the three fish farms (Table 8). The three highest recorded concentrations of heavy metals across the fish farms were magnesium (Mg) in Farm C at 5.33 ± 2.43 mg/L, lead (Pb) in Farm V at 0.32 ± 3.98 mg/L, and chromium (Cr) in Farm V at 0.64 ± 1.15 mg/L. According to (35) and (77), heavy metals are persistent pollutants in aquatic environments due to their non-biodegradable nature, allowing them to accumulate in water bodies and aquatic organisms.

Farm C exhibited the highest concentrations of Mn (0.24 ± 0.25 mg/L) and Mg (5.33 ± 2.43 mg/L), while Farm V showed elevated levels of Chromium (Cr) (0.64 ± 1.15 mg/L) and Lead (Pb) (0.32 ± 3.98 mg/L). Farm A maintained intermediate levels with Mg at 3.83 ± 1.41 mg/L, Cr at 0.51 ± 0.63 mg/L, and Pb at 0.22 ± 2.57 mg/L. These site-specific heavy metal profiles suggest different contamination sources or water management practices across the farms. As noted by (40), the primary sources of heavy metal contamination in aquaculture systems include industrial discharges, agricultural runoff, domestic wastewater, and atmospheric deposition.

Manganese (Mn) concentrations ranged from 0.18 ± 0.12 mg/L in Farm A to 0.24 ± 0.25 mg/L in Farm C. Zinc (Zn) levels were highest in Farm V at 0.28 ± 0.21 mg/L and lowest in Farm A at 0.20 ± 0.11 mg/L. Nickel (Ni) concentrations remained relatively consistent across all farms, ranging from 0.13 ± 0.11 mg/L to 0.16 ± 0.14 mg/L. Cadmium (Cd) was similar across farms, with 0.10 ± 0.05 mg/L in Farm V and 0.12 ± 0.12 mg/L in both Farms A and C.

The presence of toxic metals like Lead (Pb) and Cadmium (Cd) in all three farms raises concerns about potential bioaccumulation in fish tissues and subsequent human health risks through consumption. (39) highlighted that lead exposure from contaminated seafood has been associated with developmental disorders, neurological dysfunction, and reduced cognitive abilities. Similarly, (33) linked long-term exposure to multiple heavy metals with chronic diseases such as Alzheimer's, Parkinson's, and multiple sclerosis.

The detection of chromium (Cr) at Farm V (0.64 ± 1.15 mg/L) is particularly concerning as (39) reported that chromium can disrupt endocrine functions, causing hormonal imbalances that affect growth and reproductive capabilities in fish. Furthermore, (75) noted that once inside organisms, heavy metals accumulate in tissues such as the liver, kidneys, and muscles, often exceeding safe biological limits and causing physiological and biochemical disruptions.

Comparative Water Quality Profile of Fish Farms

Comparative analysis across the three farms revealed consistent patterns in water quality issues, with all farms showing identical values for most physicochemical parameters due to similar management practices and environmental conditions. The bacterial load analysis indicated the presence of pathogenic bacteria across all farms, with *E. coli* being the most prevalent organism.

Site-specific characteristics were evident in heavy metal concentrations and bacterial species distribution. Farm C showed the highest concentrations of Mn and Mg, while Farm V exhibited higher levels of potentially toxic metals (Cr and Pb). The bacterial species distribution varied across farms, with Farm 1 showing 50.0% *E. coli* prevalence, Farm 2 showing 70.0%, and Farm 3 showing 60.0%. Farm 2 also had the most diverse bacterial community with representatives of *Staphylococcus* sp., *Campylobacter* sp., *Proteus* sp., *Enterococcus* sp., and *Salmonella* sp., while Farm 3 showed presence of *Shigella* sp. and *Enterobacter* sp.

The consistent pattern of deficient dissolved oxygen, acidic pH, elevated COD, and bacterial contamination across all farms suggests systemic issues in urban aquaculture management that require comprehensive intervention strategies. As emphasized by (8) and (82), technological advancements in water quality monitoring and management could significantly improve aquaculture sustainability by ensuring optimal conditions for fish health while minimizing environmental impacts.

Conclusion

This study investigated water quality parameters across three fish farms in Abuja, examining physicochemical properties, bacterial populations, and heavy metal contamination. The findings reveal critical concerns for the sustainability of urban aquaculture operations in the region.

Water quality analysis showed severely deficient dissolved oxygen levels (0.18 ± 0.07 mg/L), consistently acidic pH values (6.4 ± 0.41), and elevated COD measurements (98.72 ± 19.90 mg/L) across all farms. These parameters fall below regulatory standards and impose physiological stress on fish, as noted by (19) and (25), leading to reduced growth rates and increased disease susceptibility.

Bacteriological analysis revealed predominance of E. coli (60.0% of total isolates) and pathogenic species including *Salmonella sp*. and *Shigella sp*., indicating fecal contamination and potential public health risks, consistent with observation by (67, 26) regarding the role of contaminated water sources, poor waste management, and unsanitary handling practices in bacterial proliferation..

Heavy metal analysis showed site-specific contamination with elevated levels of Mn, Mg, Cr, and Pb, with toxic metals like Pb and Cd present in all farms, suggesting bioaccumulation risks as highlighted by (47) and (69).

A positive finding was that all bacterial species demonstrated sensitivity to all ten antibiotics tested, indicating absence of antimicrobial resistance. However, the consistent pattern of deficient water quality parameters across all farms suggests systemic issues in urban aquaculture management requiring comprehensive intervention strategies. As emphasized by (8) and (82), technological advancements in water quality monitoring could significantly improve aquaculture sustainability while minimizing environmental impacts.

