http://microbescipublisher.com/index.php/ija
ISSN 1927-5773

Aqua Publisher®

International Journal of AQUACULTURE

Vol.16 No.3 2026

2026
03

Publisher
Aqua Publisher

Edited by
Editorial Team of International Journal of Aquaculture
Email: edit@ija.aquapublisher.com
Website: http://www.aquapublisher.com/index.php/ija

Address:
11388 Stevenston Hwy,
PO Box 96016,
Richmond, V7A 5J5, British Columbia
Canada

International Journal of Aquaculture (ISSN 1927-5773) is an open access, peer reviewed journal published online by AquaPublisher.

The journal publishes all the latest and outstanding research articles, letters and reviews in all working and studying within varied areas of aquaculture, containing the latest developments and techniques for practice in aquaculture; information about the entire area of applied aquaculture, including breeding and genetics, physiology, aquaculture-environment, hatchery design and management, utilization of primary and secondary resources in aquaculture, production and harvest, the biology and culture of aquaculturally important and emerging species.

Aqua Publisher is an international Open Access publisher specializing in the field of marine science and aquaculture registered at the publishing platform that is operated by Sophia Publishing Group (SPG), founded in British Columbia of Canada.

International Journal of Aquaculture (online), 2026, Vol. 16, No. 3
ISSN 1927-6648
http://aquapublisher.com/index.php/ija

Latest Content

Ecotoxicological Impacts and Biotransformation of Xenobiotic Pollutants in Aquatic Ecosystems: Implications for Fish Bioindicators and Environmental Remediation

Ninawe A.S., Shakir C., Subhash S.K., Delisho F.D.

International Journal of Aquaculture, 2026, Vol. 16, No. 3, 141-148

Haematological Parameters and Behavioural Responses of *Clarias gariepinus* Exposed to Sub-Acute *Senna occidentalis* Ethanol Leaf Extract

Adesanya Oluwatosin Emmanuel, Idowu Adekunle Adedoyin, Adekola Mukaila B., Towolawi Adeleke Taofik, Odukoya Abimbola Erastus

International Journal of Aquaculture, 2026, Vol. 16, No. 3, 149-155

Efficacy of a Closed-Water Depuration System in Reducing Bacterial Load in Bivalve Shellfish

K. Kavya, A. Raj, A. Suresh, A. A. Mohamed Hatha

International Journal of Aquaculture, 2026, Vol. 16, No. 3, 156-165

Assessment of Genetic Diversity in Germplasm Resources of Cultured Marine Groupers

Hejun Peng

International Journal of Aquaculture, 2026, Vol. 16, No. 3, 166-183

Antimicrobial Susceptibility, Microbial Loads and Isolation of *Plesiomonas shigelloides* from African Sharptooth Catfish (*Clarias gariepinus*) Juveniles and Experimental Pond Water

Sunday Emmanuel Olusola, Temitope Deborah Agboola, Tolulope Martins Ogunmakinwa, Folasade Damilola Amulejoye, Mariam Iyabo Adeoba, Omotola Victoria Ayebidun

International Journal of Aquaculture, 2026, Vol. 16, No. 3, 184-195

Effects of *Saccharomyces cerevisiae* Supplementation on Growth Performance and Nutrient Utilization of African Catfish *Clarias gariepinus* (Burchell, 1822)

Abdurrazzaq Ibrahim Abdullahi, Maryam Abdullahi Sanda, Tukur Mohammed, Abubakar Mohammed Mohammed, Yakubu Ibrahim, Sani Maiwada, Goni Bukar Lawan

International Journal of Aquaculture, 2026, Vol. 16, No. 3, 196-202

International Journal of Aquaculture, 2026, Vol.16, No.3, 141-148
http://www.aquapublisher.com/index.php/ija

Review Article Open Access

Ecotoxicological Impacts and Biotransformation of Xenobiotic Pollutants in Aquatic Ecosystems: Implications for Fish Bioindicators and Environmental Remediation

Ninawe A.S.[1], Shakir C.[2], Subhash S.K.[3], Delisho F.D.[4]

1 Ex-Scientist"G"Department of Biotechnology, Government of India, New Delhi-110 003, India

2 Department of Biochemistry and Industrial Microbiology, PMSA PTM Arts and Science College, (affiliated to Kerala University), Kottappuram, Kuttikkadu. P.O, Kadakkal, Kollam, Kerala, India

3 Department of Biochemistry and Microbiology, Sree Narayana College for Women, (affiliated to Kerala University), Kollam, India

4 Department of Biology, Arba Minch University, Arba Minch, Ethiopia

Corresponding email: ninawe@gmail.com

International Journal of Aquaculture, 2026, Vol.16, No.3 doi: 10.5376/ija.2026.16.0011

Received: 08 Mar., 2026

Accepted: 30 Apr, 2026

Published: 15 May., 2026

Preferred citation for this article:

Ninawe A.S., Shakir C., Subhash S.K., and Delisho F.D., 2026, Ecotoxicological impacts and biotransformation of xenobiotic pollutants in aquatic ecosystems: implications for fish bioindicators and environmental remediation, International Journal of Aquaculture, 16(3): 141-148 (doi: 10.5376/ija.2026.16.0011)

Abstract The persistence of biologically active xenobiotic compounds in aquatic environments poses a significant threat to ecosystem health and human safety. This review synthesizes current knowledge on the interactions and ecological impacts of xenobiotics in aquatic systems, with particular emphasis on fish as sensitive bioindicators of environmental contamination. Xenobiotics, including heavy metals and synthetic chemicals, induce a wide spectrum of biological responses in fish, ranging from synergistic or antagonistic interactions to alterations in mortality, behaviour, physiology, and cellular integrity. Bioaccumulation of these contaminants in fish tissues not only disrupts aquatic biodiversity but also facilitates their transfer through trophic levels into the human food chain, thereby posing serious public health concerns. Recent investigations highlight the significance of cellular-level xenobiotic interactions in processes such as carcinogenesis and chronic toxicity. Freshwater fish species are therefore increasingly employed as ecological sentinels for early detection of environmental contamination. In addition, this review discusses emerging remediation strategies, including bacterial bioremediation and phytoremediation, which utilize natural biological processes to degrade xenobiotic compounds. Advancements in the understanding of xenobiotic biotransformation pathways provide promising opportunities for mitigating environmental pollution and protecting aquatic ecosystems.