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RESEARCH

Optimising site selection for ecosystem approaches to shrimp aquaculture in mangrove systems

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Abstract
Indonesia has experienced significant mangrove loss due to aquaculture expansion, particularly for shrimp farming, leading to the degradation of habitats and critical ecosystem services such as carbon storage, coastal protection, and fish spawning grounds. Ecosystem based aquaculture approaches offer a pathway to both mitigate environmental impacts and support sustainable seafood production. Here we explore the implementation of the Shrimp-Carbon Aquaculture (SECURE) approach in Berau Regency, East Kalimantan, Indonesia, which integrates mangrove restoration with organic shrimp farming to achieve sustainable aquaculture. Using environmental DNA (eDNA) metabarcoding to detect species from DNA shed into the environment, we assessed impacts of SECURE intervention compared to traditional ponds and found increased abundance of key taxonomic groups associated with healthy aquatic ecosystems, such as phytoplankton Chaetoceros, Chlorophyceae, and Cryptomonadales and zooplankton Calanoida and Cyclopoida. We then conducted a spatial prioritisation analysis to identify additional areas for SECURE implementation, considering mangrove restoration and protection potential, profitability, and intervention costs. High-priority ponds for restoration were typically set back from riverbanks, large, and spatially clustered, indicating opportunities for cost-effective, strategic expansion. This study underscores how spatial prioritisation can support strategic implementation of aquaculture to balance ecosystem-based aquaculture development with environmental conservation, offering a replicable framework for other regions facing similar challenges. This approach provides a pathway for achieving long-term sustainability in aquaculture, contributing to global food security and ecological resilience.

Keywords Shrimp · Aquaculture · Mangrove · Prioritisation · Silvofisheries

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Introduction

Indonesia has lost around 40% of its mangrove cover since the mid-1980s, due to aquaculture development, deforestation, timber harvesting, mining, and land reclamation (Arifanti et al. 2022). Aquaculture has been the primary driver as mangroves are clear-cut to create space for fish and shrimp farming ponds (Godoy and De Lacerda 2015; Murdiyarso et al. 2015; Ashton 2022; Mohd Razali et al. 2022). As a consequence, important mangrove ecosystem services, including carbon storage, fish spawning grounds, and coastal protection, have deteriorated (Polidoro et al. 2014). The rate of mangrove deforestation has decreased in recent years to a third of its peak between 1980 and 2005, but is still twice as high as the overall rate of mangrove loss across Southeast Asia (Arifanti et al. 2021). In response, the Government of Indonesia pursued an ambitious programme of restoring 600,000 ha of mangroves between 2020 and 2024 (Sasmito et al. 2023). At the same time, the government has announced growth targets for most aquaculture species, including brackish water shrimp species, to meet growing national demands and global exports (Henriksson et al. 2019). Ecosystem approaches to aquaculture (i.e., implementation of aquaculture practices that balance environmental health, social equity, and economic viability within the wider ecosystem) provide an opportunity to balance trade-offs in such situations, outlining methods which can both protect mangroves and support farmers engage in sustainable aquaculture (Soto et al. 2008).

The Shrimp-Carbon Aquaculture approach (SECURE) developed by Yayasan Konservasi Alam Nusantara (YKAN) in Ogan Komering Ilir, South Sumatra, and Berau Regency, East Kalimantan, promotes sustainable aquaculture practices which mitigate negative environmental impacts. Mangroves are restored in approximately 60–80% of an active shrimp pond through hydrological restoration, planting of seedlings, and natural regeneration. The remaining area is used for organic shrimp farming free of external inputs such as artificial fertilisers and feed. SECURE also aims to prevent further pond expansion, thereby reducing carbon emissions from continued mangrove conversion, and to increase atmospheric carbon removal by restoring and reconnecting mangroves areas. In general, it aims to contribute to both climate mitigation and biodiversity recovery by demonstrating that aquaculture productivity and ecosystem restoration can coexist and reinforce each other within a carbon-positive framework.

SECURE shares conceptual similarities with silvofishery systems, in that both aim to integrate aquaculture production with mangrove conservation. However, unlike traditional silvofishery practices where mangroves and shrimp ponds coexist within the same physical compartment, the SECURE approach spatially separates the aquaculture and mangrove components into distinct but hydrologically connected zones, allowing each compartment to be optimised for its primary function. Farmers can benefit from using fewer resources while also gaining access to eco-certification and higher premium prices for their organic shrimp (Paul and Vogl 2012; Cong and Khanh 2022). The presence of low-density mangroves inside ponds increases shrimp production (Anggoro et al. 2025). Meanwhile the increase in regional mangrove cover through restoration provides a range of biodiversity, ecosystem, and community benefits (Sasmito et al. 2023). While a number of SECURE pilot sites have been started since 2020, the region would benefit from wider adoption of SECURE in additional mangrove shrimp farming ponds.

Compared to another prominent approach, the biofloc system (Khanjani and Sharifinia 2020), the SECURE system offers a nature-based alternative that integrates mangrove restoration to improve surrounding hydrology and water filtration. While biofloc systems

may achieve higher productivity, they are energy-intensive and costly to maintain. The SECURE approach, contrastingly, prioritises ecological balance and resilience, offering moderate productivity gains while enhancing ecosystem services and reducing environmental impact.

To expand implementation of SECURE across Berau, East Kalimantan, a spatial planning process can help ensure long-term, sustainable success of aquaculture ponds and mangrove restoration (Zavalloni et al. 2014; Petrosillo et al. 2023). Spatial planning is a holistic framework for managing marine and coastal resources that integrates ecological, social, economic, and institutional systems to ensure long-term sustainability (Domínguez-Tejo et al. 2016). To reduce further encroachment of intensive aquaculture farms into intact mangrove areas, spatial planning can identify how resources should be spatially allocated for effective and efficient restorative actions. As additional SECURE ponds are implemented in the region, we use spatial planning to identify which other existing ponds are priority candidates for SECURE conversion. In Berau, spatial planning involves systematic assessment, zoning, and regulation of land use to ensure sustainable development, prevent conflicts, and enhance resilience against climate change. Implementation of spatial planning is hampered by weak regulations, poor law enforcement, and conflicts with other land uses, such as forest zoning status (Rusdi et al. 2022). Many traditional ponds operate informally without proper zoning and are often located in protected zones, leading to environmental degradation, including biodiversity loss and reduced water catchment capacity. Additionally, limited government oversight and low awareness among farmers contribute to a lack of interest in implementing spatial planning, further worsening the condition of mangrove ecosystems in the area.