Keywords Xenobiotic contamination; Biotransformation pathways; Aquatic biomarkers; Fish ecotoxicology; Pollution remediation

1 Introduction

Environmental pollution affecting aquatic and terrestrial ecosystems represents one of the most significant global environmental challenges. Contaminants released from both natural and anthropogenic activities accumulate in environmental compartments and frequently enter food chains, where they exert harmful effects on plants, animals, and human populations. Aquatic ecosystems are particularly vulnerable to contamination by xenobiotic compounds originating from agricultural runoff, industrial effluents, pharmaceutical residues, and municipal wastewater discharges (Gavrilescu et al., 2015; Richardson and Kimura, 2017). Through processes such as bioaccumulation and biomagnification, these pollutants progressively concentrate within higher trophic levels, leading to serious ecological and toxicological consequences (Schwarzenbach et al., 2006; Kumar et al., 2023).

The term xenobiotic is derived from the Greek words *xenos* meaning "foreign" and *bios* meaning "life," referring to chemical substances that are not naturally synthesized or expected to occur within biological systems. Xenobiotics encompass a broad spectrum of compounds including agrochemicals, pharmaceuticals, petrochemicals, dyes, preservatives, adhesives, and personal care products (Daughton and Ternes, 1999; Fent et al., 2006; Aus der Beek et al., 2016; Wilkinson et al., 2022). The extensive global production and use of these compounds have resulted in widespread contamination of aquatic environments.

Among these pollutants, heavy metals represent a particularly hazardous group because of their persistence, toxicity, and ability to accumulate within biological tissues. Heavy metals such as lead, cadmium, mercury, and arsenic are

among the most hazardous environmental pollutants due to their persistence, bioaccumulation, and toxic effects on aquatic organisms and human health (Tchounwou et al., 2012; Jaishankar et al., 2014; Ali et al., 2019). These metals accumulate in fish tissues and may threaten aquatic biodiversity while posing potential health risks to humans through seafood consumption (Kumar et al., 2023).

Fish species are widely recognized as valuable model organisms in ecotoxicological research because their physiological and biochemical responses often reflect the overall health status of aquatic ecosystems. Exposure to xenobiotic pollutants can induce biochemical, physiological, and metabolic disturbances in fish, including oxidative stress, enzyme induction, cellular damage, and disruption of metabolic pathways (Authman et al., 2015; Rai et al., 2021).

Various strategies have been developed to remove xenobiotic contaminants from aquatic environments. Conventional physicochemical treatment methods, including precipitation, adsorption, and advanced oxidation processes, are often costly and may generate secondary pollutants. Consequently, environmentally sustainable approaches such as microbial bioremediation and phytoremediation are increasingly explored because of their ecological compatibility and cost effectiveness (Varjani et al., 2020; Sharma et al., 2022).

Despite extensive research on xenobiotic contamination and its ecological impacts, existing reviews often address these aspects in isolation, focusing either on environmental occurrence, toxicological mechanisms, or remediation technologies. There remains a critical lack of integrated perspectives that connect xenobiotic biotransformation processes with ecotoxicological responses in aquatic organisms, particularly fish, and their application as bioindicators in environmental monitoring. Furthermore, limited attention has been given to linking biomarker responses with emerging remediation strategies in a unified framework. This review aims to bridge these gaps by providing a comprehensive synthesis of xenobiotic distribution, bioaccumulation, and biotransformation pathways alongside fish-based ecotoxicological responses, while also highlighting the role of bioindicators in assessing environmental health and guiding sustainable remediation approaches.

2 Classification and Environmental Distribution of Xenobiotic Pollutants

Xenobiotic compounds present in aquatic environments can be categorized based on their origin, chemical composition, and environmental behavior. These substances include both naturally occurring bioactive compounds synthesized by living organisms and synthetic chemicals produced through industrial and agricultural activities (Richardson and Kimura, 2017; Wilkinson et al., 2022).

2.1 Natural and anthropogenic xenobiotic compounds

Xenobiotic substances can broadly be classified into natural and synthetic categories depending on their origin. Synthetic xenobiotics are artificially produced chemicals associated primarily with anthropogenic activities such as industrial manufacturing, agricultural practices, and pharmaceutical production. Examples include pesticides, industrial solvents, pharmaceutical residues, synthetic dyes, preservatives, and plastic additives (Schwarzenbach et al., 2006; Aus der Beek et al., 2016).

Natural xenobiotics, on the other hand, are compounds synthesized by plants, microorganisms, or animals as part of their chemical defense systems. These compounds include plant-derived alkaloids, microbial toxins, and naturally occurring antibiotics. Examples include pyrethrins produced by *Chrysanthemum* species and nicotine synthesized by plants belonging to the *Solanaceae* family (Rai et al., 2021; Wilkinson et al., 2022).

2.2 Biochemical classification of xenobiotic substances

Xenobiotic compounds may also be categorized according to their biochemical origin and metabolic behavior within biological systems.

2.2.1 Externally introduced xenobiotic chemicals

Exogenous xenobiotics are foreign chemical substances introduced into biological systems from external environmental sources. These compounds may enter organisms through contaminated food, water, inhalation, or

pharmaceutical administration. Examples include pesticides, pharmaceutical residues, food additives, industrial chemicals, and emerging contaminants such as microplastics and personal care products. Because these substances are foreign to the body, they are typically subjected to detoxification processes mediated by metabolic enzymes, particularly cytochrome P450 monooxygenases, which play a central role in the biotransformation and elimination of xenobiotics (Varjani et al., 2020; Rai et al., 2021; Wilkinson et al., 2022).

2.2.2 Endogenously generated toxic metabolites

Endogenous compounds are substances naturally synthesized within living organisms during metabolic processes but may exhibit toxic properties when accumulated at elevated concentrations. Examples include bile acids, steroid hormones, bilirubin, eicosanoids, and certain fatty acids. Although these compounds are physiologically produced, excessive accumulation can lead to toxic effects similar to those produced by external xenobiotic substances (Rai et al., 2021).