Here, we aim to outline the background and benefits of not only SECURE ponds, but to also create a spatial planning framework for ecosystem-based shrimp aquaculture. We performed a spatially explicit, biogeographic-economic prioritisation analysis to help inform the wider adoption of sustainable aquaculture practices in Berau regency, Kalimantan, Indonesia. First, we assessed the biodiversity benefits from existing SECURE pilot sites by measuring changes in species abundance before and after interventions. We surveyed biodiversity using environmental DNA metabarcoding, whereby species were detected from DNA shed into water and sediment samples. Next, we performed a spatial prioritisation analysis to rank existing shrimp farming ponds according to their suitability for implementing SECURE. The suitability of each pond was determined according to three criteria: (1) the capacity to restore new mangroves or protect existing mangroves, (2) the expected profitability from shrimp farming, and (3) the cost of implementing SECURE. The ranking indicates where resources may best be allocated to achieve the greatest return on both conservation and farming benefits.

Methods

We focus our work and data collection in Berau Regency, East Kalimantan, Indonesia. Berau is part of Borneo Island and has total area of 34,127 km2, consisting of 21,240 km2 of land and 12,887 km2 of water bodies. Berau Regency is home to the largest mangrove ecosystem in East Kalimantan, spanning 86,043 hectares. However, mangrove deforestation due to shrimp pond expansion is a growing concern. In 2019 alone, 11,237 hectares (13%) of mangroves were converted into traditional shrimp ponds, posing a serious threat to coastal ecosystems. Shrimp species farmed are *Panaeus monodon*,

Panaeus indicus, and *Litopenaeus vannamei*. One of the key areas of shrimp farming is Pegat Batumbuk Village, which features around 7000 hectares of shrimp ponds within a 20,000-hectare mangrove area. Shrimp pond sizes range from 5 to 25 hectares, but productivity remains low, averaging 36 kg/ha per cycle. This low yield often drives farmers to clear additional mangrove areas to maintain or improve their income. As of November 2023, there were 968 mangrove shrimp farming ponds across Berau Regency in East Kalimantan, Indonesia, covering 12,476 ha. The number of productive (i.e. actively engaged in shrimp production) and unproductive ponds was 724 and 244, respectively, with 14 pilot ponds implementing SECURE (Fig. 1).

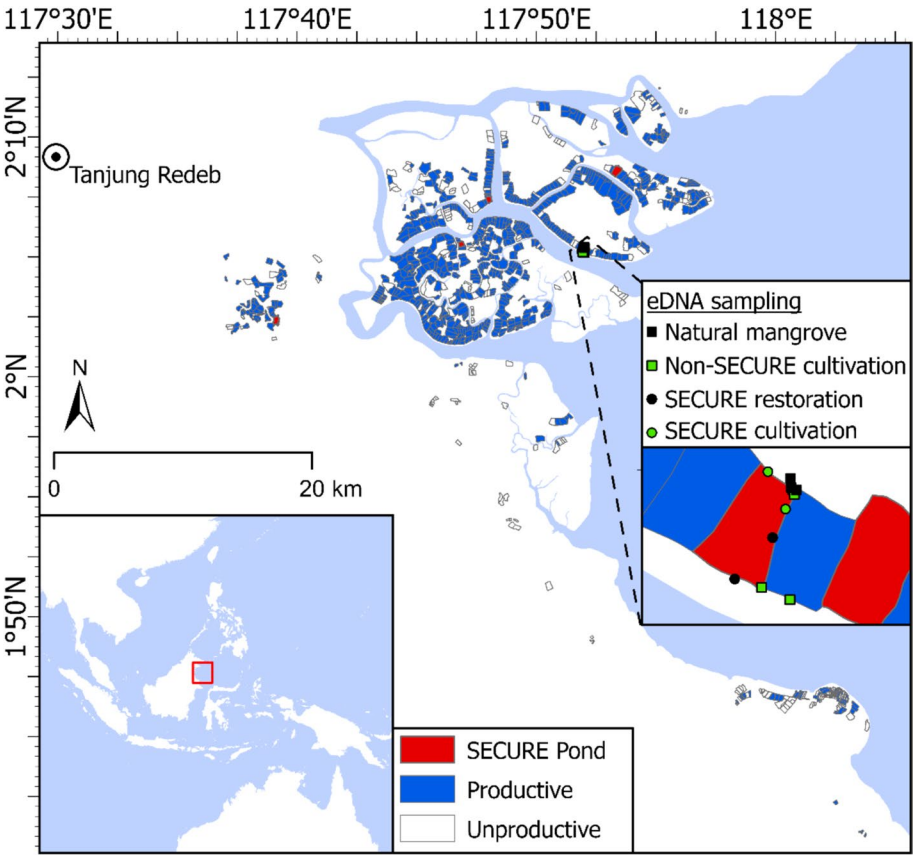

Fig. 1 Shrimp farming ponds in Berau Regency, Kalimantan, Indonesia, classified into productive ponds implementing SECURE (red), productive ponds not implementing SECURE (blue), and unproductive ponds (white). The nearest city of Tanjung Redeb is shown in the top-left. The inset on the left shows the locations where environmental DNA was sampled to assess change in species abundance before and after SECURE implementation. The SECURE pond contains two areas: restoration where mangroves are restored and cultivation where shrimp are reared. The non-SECURE pond contains only a cultivation area

Assessing biodiversity benefits

We employed environmental DNA (eDNA) metabarcoding via nanopore sequencing ONT MinION Mk1C (Oxford, UK) to identify species composition from water and sediment samples. eDNA metabarcoding is an approach for identifying species from the DNA they naturally release into the environment (Bohmann et al. 2014). We used the universal primer 18S to amplify the 18S rRNA gene in eukaryotic organisms (Hadziavdic et al. 2014). We took three sediment and water samples each across four habitat types both before and after SECURE implementation: the restoration area of a SECURE pond, the cultivation area of a SECURE pond, the cultivation area of a non-SECURE pond, and non-SECURE natural mangrove area (Fig. 1). We used the non-SECURE locations as a counterfactual to the SECURE locations to disentangle effects between the intervention and background effects. We chose one representative SECURE and non-SECURE pond each to control for environmental variability and to focus on temporal changes before and after SECURE implementation. Both ponds are influenced by similar tidal and salinity regimes but have independent water gates connected to separate drainage channels to prevent cross-contamination.