2.3 Major environmental seources and transport pathways of xenobiotics

Anthropogenic activities represent the principal sources of xenobiotic contaminants in aquatic ecosystems. Industrial activities such as pharmaceutical manufacturing, chemical processing, mining operations, and petroleum refining contribute substantially to environmental pollution. Agricultural practices involving intensive application of pesticides, herbicides, and fertilizers also introduce large quantities of xenobiotic compounds into aquatic systems through surface runoff (Varjani et al., 2020; Sharma et al., 2022).

Persistent organic pollutants such as polychlorinated biphenyls and pharmaceutical residues have been widely detected in aquatic ecosystems worldwide (Aus der Beek et al., 2016; Wilkinson et al., 2022). These compounds can be absorbed by primary producers such as algae and plankton and subsequently transferred through aquatic food webs, eventually accumulating in higher organisms including fish and aquatic mammals (Richardson and Kimura, 2017). Microbial degradation processes play a crucial role in the transformation and detoxification of xenobiotic compounds in the environment. Through biodegradation and biotransformation reactions, microorganisms convert toxic chemicals into intermediate metabolites that may eventually be mineralized into inorganic products (Varjani et al., 2020; Singh et al., 2021).

3 Bioaccumulation and Toxicological Impacts of Xenobiotics in Fish

Aquatic ecosystems frequently receive a wide range of xenobiotic pollutants, including heavy metals, pharmaceuticals, pesticides, and endocrine-disrupting compounds, originating from industrial discharges, agricultural runoff, atmospheric deposition, and natural geological processes. Once introduced into aquatic systems, these contaminants can be absorbed by aquatic organisms and progressively accumulate in biological tissues through bioaccumulation and biomagnification processes (Luoma and Rainbow, 2015; Kumar et al., 2019; Kumar et al., 2023). In addition to heavy metals, emerging contaminants such as pharmaceutical residues and personal care products are increasingly detected in aquatic environments and are known to persist and exert chronic toxic effects on aquatic biota.

Fish absorb xenobiotic contaminants primarily through their gills, digestive system, and skin. After entering the bloodstream, these substances may accumulate in vital organs such as the liver, kidney, and muscle tissues. The extent of accumulation depends on species-specific traits, environmental conditions, exposure duration, and pollutant concentration (Authman et al., 2015; Luoma and Rainbow, 2015; Kumar et al., 2023). While heavy metals remain a major concern, organic xenobiotics such as pesticides and pharmaceuticals can also bioaccumulate and interfere with metabolic and physiological processes in fish.

Chromium represents one of the most extensively studied heavy metals due to its widespread industrial application and high toxicity. Exposure to chromium can induce physiological, biochemical, histological, enzymatic, and genetic alterations in fish species (Jaishankar et al., 2014; Rai et al., 2021). Similarly, arsenic contamination is a major environmental concern, typically occurring in aquatic environments as arsenate or arsenite ions, which can disrupt metabolic processes and accumulate in aquatic organisms (Ali et al., 2019).

Beyond heavy metals, pesticides and their degradation products persist in aquatic environments and accumulate within sediments and food chains, thereby threatening aquatic biodiversity and human health (Sarkar et al., 2019; Sharma et al., 2022). In addition, endocrine-disrupting chemicals such as synthetic hormones and industrial compounds can interfere with hormonal regulation in fish, leading to reproductive abnormalities, altered growth patterns, and population-level effects. Pharmaceutical contaminants, including antibiotics and analgesics, have also been shown to induce sub-lethal toxicity, behavioral changes, and antimicrobial resistance in aquatic organisms, further highlighting the complexity of xenobiotic pollution in aquatic ecosystems.

4 Biomarkers and Receptor-Mediated Toxicological Responses in Fish

Exposure to xenobiotic compounds can trigger a wide range of molecular and physiological responses in aquatic organisms. Many xenobiotics interact with intracellular receptor proteins that function as ligand-activated transcription factors, resulting in altered gene expression and metabolic activity (Whyte et al., 2000; Wilkinson et al., 2022). One of the most extensively studied receptor systems involved in xenobiotic toxicity is the aryl hydrocarbon receptor signaling pathway. Activation of this receptor leads to the induction of detoxification enzymes and plays an important role in mediating toxicity associated with polycyclic aromatic hydrocarbons and related compounds (Whyte et al., 2000).

Fish species are widely used as bioindicators of environmental pollution because biochemical changes within their tissues provide early warning signals of ecosystem contamination (Moore et al., 2004; Authman et al., 2015; Rai et al., 2021). Biochemical biomarkers such as cytochrome P450 enzymes, particularly ethoxyresorufin-O-deethylase (EROD) activity, serve as important indicators of xenobiotic exposure. Changes in enzyme activity levels are widely used in environmental monitoring programs to assess pollution levels in aquatic ecosystems (Whyte et al., 2000; Wilkinson et al., 2022).

In practical applications, biomarker-based monitoring has been incorporated into several environmental assessment programs worldwide. For example, EROD activity and other biochemical biomarkers have been used in river monitoring studies to evaluate contamination from industrial effluents and urban wastewater discharges. Similarly, integrated biomarker responses in fish have been applied in ecological risk assessment frameworks to assess the impact of complex pollutant mixtures in aquatic environments. Monitoring programs in contaminated rivers and coastal ecosystems have demonstrated that biomarker responses in fish can provide early detection of sub-lethal toxicity before visible ecological damage occurs, thereby supporting timely environmental management and remediation strategies.

5 Metabolic Biotransformation and Microbial Degradation of Xenobiotics

Xenobiotic compounds undergo metabolic transformation within living organisms through complex detoxification pathways that are critical for detoxification and elimination. In aquatic organisms such as fish, xenobiotic biotransformation primarily occurs in the liver, which serves as the major detoxification organ, but is also significantly influenced by gill uptake and direct exposure to contaminants in water. Unlike terrestrial organisms, fish are continuously exposed to dissolved pollutants, making gill tissues an important site for both uptake and initial metabolic processing.