The baseline sampling before intervention was conducted in January 2023 to establish the pre-intervention eDNA profile before the construction of the SECURE pond. This baseline dataset served as a reference to assess changes in eDNA composition after the SECURE design was implemented. The sampling following intervention was carried out in October 2023 when the SECURE pond cultivation cycle had been running for 10 weeks. The SECURE pond cultivation cycle runs for approximately 3–4 months, and sampling was conducted at week 10 to represent the biologically stable mid-culture phase, which corresponds to periods of maximum biological activity and ecological equilibrium in shrimp aquaculture systems (Astutik et al. 2025; Chainark et al. 2025; Zhao et al. 2025). Sampling closer to harvest was avoided to minimise potential bias from water quality deterioration, organic load accumulation, and community shifts, conditions that are frequently observed in intensive shrimp pond cycles (Astutik et al. 2025; Chainark et al. 2025). Similar sampling design has been widely used in eDNA diversity studies for coastal and aquaculture environments to capture within-site variation while representing the main ecological gradients across the study area (Thomsen et al. 2012; Deiner et al. 2017).

Molecular Analysis

Metagenomic DNA was isolated using DNeasy PowerWater Kit (for water samples) and DNeasy PowerSoil Pro Kit (for sediment samples) (Qiagen, Hilden, Germany), following the manufacturer's instructions. Eukaryota in the water were captured by filtering 1 L using a sterile 0.22 µm vacuum filtration system (Merck Millipore, Massachusetts, USA). After genomic DNA was obtained from eDNA samples, PCR amplification was conducted to obtain a specific DNA locus target 18S (Hadziavdic et al. 2014). The amplification process was carried out for 25 cycles consisting of melting at 95 °C for 30 s, annealing at 44 °C for 30 s, and extension at 72 °C for 1 min, followed by a 10 min final extension at 72 °C. The PCR amplicon product was sequenced using the Oxford Nanopore Technology (ONT) MinION sequencing template. The DNA library was prepared following the manufacturer's protocols for Native Barcoding Kit 24 V14 (SQK-NBD114.24). Sequencing was done using the R10.4.1 flow cell (FLO-MIN114; Oxford Nanopore Technologies) for a total of 24 h per run.

Bioinformatics Analysis

NanoPlot v1.42.0 (https://github.com/wdecoster/NanoPlot) was used to assess the quality of reads obtained from nanopore sequencing (De Coster and Rademakers 2023). The bioinformatics workflow proceeded with the raw FastQ files, which served as input for downstream analyses. Taxonomic profiling was primarily conducted using Kraken2 v2.1.3 (https://github.com/DerrickWood/kraken2), a fast and accurate tool for assigning taxonomic labels to metagenomic sequences, utilising a reference database derived from SILVA (Wood et al. 2019). To refine taxonomic abundance estimates at specific ranks, Bracken v2.9 (https://github.com/jenniferlu717/Bracken) was applied to the Kraken2 output (Lu et al. 2017). Krona Tools v2.8.1 (https://github.com/marbl/Krona) was then employed to visualise the taxonomic profiles in the form of interactive pie charts (Ondov et al. 2011). Lastly, the MicroEco package in R (https://chiliubio.github.io/microeco/) was utilised to perform comprehensive microbiome analysis through efficient and integrative data mining techniques (Liu et al. 2021).

Operational Taxonomic Units (OTUs), the clusters of closely related organisms identified by their DNA, were matched to known databases and assigned to genus-level where possible or the next lowest known taxonomic rank. We rarefied OTU tables to the same minimum number of counts across sites and years to standardise comparisons. The expected benefits of SECURE intervention were quantified as the change in relative abundance of taxonomic groups.

Spatial planning for priority ranking

We assessed the suitability of productive ponds for SECURE implementation using spatial prioritisation, a biogeographic-economic analysis using quantitative criteria and objectives to aid decision-making (Moilanen et al. 2009; Kukkala and Moilanen 2013; Hanson et al. 2025). Spatial prioritisation provides a transparent, flexible framework that allows users to explore trade-offs using spatially explicit information. Ponds were ranked by prioritisation based on the criteria to minimise implementation costs and maximise the following desirable features: shrimp production profitability, mangrove restoration potential, and mangrove protection. Features and costs were established by expert consultation with Yayasan Konservasi Alam Nusantara based on prior experiences in developing sustainable shrimp aquaculture. Priority or highest rank was given to ponds with high shrimp production profitability and either high potential for mangrove restoration or a large existing mangrove area, yielding high economic and environmental benefits. The cost component represented the financial investment required to implement SECURE, ensuring an efficient allocation of resources.

Restoration potential and protection of mangroves

We used a 30 m resolution remote sensing habitat map of mangroves (Prakoso et al. 2023) to determine the mangrove cover inside each pond. We assigned a high suitability ranking for SECURE to two types of ponds. The first consisted of ponds with low existing mangrove cover which have a high potential for mangrove restoration (Fig. S1). The second consisted of ponds with high existing mangrove cover which have a high potential for mangrove protection (Fig. S2). For each pond i having $< 60\%$ mangrove cover, we calculated

the total potential area of mangrove restoration (R_i) as 60% of the pond size (A_i) minus any area of existing mangrove in hectares (M_i). For each pond i having ≥ 60% mangrove cover, we calculated the area of existing mangrove within that pond in hectares (M_i).

The restoration to 60% mangrove cover was determined based on site-specific conditions and agreements with pond owners. The SECURE model is currently implemented as a prototype approach, intended to explore how different configurations of aquaculture and mangrove restoration can co-exist within the same management unit. This percentage was derived from practical considerations developed jointly with local farmers, balancing the need to maintain shrimp production for economic viability, the restoration of hydrological and ecological functions to enhance biodiversity, and the improvement of blue carbon storage within the pond landscape. The 60% configuration thus represents a context-specific compromise reflecting pond-owner willingness, land suitability, and the restoration objectives of the SECURE design.

Profitability of shrimp production

The expected profitability of each shrimp pond (S_i) was determined by a pond's Euclidean distance to the nearest village (V_i), shared perimeter length with a 100 m buffer zone of the river (B_i), and pond size (A_i) (Fig. S3). Ponds closer to the village are more profitable due to shorter transportation distances and greater ease of pond maintenance. Ponds sharing shorter boundaries with the river are more profitable as they require less operating maintenance to repair damages from river pressure. Larger ponds are more profitable as they can stock more shrimp. We rescaled each of the three profitability parameters to range between 0.01 and 1 and multiplied them together into a single profitability metric for each pond (Fig. S3).

Cost of intervention

The cost of establishing a SECURE pond i (C_i) was determined by a pond's shared perimeter length with a 100 m buffer zone of the river (B_i), Euclidean distance to the main city of Tanjung Redeb (D_i), and proportion of mangrove (P_i) (Fig. S4). Ponds sharing longer boundaries with the river are more costly as they require greater construction of protective levees between the pond and the river when establishing SECURE. Ponds further from the city are more costly as construction materials are transported over longer distances. Ponds with lower mangrove cover are more costly as more labour and materials are required to restore mangroves. Ponds with ≥ 60% mangrove cover were assigned identical mangrove cover costs, as no additional mangrove restoration efforts are needed if a pond is already exceeding the minimum mangrove cover threshold. We rescaled each of the three cost parameters to range between 0.01 and 1 and multiplied them together into a single cost metric for each pond (Fig. S4).

Prioritisation analysis

We used spatial prioritisation to rank productive ponds which have not yet implemented SECURE (Table 1). We set a range of targets for the three features to be maximised, shrimp production profitability, mangrove restoration potential, and mangrove protection, from 10 to 100% by increments of 10%, with the objective of minimising overall cost. The most suitable ponds with highest priority for SECURE implementation are selected when

Table 1 Summary of prioritisation analysis used to rank productive shrimp ponds for SECURE implementation

Step	Description	Variables	Computation	Output
1. Define planning units	Identify productive shrimp ponds not yet implementing SECURE	Pond spatial polygons	Ponds divided into northern and southern clusters by 1° 48′ N	Planning units
2. Select features to maximise	Maximise three desirable criteria while minimising cost	(i) Shrimp production profitability	Each feature scaled 0.01–1	Feature layers for analysis
		(ii) Mangrove restoration potential		
		(iii) Mangrove protection		
3. Define cost layer	Represent financial investment required for SECURE implementation	(i) Shared perimeter with river buffer	Each variable rescaled 0.01–1; combined multiplicatively	Cost surface
		(ii) Distance to city		
		(iii) Proportion of mangrove cover		
4. Calculate restoration potential (< 60% mangrove cover)	Identify ponds suitable for mangrove restoration	Pond size and existing mangrove area	Difference between existing mangrove and 60% of pond area	Restoration potential feature layer
5. Calculate protection potential (≥ 60% mangrove cover)	Identify ponds suitable for mangrove protection	Existing mangrove area	Existing mangrove area as is	Protection potential feature layer
6. Compute profitability	Estimate expected shrimp farming profitability	(i) Distance to nearest village	Each variable rescaled 0.01–1; combined multiplicatively	Profitability feature layer
		(ii) Shared river boundary		
		(iii) Pond size		
7. Set targets	Define percentage of feature to achieve	Target levels from 10%–100% (10% increments)	Calculate percentage increments of total	Targets
8. Run prioritisation model	Identify ponds that minimise cost while achieving feature targets	Features + cost layers	Performed using prioritizr package (Hanson et al. 2025)	Ranked ponds by suitability for SECURE implementation

targets are 10%. We did not assign rankings to unproductive ponds, as there is a high financial cost involved in recommissioning inactive ponds. We divided the planning region into two clusters of northern and southern villages and ran separate spatial prioritisations on ponds located above or below 1° 48′ N latitude. We performed this split to account for a large ~ 15 km gap between the two clusters of ponds and to create a more equitable ranking of ponds across villages in the south which are further away from the city. We ran the prioritisation analysis using the package *prioritizr* (Hanson et al. 2025) in R v4.3.1 (R Core Team 2023).

Results

Biodiversity benefits from SECURE implementation

eDNA sequencing revealed 4291 OTUs across all sites both before and after SECURE implementation in Pegat Batumbuk. The percentage of OTUs assigned to phylum, class, order, family, and genus were 100%, 97%, 93%, 83%, and 75%, respectively. The taxonomic groups which increased the most as a result of SECURE implementation in restoration ponds (Fig. 2A) belonged predominantly to the phyla of Ascomycota (26 OTU groups), Chlorophyta (15 OTU groups), Cercozoa (11 OTU groups), Bacillariophyta (10 OTU groups), and Ciliophora (5 OTU groups). In cultivation ponds (Fig. 2B), these were instead Ascomycota (8 OTU groups), Chlorophyta (7 OTU groups), Cercozoa (5 OTU groups), Ciliophora (4 OTU groups), and Bacillariophyta (3 OTU groups). However, despite similar phyla increasing in abundance, there was a weak negative correlation

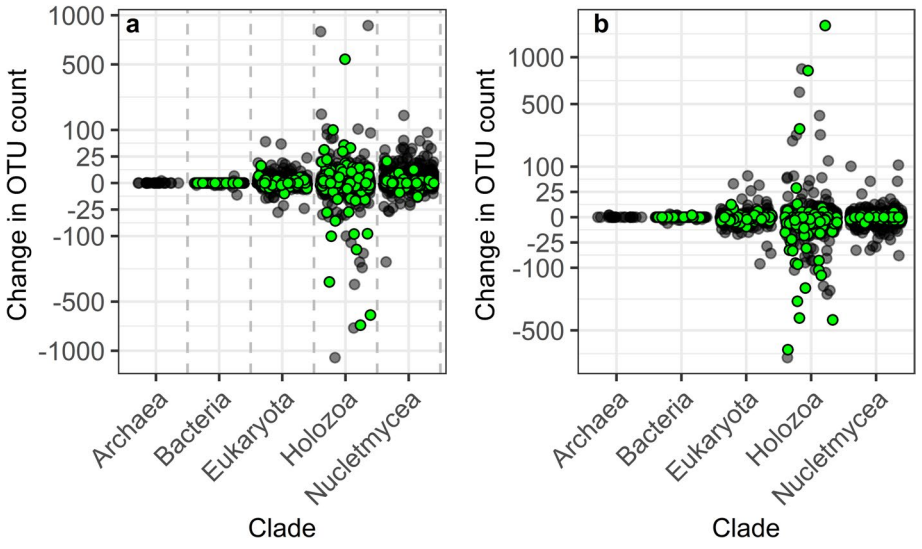

Fig. 2 Change in count of OTUs comparing **a** SECURE restoration ponds and non-SECURE mangroves and **b** SECURE cultivation ponds and non-SECURE ponds. Each point represents an OTU grouped to the lowest-known taxonomic rank. Green points are groups which may be indicators of healthy aquaculture management (Table 2). The change is the average effect of SECURE intervention in one pond