Phase I reactions involve oxidation, reduction, and hydrolysis processes that introduce reactive functional groups into xenobiotic molecules, primarily mediated by cytochrome P450 monooxygenases. However, compared with mammals, fish often exhibit lower activity and diversity of certain cytochrome P450 isoforms, which may result in slower metabolic transformation and prolonged persistence of xenobiotics in tissues. Phase II reactions involve conjugation processes such as glucuronidation, sulfation, methylation, glutathione conjugation, and amino acid conjugation, which enhance the water solubility of xenobiotics and facilitate their excretion (Wilkinson et al., 2022).

The efficiency of these biotransformation processes in fish is influenced by environmental factors such as temperature, dissolved oxygen, and pollutant concentration, which can further modulate enzymatic activity and detoxification capacity. For example, polycyclic aromatic hydrocarbons (PAHs) are metabolized in fish through

Phase I oxidation to reactive intermediates, which can bind to cellular macromolecules and induce toxic effects if not effectively conjugated during Phase II reactions. This incomplete detoxification can lead to oxidative stress, DNA damage, and carcinogenic outcomes in aquatic organisms.

In addition to organism-level metabolism, microbial biodegradation plays a crucial role in the environmental transformation of xenobiotics. Microorganisms in aquatic systems can metabolize a wide range of pollutants, including hydrocarbons, pesticides, and pharmaceutical residues, thereby contributing to the natural attenuation of contaminants (Wang and Wang, 2016; Varjani et al., 2020; Singh et al., 2021). The interaction between microbial processes and fish metabolism ultimately determines the persistence, bioavailability, and ecological impact of xenobiotic compounds in aquatic ecosystems.

6 Oxidative Stress and Cellular Damage Induced by Xenobiotics

One of the most significant mechanisms through which xenobiotics exert toxic effects in aquatic organisms is the induction of oxidative stress. Xenobiotic compounds such as heavy metals, pesticides, and pharmaceutical residues can stimulate the excessive production of reactive oxygen species (ROS) including superoxide radicals, hydrogen peroxide, and hydroxyl radicals. These reactive molecules can damage cellular macromolecules such as proteins, lipids, and nucleic acids, ultimately leading to impaired physiological functions in aquatic organisms (Livingstone, 2001; Valavanidis et al., 2006). Fish exposed to xenobiotic pollutants frequently exhibit increased lipid peroxidation, DNA damage, and enzyme inhibition due to oxidative stress. Antioxidant defense systems such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferase play essential roles in mitigating oxidative damage. However, prolonged exposure to pollutants can overwhelm these defense mechanisms, resulting in cellular dysfunction and tissue damage (Monteiro et al., 2010; Lushchak, 2011). Recent studies have demonstrated that oxidative stress biomarkers in fish can be used as sensitive indicators of environmental contamination. Monitoring antioxidant enzyme activity and oxidative damage products therefore provides valuable information for assessing the ecological impact of xenobiotic pollutants in aquatic ecosystems (Valavanidis et al., 2006).

7 Endocrine Disruption and Reproductive Toxicity in Aquatic Organisms

Certain xenobiotic pollutants function as endocrine-disrupting chemicals (EDCs) that interfere with hormonal signaling pathways in aquatic organisms. These substances can mimic, block, or alter the synthesis, transport, and metabolism of natural hormones, resulting in disturbances in growth, reproduction, and development. Endocrine-disrupting compounds commonly detected in aquatic environments include industrial chemicals, pesticides, plasticizers, and pharmaceutical residues (Sumpter and Johnson, 2005; Diamanti-Kandarakis et al., 2009).

At the molecular level, many EDCs exert their effects by binding to nuclear hormone receptors such as estrogen receptors (ERs), androgen receptors (ARs), and thyroid hormone receptors, thereby altering transcriptional regulation of target genes involved in reproductive and developmental processes. This receptor-mediated interaction can lead to changes in gene expression, protein synthesis, and endocrine feedback mechanisms, ultimately disrupting physiological homeostasis. In fish populations, exposure to endocrine-disrupting xenobiotics has been associated with abnormalities such as reduced fertility, altered sex ratios, intersex conditions, and impaired reproductive behavior. Synthetic estrogens, for example, have been shown to induce feminization of male fish in contaminated aquatic environments (Jobling et al., 2003).

Recent studies have highlighted the growing significance of emerging endocrine disruptors, including pharmaceutical residues and microplastics, in aquatic ecosystems. Pharmaceutical compounds such as synthetic hormones and antidepressants can interact with endocrine signaling pathways even at low concentrations, while microplastics may act as carriers for adsorbed EDCs or directly interfere with endocrine function. These contaminants have been shown to modulate gene expression related to reproductive development and endocrine regulation, leading to sublethal but ecologically significant effects in fish populations (Sharma et al., 2022; Wilkinson et al., 2022).

The increasing detection of endocrine disruptors in rivers and lakes worldwide has raised serious concerns regarding the long-term sustainability of aquatic ecosystems. Consequently, monitoring endocrine biomarkers such as vitellogenin expression in fish has become an important tool in environmental toxicology and ecological risk assessment (Sumpter and Johnson, 2005). Advances in molecular biomarkers, including gene expression profiling and omics-based approaches, are further enhancing the sensitivity and specificity of detecting endocrine disruption in aquatic environments.

8 Role of Nanotechnology in Xenobiotic Remediation

Nanotechnology has recently emerged as a promising approach for the remediation of xenobiotic pollutants in aquatic environments. Nanomaterials such as metal nanoparticles, carbon nanotubes, and nano-adsorbents possess unique physicochemical properties including high surface area and enhanced catalytic activity, making them highly effective for pollutant removal (Qu et al., 2013).

Nanoparticles can facilitate the adsorption, degradation, or transformation of xenobiotic compounds including pesticides, dyes, pharmaceuticals, and heavy metals. For instance, nano-scale zero-valent iron has been widely studied for its ability to degrade chlorinated organic pollutants in contaminated water systems (Zhang, 2003).

Despite their promising applications, the environmental safety of nanomaterials remains an important concern. Further research is required to evaluate the ecological risks associated with nanoparticle release into aquatic ecosystems while optimizing their use in environmental remediation technologies (Qu et al., 2013).