between the change in OTU count in restoration and cultivation ponds (Spearman rank correlation, $r_{4112} = -0.04$, $p = 0.02$).

Certain taxonomic groups are indicators of healthy, well-managed shrimp aquaculture (Table 2). These range from providing services such as nutrient cycling and water filtering, contributing to the diets of shrimp, or being sensitive to conditions such as eutrophication or low oxygen levels. Notable indicator groups which increased in SECURE restoration ponds were the phytoplankton Chaetoceros, Chlorophyceae, and Cryptomonadales and zooplankton Calanoida and Cyclopoida. Of these, only Calanoida also increased in SECURE cultivation ponds (Table 2).

Mangrove spatial planning priorities

The prioritisation analysis ranked 718 productive ponds which had not yet implemented SECURE, 672 of which were located in the north and 46 of which were located in the south (Figs. 3 and 4). The highest priority ranking, i.e. most suitable ponds, were selected at 10% of the target increment.

Five hundred fifty-one of productive ponds had an existing mangrove cover < 60% and the planned management intervention for implementing SECURE for these was to restore mangrove area to 60%. Lower priority ponds were generally located directly on the riverbank, situated to the east and closer to the ocean, and smaller. Higher priority ponds were generally set back from the riverbank, situated to the west, and larger. Priority ranks were often spatially clustered, such that adjacent ponds had similar rankings (Fig. 3).

One hundred sixty-seven of productive ponds had an existing mangrove cover ≥ 60% and the planned management intervention for implementing SECURE for these was to protect existing mangrove areas (Fig. 4). Unlike the ranking of ponds for restoration (Fig. 3), the ranking of ponds for protection showed less clear spatial patterns. The size of the pond was not strongly related to its ranking, as there were a mix of both small and large ponds with either high or low existing mangrove cover (Fig. S2). Ponds closer to the riverbank had slightly lower ranks, and ranks were not strongly spatially correlated (Fig. 4).

Discussion

Ecosystem-based aquaculture approaches are essential for balancing shrimp production with environmental sustainability (Soto et al. 2008), a challenge particularly acute in coastal countries like Indonesia where aquaculture is critical for food security (Wasik et al. 2025). Combining sustainable aquaculture practices with spatial planning offers a strategic approach to maximising both ecological and economic benefits in shrimp farming. Spatial prioritisation has previously been used to identify priority mangrove areas for providing ecosystem services (Atkinson et al. 2016; Trialfhianty et al. 2022), but this is, to our knowledge, the first application of a prioritisation approach to identify suitable areas for expansion of sustainable management practices. Our study demonstrates that incorporating restored mangroves into shrimp ponds can enhance abundance of key taxonomic groups which provide key ecosystem functions including improved water quality, increased habitat complexity, and carbon sequestration. While these benefits do not fully replicate those of intact mangrove forests, they indicate that targeted restoration and sustainable management practices can partially offset aquaculture's ecological footprint.

Table 2 Taxonomic groups as potential indicators of sustainable management and ecosystem health. Changes in OTU count are differences between SECURE and non-SECURE restoration ponds and SECURE and non-SECURE cultivation ponds

Change in OTU count		Taxonomic group	Indicator	Reference
Restoration	Cultivation			
Beneficial Algae & Phytoplankton				
545.67	−412	Chaetoceros	Contribute to shrimp diets and nutrient cycling in biofilms and sediment	(Arifin et al. 2017)
14	−25.67	Bacillariophyceae	Contribute to shrimp diets and nutrient cycling in biofilms and sediment	(Gatune et al. 2017)
15.67	−73.33	Chlorophyceae	Contribute to primary productivity and water quality	(Akbarurrasyid et al. 2024)
30.17	−19.33	Cryptomonadales	Sensitive to environmental changes	(Monsalve and Vergara 2023)
Benthic Organisms & Bioindicators of Stability				
−0.17	−2	Polychaeta	e.g. Spionida indicate better ecological conditions in mangrove-planted compared to traditional ponds	(Fujioka et al. 2007)
−0.67	0	Gastropoda	Indicate better ecological conditions in mangrove-planted compared to traditional ponds	(Fujioka et al. 2007)
0	0	Ostracoda	Indicate better ecological conditions in mangrove-planted compared to traditional ponds	(Fujioka et al. 2007)
−10	−7.17	Chromadorea	e.g. Chromadorida and Monhysterida contribute to the benthic food web	(Yến et al. 2018)
Key Bacterial Taxa for Nutrient Cycling				
0	0	Actinobacteria	Linked to healthy shrimp ponds and good water quality parameters such as nitrite and phosphate levels	(Wu et al. 2016)
0	0.08	Flavobacteriia	Linked to healthy shrimp ponds and good water quality parameters such as nitrite and phosphate levels	(Wu et al. 2016)
0	0	Bacilli	Linked to healthy shrimp ponds and good water quality parameters such as nitrite and phosphate levels	(Wu et al. 2016)
Zooplankton & Crustaceans Supporting Trophic Dynamics				
100	837.67	Calanoida	Contribute to food web balance and shrimp larval nutrition	(Cardozo et al. 2007)
39.17	−42.33	Cyclopoida	Food web balance and shrimp larval nutrition	(Cardozo et al. 2007)
0	−3.33	Sessilia	Filter feeders improving water quality	(Kohan et al. 2019)

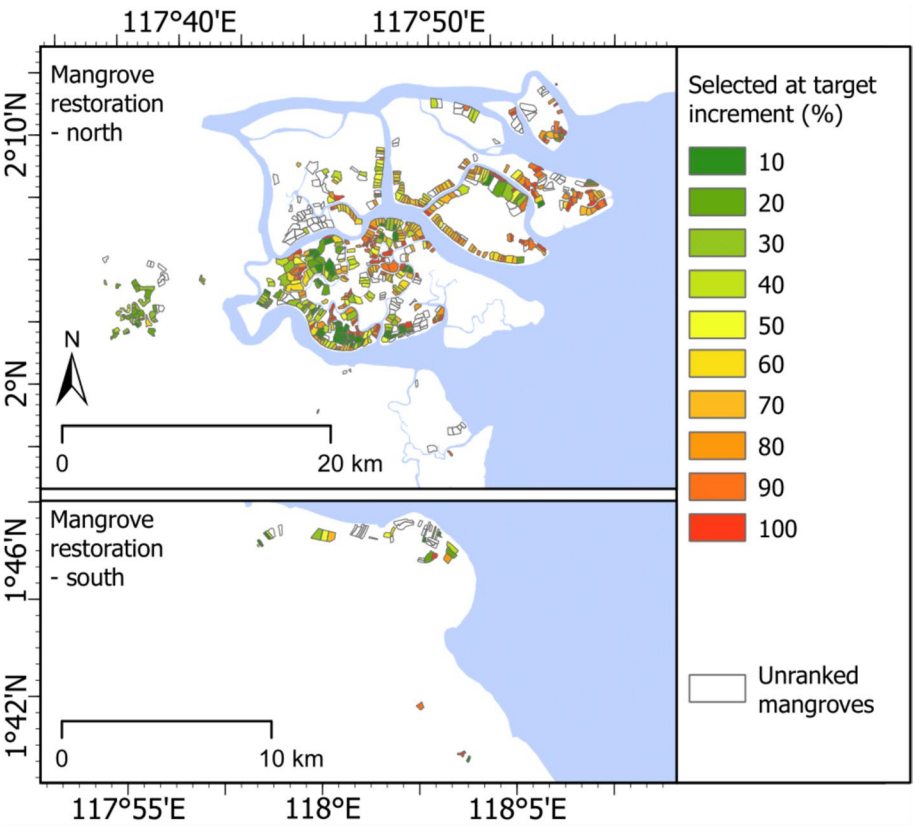

Fig. 3 Results of priority ranking analysis to determine the suitability of shrimp farming ponds for SECURE implementation with a focus on mangrove restoration. Highest priority ponds are selected at 10% targets (dark green), lowest priority ponds are selected at 100% targets (red). Only ponds with < 60% mangrove cover are assigned priority rankings, where the management intervention is to increase mangrove cover to 60%

The challenge of balancing shrimp farming productivity with environmental conservation remains a key issue in coastal resource management. Intensive farming systems yield high shrimp production per unit area in the short-term but require significant external inputs, such as feed and aeration, which contribute to eutrophication and waste accumulation (Shang et al. 1998). As a consequence, they have a relatively short lifespan due to accumulation of pollutants and disease that lead to their abandonment and encroachment into new mangrove areas (Aslan et al. 2021). On the other hand, ecosystem-based systems generally have lower environmental impacts but also lower yields, although there are exceptions (Paul and Vogl 2012). Our study suggests that the healthier communities in SECURE ponds should enable longer pond lifespans. Future aquaculture development should focus on long-term pond health and production efficiency rather than expanding the footprint of shrimp ponds into mangrove ecosystems. Policy incentives, certification programmes, and financial support for small-scale farmers transitioning to sustainable practices could help bridge the gap between sustainability and economic viability (Gambelli et al. 2019).

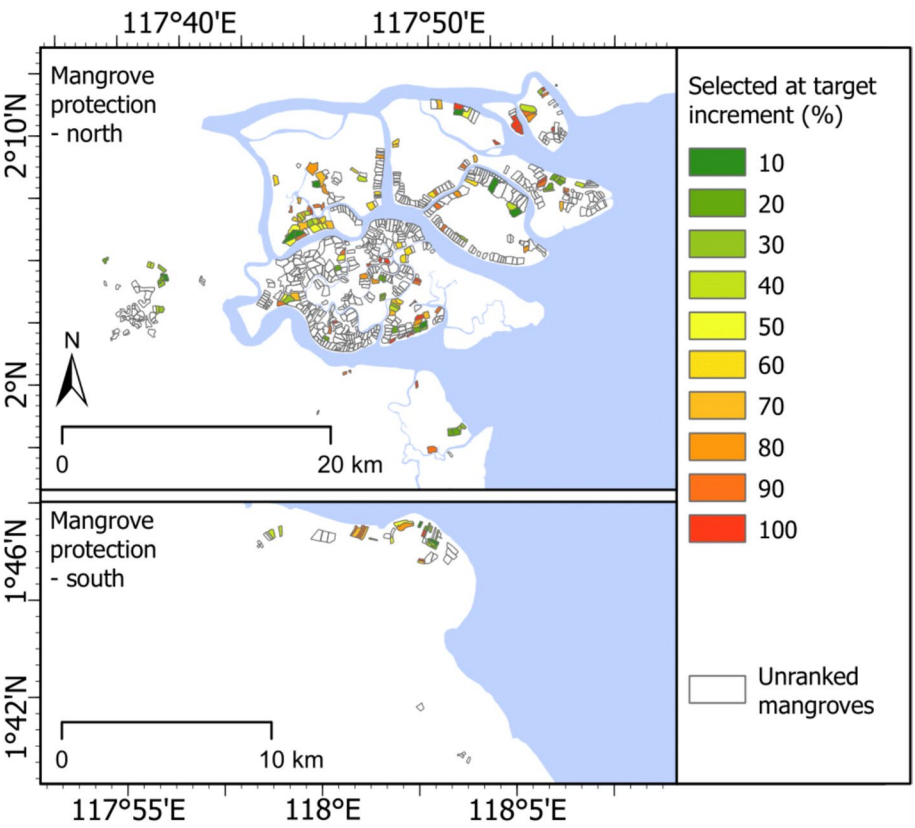

Fig. 4 Results of priority ranking analysis to determine the suitability of shrimp farming ponds for SECURE implementation with a focus on mangrove protection. Highest priority ponds are selected at 10% targets (dark green). Lowest priority ponds are selected at 100% targets (red). Only ponds with ≥ 60% mangrove cover are assigned priority rankings, where the management intervention is to protect existing mangrove areas