9 Climate Change and Its Influence on Xenobiotic Toxicity

Climate change is increasingly recognized as an important factor influencing the environmental behavior and toxicity of xenobiotic pollutants. Rising water temperatures, changes in pH, and altered hydrological cycles can significantly modify the transport, transformation, and bioavailability of contaminants in aquatic ecosystems (Noyes et al., 2009).

Elevated temperatures can increase metabolic rates in aquatic organisms, which may enhance the uptake and toxicity of xenobiotic compounds. In addition, climate-driven changes in precipitation patterns may increase the transport of agricultural pesticides and industrial pollutants into aquatic environments (Noyes et al., 2009).

Understanding the combined effects of climate change and chemical pollution is therefore essential for predicting future environmental risks and developing effective strategies for aquatic ecosystem protection.

10 Concluding Perspectives and Future Research Directions

Xenobiotic contamination of aquatic ecosystems remains a critical global environmental challenge driven by the persistence, toxicity, and bioaccumulative nature of anthropogenic pollutants. The continuous release of complex mixtures of chemicals—including heavy metals, pesticides, pharmaceuticals, and industrial compounds—has significantly impacted aquatic biodiversity and poses risks to human health through trophic transfer. Fish serve as sensitive and reliable bioindicators of environmental contamination, reflecting the integrated effects of xenobiotic exposure at biochemical, physiological, and molecular levels, with key mechanisms of toxicity including oxidative stress and endocrine disruption that impair cellular function, reproduction, and overall organismal health. While advances in bioremediation and emerging technologies such as nanomaterials offer promising strategies for mitigating xenobiotic pollution, their long-term ecological safety requires careful evaluation, particularly under the influence of climate change, which can alter pollutant distribution, bioavailability, and toxicity in aquatic systems. Moving forward, research should prioritize the development of species-specific biomarker systems for early detection of xenobiotic exposure in fish, alongside the integration of omics-based approaches with ecological modeling to better predict long-term ecosystem responses. Greater emphasis is also needed on understanding the environmental fate and toxicological impacts of emerging contaminants such as pharmaceutical residues and microplastics, as well as elucidating species-specific biotransformation pathways and their implications for ecological risk and trophic transfer. In addition, evaluating the combined effects of xenobiotics under changing climate conditions and optimizing sustainable remediation strategies—including microbial, phytoremediation, and

nanotechnology-assisted approaches—will be essential. Finally, strengthening the linkage between scientific research and environmental policy through standardized monitoring frameworks and risk assessment tools will be critical for effective management and long-term protection of aquatic ecosystems.

Conflict of Interest

Author declares that there is no conflict of interest.

Acknowledgements

The authors acknowledges the research institutes working in India and elsewhere for gathering the data, referring publications on the subject and Google research scholars, research reports pertaining the reviews on the subject including the web based information for preparing the manuscript.

References

Ali H., Khan E., and Ilahi I., 2019, Environmental chemistry and ecotoxicology of hazardous heavy metals, Chemosphere, 218: 442-457. https://doi.org/10.1155/2019/6730305

Aus der Beek T., Weber F.A., Bergmann A., Hickmann S., Ebert I., Hein A., and Küster A., 2016, Pharmaceuticals in the environment-Global occurrences and perspectives, Environmental Toxicology and Chemistry, 35(4): 823-835. https://doi.org/10.1002/etc.3339

Authman M.M.N., Zaki M.S., Khallaf E.A., and Abbas H.H., 2015, Use of fish as bio-indicators of the effects of heavy metals pollution, Ecotoxicology and Environmental Safety, 112: 1-10.

Daughton C.G., and Ternes T.A., 1999, Pharmaceuticals and personal care products in the environment: Agents of subtle change?, Environmental Health Perspectives, 107(Suppl 6): 907-938. https://doi.org/10.1289/ehp.99107s6907

Diamanti-Kandarakis E., Bourguignon J. P., Giudice L.C., Hauser R., Prins G.S., Soto A.M., Zoeller R.T., and Gore A.C., 2009, Endocrine-disrupting chemicals: an endocrine society scientific statement, Endocrine reviews, 30(4): 293-342. https://doi.org/10.1210/er.2009-0002

Fent K., Weston A.A., and Caminada D., 2006, Ecotoxicology of human pharmaceuticals, Aquatic Toxicology, 76(2): 122-159. https://doi.org/10.1016/j.aquatox.2005.09.009

Gavrilescu M., Demnerová K., Aamand J., Agathos S., and Fava F., 2015, Emerging pollutants in the environment: Present and future challenges in biomonitoring, ecological risks and bioremediation, New Biotechnology, 32(1): 147-156. https://doi.org/10.1016/j.nbt.2014.01.001

Jaishankar M., Tseten T., Anbalagan N., Mathew B., and Beeregowda K.N., 2014, Toxicity, mechanism and health effects of heavy metals, Interdisciplinary Toxicology, 7(2): 60-72. https://doi.org/10.2478/intox-2014-0009

Jobling S., Nolan M., Tyler C.R., Brighty G., and Sumpter J.P., 2003, Widespread sexual disruption in wild fish, Environmental Science and Technology, 37(6): 1053-1061.

Kumar V., Parihar R.D., Sharma A., Bakshi P., Sidhu G.P.S., Bali A.S., Karaouzas I., Bhardwaj R., Thukral A.K., and Gyasi-Agyei Y., 2023, Heavy metal contamination in aquatic ecosystems and associated human health risks, Science of the Total Environment, 857: 159369.

Kumar V., Parihar R.D., Sharma A., Bakshi P., Sidhu G.P.S., Bali A.S., Karaouzas I., Bhardwaj R., Thukral A.K., and Gyasi-Agyei Y., 2019, Global evaluation of heavy metal contamination in aquatic ecosystems, Chemosphere, 216: 561-576.

Livingstone D.R., 2001, Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms, Marine Pollution Bulletin, 42(8): 656-666. https://doi.org/10.1016/S0025-326X(01)00060-1

Luoma S.N., and Rainbow P.S., 2015, Why is metal bioaccumulation so variable? Biodynamics as a unifying concept, Environmental Science and Technology, 49(4): 1936-1943.