The application of environmental DNA metabarcoding provides a powerful tool for monitoring biodiversity in mangroves and aquaculture landscapes (Peters et al. 2018; Wee et al. 2023). Traditional biodiversity assessments rely on direct observations and physical sampling, which can be labour intensive and biased toward certain taxa. In contrast, eDNA allows for the cost-effective detection of a broad range of organisms, including rare and cryptic species (Bohmann et al. 2014). Our results highlight shifts in taxonomic composition between sustainably managed shrimp ponds and traditional systems, with higher relative abundances of indicator taxa associated with healthier aquatic environments. This effect was stronger in the rehabilitation ponds compared to the cultivation ponds. As the time between our two sampling events was relatively short, it may be that more time is necessary for conditions to stabilise and other improvements to become evident. Newly planted mangroves need time to form a functional canopy and root system with a stable soil microbial system, which may become more prominent in successive years as mangroves mature. This underscores the potential for eDNA to inform adaptive management strategies in aquaculture, ensuring that biodiversity metrics are integrated into decision-making

processes (Chouhan et al. 2023). Despite showing a good contrast between treatments, our conclusion is obscured by the resolution of the 18S primer, which mainly targets broad taxonomic coverage including fungi, protists, and algae. As there is no species-level resolution of animals, such as fish and invertebrates, there might a loss of signal in ecosystem improvement in shrimp and mangrove ecosystem (Hadziavdic et al. 2014; Wang et al. 2014). Future work should explore a combinatory use of multiple primers (e.g., COI and 12S) to establish long-term monitoring frameworks for aquaculture operations and coastal habitat restoration projects. As aquaculture impacts can extend to areas outside of those designated for farming activities through transmission of nutrients, pollutants, or disease, or escape of stock, monitoring will need to consider environmental impacts at appropriate spatial scales (Cheshire 2006).

We used readily available remote-sensing and spatial data to rank ponds according to the three criteria of mangrove protection or restoration, profitability from farming, and cost of implementing sustainable practices. We used spatial proxies due to an unavailability of detailed economic and other financial data. Although limited census data on the cost and profitability of the 14 pilot SECURE ponds was available, there were too few data points to extrapolate to the other 724 productive ponds. We did not account for all of the impacts of differences in hydrology between ponds due to a lack of hydrological data, such as hydrological connection with adjacent water bodies or exposure to water flow from tides and ocean circulation which can determine success rates of rehabilitation and restoration (López-Portillo et al. 2017). The hydrological characteristics among ponds were highly variable, making it difficult to draw generalisations across sites. Subsequent phases will integrate hydrological and ecological criteria to optimise SECURE placement and performance at the landscape scale. As integrated mangrove aquaculture expands, it is important to note that its fragmented nature limits the ecosystem functions and biodiversity benefits it provides. Ecosystem-based practices cannot fully restore fish nursery habitat, carbon sequestration, or coastal protection functions at levels comparable to undisturbed mangrove ecosystems, and should not be used in a misleading narrative that allows for further mangrove conversion (McSherry et al. 2023).

We excluded unproductive ponds from our analysis as we assumed that the cost of restoring these ponds for active shrimp farming exceeded their expected profitability. We assumed instead that the greatest net benefits could be achieved by concentrating resources on mitigating the negative impacts of productive ponds. As unproductive ponds are relatively undisturbed, they may already be serving as refugia for wildlife and could be experiencing natural mangrove regeneration (Stevenson 1997). On the other hand, the reason for pond abandonment is often due to severe degradation or pollution of the soil, which would require active rehabilitation before any natural regeneration can occur (Aslan et al. 2021). Future work that quantifies biodiversity and ecosystem service provision of these unproductive ponds and costs of rehabilitation can reveal whether resources should be allocated for their management. We excluded levels of knowledge or acceptability as a spatial planning criteria, as awareness-raising and capacity-building activities are ongoing within the project area to improve understanding of the SECURE system and its potential benefits. Preliminary results from surveys show that community acceptance is high when improvements in productivity and biomass are demonstrated. This suggests that social readiness is dynamic and can be enhanced through participatory engagement and evidence-based demonstrations.

In future iterations, the spatial prioritisation can be updated as some ponds implement SECURE or change status between productive and unproductive. Additional data can also be added onto the prioritisation analysis. For example, if regional biodiversity surveys are

conducted and identify biodiversity hotspots in certain areas, these may be included into the ranking criteria such that ponds in these hotspots are given higher priorities. Outputs of hydrological models may be included to show which areas are likely to regenerate naturally, and which areas require active intervention. Areas of risk in which to avoid SECURE implementation may also inform the priority ranks, for example from infrastructure developments which may become sources of pollutants. Our coupling of eDNA monitoring and spatial planning of ecosystem-based aquaculture highlights the potential to reconcile aquaculture productivity with environmental conservation, offering a replicable model for sustainable coastal resource management in other regions facing similar challenges.

Conclusion

This study presents a replicable framework that integrates environmental DNA metabarcoding and spatial prioritisation to inform sustainable aquaculture development in mangrove landscapes. Our findings show that the SECURE approach can enhance biodiversity indicators and offer ecological and economic benefits through targeted pond-level restoration and management. By prioritising ponds based on profitability, mangrove restoration or protection potential, and implementation costs, spatial planning can support more strategic and equitable expansion of ecosystem-based aquaculture. While ecosystem services from restored systems do not fully match those of intact mangroves, they provide a meaningful compromise between conservation and production. Future work should incorporate broader ecological and hydrological data, account for unproductive ponds, and expand biodiversity monitoring using multiple eDNA markers. Our approach offers a scalable decision-support tool for balancing aquaculture productivity and mangrove conservation, supporting long-term sustainability in coastal resource management.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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