Lushchak V.I., 2011, Environmentally induced oxidative stress in aquatic animals, Aquatic Toxicology, 101(1): 13-30. https://doi.org/10.1016/j.aquatox.2010.10.006

Monteiro D.A., Almeida J.A., Rantin F.T., and Kalinin A.L., 2010, Oxidative stress biomarkers in fish exposed to environmental pollutants, Comparative Biochemistry and Physiology Part C, 151(3): 356-364.

Moore M.N., Depledge M.H., Readman J.W., and Leonard D.R.P., 2004, An integrated biomarker-based strategy for ecological risk assessment, Marine Environmental Research, 58: 351-356.

Noyes P.D., McElwee M.K., Miller H.D., et al., 2009, The toxicology of climate change, Environment International, 35(6): 971-986. https://doi.org/10.1016/j.envint.2009.02.006

Qu X., Alvarez P.J., and Li Q., 2013, Applications of nanotechnology in water and wastewater treatment, Water Research, 47(12): 3931-3946. https://doi.org/10.1016/j.watres.2012.09.058

Rai P., Kumar V., Lee S., and Kim K., 2021, Environmental fate and ecotoxicology of heavy metals in aquatic ecosystems, Environmental Chemistry Letters, 19: 343-357.

Rai P.K., Lee S.S., Zhang M., Tsang Y.F., and Kim K.H., 2019, Heavy metals in food crops: Health risks, fate, mechanisms and management, Environmental Chemistry Letters, 17: 147-163.

Richardson S.D., and Kimura S.Y., 2017, Emerging environmental contaminants: Challenges facing our next generation, Analytical Chemistry, 89(1): 546-570.

Sarkar A., Kumar V., Prasad R.K., Choudhury B.U., and Singh J., 2019, Environmental contamination and ecological risk assessment of pesticides in aquatic ecosystems, Environmental Science and Pollution Research, 26: 13620-13638.

Schwarzenbach R.P., Escher B.I., Fenner K., Hofstetter T.B., Johnson C.A., von Gunten U., and Wehrli B., 2006, The challenge of micropollutants in aquatic systems, Science, 313(5790): 1072-1077.
https://doi.org/10.1126/science.1127291

Sharma B., Dangi A.K., and Shukla P., 2022, Contemporary enzyme-based technologies for bioremediation: A review, Bioresource Technology, 344: 126290.

Singh R., Kumar M., Mittal A., and Mehta P.K., 2021, Microbial enzymes: Industrial progress in the 21st century, Frontiers in Microbiology, 12: 698735.

Sumpter J.P., and Johnson A.C., 2005, Lessons from endocrine disruption in fish, Environmental Science and Technology, 39(12): 4321-4332.
https://doi.org/10.1021/es048504a

Tchounwou P.B., Yedjou C.G., Patlolla A.K., and Sutton D.J., 2012, Heavy metal toxicity and the environment, EXS, 101: 133-164.
https://doi.org/10.1007/978-3-7643-8340-4_6

Valavanidis A., Vlahogianni T., Dassenakis M., and Scoullos M., 2006, Molecular biomarkers of oxidative stress in aquatic organisms, Ecotoxicology and Environmental Safety, 64(2): 178-189.
https://doi.org/10.1016/j.ecoenv.2005.03.013

Varjani S., Rakholiya P., Ng H.Y., You S., and Teixeira J.A., 2020, Microbial degradation of xenobiotic compounds, Bioresource Technology, 302: 122811.

Wang J., and Wang S., 2016, Removal of pharmaceuticals and personal care products from wastewater, Chemical Engineering Journal, 302: 175-187.

Whyte J.J., Jung R.E., Schmitt C.J., and Tillitt D.E., 2000, Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure, Aquatic Toxicology, 50(1-2): 73-82.

Wilkinson J.L., Boxall A.B.A., Kolpin D.W., Leung K.M.Y., Lai R.W.S., Galbán-Malagón C., Adell A.D., Mondon J., Metian M., Marchant R.A., Bouzas-Monroy A., Cuni-Sanchez A., Coors A., Carriquiriborde P., Rojo M., Gordon C., Cara M., Moermond C., Luarte T., Petrosyan V., Perdomo E., Segura P.A., et al., 2022, Pharmaceutical pollution of the world's rivers, Nature Reviews Earth and Environment, 3: 192-208.

Zhang W.X., 2003, Nanoscale iron particles for environmental remediation, Journal of Nanoparticle Research, 5: 323-332.
https://doi.org/10.1023/A:1025520116015

Zhang Y., Geißen S.U., and Gal C., 2008, Carbamazepine and diclofenac in wastewater, Chemosphere, 73: 1151-1161.
https://doi.org/10.1016/j.chemosphere.2008.07.086

International Journal of Aquaculture, 2026, Vol.16, No.3, 149-155
http://www.aquapublisher.com/index.php/ija

Review Article Open Access

Haematological Parameters and Behavioural Responses of *Clarias gariepinus* Exposed to Sub-Acute *Senna occidentalis* Ethanol Leaf Extract

Adesanya Oluwatosin Emmanuel [1], Idowu Adekunle Adedoyin [1], Adekola Mukaila B. [2], Towolawi Adeleke Taofik [3], Odukoya Abimbola Erastus [4]

1 Department of Aquaculture and Fisheries Management, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria

2 Department of Environmental Management and Toxicology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria

3 Department of Environmental Health Science, Fountain University, Osogbo, Osun State, Nigeria

4 Department of Aquaculture and Fisheries, University of Ilorin, Kwara State, Nigeria

Corresponding email: 4desanyaoluwatosin@gmail.com, attowolawi@fuo.edu.ng

International Journal of Aquaculture, 2026, Vol.16, No.3 doi: 10.5376/ija.2026.16.0012

Received: 18 Mar., 2026

Accepted: 16 May., 2026

Published: 25 May., 2026

Preferred citation for this article:

Adesanya O.E., Idowu A.A., Adekola M.B., Towolawi A.T., and Odukoya A.E., 2026, Haematological parameters and behavioural responses of *Clarias gariepinus* exposed to sub-acute *Senna occidentalis* ethanol leaf extract, International Journal of Aquaculture, 16(3): 149-155 (doi: 10.5376/ija.2026.16.0012)

Abstract The health of fish largely depends on the quality of their blood, which is relevant in indicating the state of the fish's health. The present study was conducted to evaluate the effects of sub-acute exposure to *Senna occidentalis* ethanol leaf extract on the haematological parameters and behavioural responses of *Clarias gariepinus* juveniles. The study was carried out at the Fish Hatchery Complex, FUNAAB. One hundred and eight (108) juveniles of *Clarias gariepinus* with an initial average body weight of 16 ± 2 grams and length of 8.5 ± 0.5 cm were used. The leaves of *Senna occidentalis* were obtained at Old Bola Ahmed Tinubu Road, off Iju Road, Ifako-Ijaiye LGA, Lagos State. The experiment had four treatments and three replicates, each with 9 fish per treatment tank. During the 23-day subacute toxicity test, *C. gariepinus* exposed to graded doses of *Senna occidentalis* were closely observed for behavioural responses. Haematological parameters such as Red Blood Cell, White Blood Cell, Packed Cell Volume, Haemoglobin, and White Blood Cell Differential Counts were also carried out. Analysis of variance was used to assess the data. The results showed that the values of Mean Corpuscular Volume (115.15-112.33 fL), Hemoglobin (12.31-9.50 g/dL), Red Blood Cell (3.30-2.13 x $10^{12/L}$), White Blood Cell (15.82-10.90 x $10^{9/L}$), Packed Cell Volume (38.31%-28.11%), and Mean Cell Hemoglobin concentration (34.71-32.27 g/dL) decreased as *Senna occidentalis* concentrations increased in relation to the control treatment. These findings provide a baseline that what is natural may not be automatically safe, as the results of this research showed poor growth and fish deaths. These findings indicate that *Senna occidentalis* ethanol leaf extract may pose sub-acute toxic effects to *Clarias gariepinus*, and the results may provide useful baseline information for fish health assessment and the safe use of medicinal plant extracts in aquaculture environments.

Keywords *Clarias gariepinus*; *Senna occidentalis*; Haematology; Behavioural response; subacute toxicity; Plant extract

1 Introduction

Aquatic ecosystems are fundamental to global biodiversity, food security, and ecological stability; however, they are increasingly compromised by contamination from a broad spectrum of anthropogenic substances (Thanigaivel et al., 2023). These environmental pollutants enter the aquatic systems through runoffs and discharges (Amoatey and Baawain, 2019; Das et al., 2024) and have been widely reported to disrupt multiple physiological systems in fish (Mustafa et al., 2024) and biochemical processes (Rocha et al., 2018). Their impact includes impairments to immune balance, reproduction, and metabolism, with effects often under concurrent environmental stressors such as hypoxia and pH fluctuations.

Recent studies have increasingly examined environmental pollutants and their effects in aquatic environments (Adeleye et al., 2024; Sefali et al., 2026). While most of this research is centered on conventional contaminants such as heavy metals, pesticides, and pharmaceuticals, comparatively less research has been done on plant-derived bioactive compounds.

Plants and their derivatives serve as key sources of nutrients for humans and animals while also providing medicinal benefits (Diouf et al., 2019; Samtiya et al., 2020). They contain bioactive compounds whose effects are either

beneficial or harmful, and are largely concentration-dependent (Ali et al., 2022; Dey et al., 2022). *Senna occidentalis*, a medicinal plant widely used in traditional systems and reported to possess potent bioactive constituents, warrants toxicological evaluation in aquatic organisms such as *Clarias gariepinus*. Egharevba et al. (2010) stated that *Senna occidentalis* L. Link (Leguminosae), formerly known as *Cassia occidentalis* L., is well-known for its wide range of medical applications and is utilized locally to treat various human and animal illnesses. However, *Senna species* seeds, leaves, and roots have been shown to have a variety of toxicities despite their enormous medicinal potential (Gebrelibanos et al., 2014). In other words, exposure to some of these plants may be toxic to humans and animals, even with the wide range of medicinal potentials exhibited by many botanical products (plants) (Belay and Enyew, 2016).

Hematological analysis of peripheral blood parameters and quantitative assessment of blood cell morphology serve as practical, cost-effective tools in fish toxicology (Witeska et al., 2023). Accordingly, the present study evaluated erythrocyte counts, leukocyte differentials, hemoglobin concentration, etc., in *Clarias gariepinus* exposed to *Senna occidentalis* leaf extract to assess hematotoxic potential. Packed cell volume (PCV) and haemoglobin concentration are standard indicators of anaemia in aquaculture (Afia and Gift, 2017), while RBC indices like MCHC, MCH, and MCV aid diagnosis (Iheanacho et al., 2017). Hematological responses of fish to xenobiotics vary with the toxicant, exposure time, and biological factors such as species, age, and size (Ahmed et al., 2020), and may represent adaptation, damage, or both (Witeska et al., 2023). Given that plant-derived bioactive compounds can act as xenobiotics in aquatic systems, these haematological parameters, alongside behavioral responses, are therefore critical for evaluating sub-acute toxic effects of *Senna occidentalis* ethanol leaf extract on *Clarias gariepinus.*

2 Materials and Methods

2.1 Experimental site

The study was conducted at the Fish Hatchery Complex, Aquaculture and Fisheries Management Department, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria (FUNAAB).

2.2 Collection of juveniles of *Clarias gariepinus*

A total of one hundred and eight (108) juveniles of *Clarias gariepinus* with an initial average body weight of 16 ± 2 grams and length of 8.5 ± 0.5 cm were obtained from Path Farm, Sagamu, Remo, Ogun State, Nigeria, and transported in a 50-liter keg to the Fish Hatchery, FUNAAB.

2.3 Acclimatisation of the *C. gariepinus* juveniles

The *C. gariepinus* juveniles were acclimatised for two weeks; in that period, they were fed 1.8 mm imported Skretting feed, which contained 45% crude protein, twice a day, in the early morning and in the evening. Feeding was stopped 48 hours before the beginning of the experiment, and wastes and wasted feed were taken out daily along with replenishing water.

2.4 Collection and preparation of *Senna occidentalis* leaves

The leaves of *Senna occidentalis* were obtained from Old Bola Ahmed Tinubu Road, off Iju Road, Ifako-Ijaiye LGA, Lagos State, Nigeria, and authenticated by the Forestry and Wildlife Management Department, FUNAAB. Fresh leaves of S. occidentalis were air-dried for two weeks and ground using a Binatone BLS-360 1.5 L electronic blender; then 300 g of the powdered leaves were obtained and soaked in 1400 mL of ethanol and 600 mL of distilled water (70% ethanol); the solution was stirred continuously at intervals for 72 hr (Jun et al., 2012). The solution was filtered, subjected to a rotary evaporator (50 °C), and oven-dried for 24 hr at 40 °C using a low-temperature oven drier to achieve a more concentrated extract at the Lagos University Teaching Hospital (LUTH). The extract was then stored in a refrigerator using an airtight container.

2.5 Experimental design and procedure

The experiment had four treatments and three replicates, each with 9 fish per treatment tank. From the acclimatised fish, 108 juvenile catfish were randomly distributed into 12 plastic tanks with a capacity of 35 litres,

each filled with 10 litres of water for the experiment. A completely randomised design was adopted. From the stored extracts, 3000 mg/25 cL, 5000 mg/25 cL, and 7000 mg/25 cl of distilled water were used as a stock solution, from which 5 ml of each concentration was infused daily into the plastic tanks.

2.6 Behavioural studies

During the 23-day sub-acute toxicity test, *C. gariepinus* exposed to graded concentrations of *S. occidentalis* was closely observed for the subsequent behavioural changes: air gulping, stunned positioning, skin peeling, aggression, and erratic swimming (fast and spiral movement). Weak, moderate, and high rankings were given to the observed changes.

2.7 Haematological test

According to Erhunmwunse and Ainerua (2013), haematological parameters, including red and white blood cell counts, packed cell volume, haemoglobin, and white blood cell differential counts, were performed following standard procedures.

2.7.1 Determination of haemoglobin

Using the Randox kit, haemoglobin was measured spectrophotometrically. Potassium ferricyanide (0.61 mmol/L), potassium cyanide (0.77 mmol/L), potassium phosphate (1.03 mmol/L), and 0.1% v/v surfactant are all present in the reagent.

Procedure:

The test tubes were labelled as blank, standard, and tests. 20 µL of whole blood was added to each tube. Additionally, 5 mL of the reagent was added to each tube, which was then incubated at room temperature for 3 minutes. The absorbance of all tubes was read at 540 nm against the reagent blank.

Haemoglobin conc. (g/dL) = Abs of sample X 36.77 --- (1)

2.7.2 PCV (Packed Cell Volume)

Blood was poured into a simple capillary tube until it was about ¾ full. Plasticine was used to seal the tube's open end. The sealed tube was centrifuged at exactly 12,000 revolutions per minute for five minutes in a Hawksley micro-hematocrit centrifuge. The packed cell volume value was determined and expressed as a percentage for each tube after placing it in a micro hematocrit reader.

2.7.3 RBC (Red Blood Cell count)

The hemocytometer was used to determine the red blood cell count. Blood was diluted 1:200 with red blood cell diluting fluid using a red blood cell pipette. After mixing the dilution and waiting two minutes, the hemocytometer's counting chamber was filled, and the red blood cells were counted using a 40x microscope objective. The total number of counted cells was expressed in cubic millimetres or litres and multiplied by 10,000.

2.7.4 Total WBC (White Blood Cell Count)

The haemocytometer's white blood cell pipette was used to dilute the blood 1:20 with WBC diluting fluid. The liquid was slowly combined with the blood. The dilution was added to the counting chamber, and the WBCs were counted using a microscope's x10 objective. The total cell count was expressed in millilitres or litres and then multiplied by 50.

2.7.5 WBC differential count

A drop of blood was evenly distributed across a clean, grease-free slide to create a thin blood film using a smooth-edged spreader. After staining the blood film with aqueous stains, it was fixed in acetone-free methyl alcohol for approximately three to five minutes to prevent hemolysis upon contact with water. It was then left to dry. After applying the field, A and B stains to the blood film, 100 white blood cells were separated under a microscope with oil immersion objectives.

2.8 Statistical analysis

Data obtained from the experiments were collated and subjected to analysis of variance using SPSS version 20.0, with significance set at $p < 0.05$. Duncan's Multiple Range Test (DMRT) was used for comparison tests.

3 Results

3.1 Behavioural signs

The behavioural and morphological responses of *Clarias gariepinus* exposed to varying concentrations of *S. occidentalis* ethanolic extract are presented in Table 1.

Table 1 Results of behavioural responses

Behavioural response	Control	Treatment A	Treatment B	Treatment C
	0 mg/ 25 cL	3000 mg/25 cL	5000 mg/25 cL	7000 mg/25 cL
Fin deformation	-	+	+++	+++++
Barbel whitening	-	+++	+++	+++++
Air gulping	-	+	+++++	+++++
Stunned positioning	-	+	+++	+++
Aggression	-	-	+++	+++
Erratic swimming	-	+	+	+++
Loss of balance	-	-	+	+++

-: Normal; +: weak; +++: moderate; +++++: high

Throughout the experiment, behavioural and morphological changes in the test fish were noted at 12-hour intervals. While there was a slight whitening of the barbels in the fish in the treatment tanks, the treatment tanks showed distinct morphological and behavioural changes. For instance, the fish in treatment one showed modest alterations in the shape and orientation of their fins, and their barbels lightened somewhat. In contrast, the fish in treatment two gulped due to insufficient dissolved oxygen, turned hostile, displayed a stunned posture, and showed mild fin deformation. The fish in treatment three exhibited moderate stunned positioning, aggression, erratic swimming, loss of body balance, and a high degree of fin deformation, barbel whitening, and gill damage due to insufficient dissolved oxygen.

3.2 Results of haematological parameters

According to Iheanacho et al. (2017), haematological indices are vital health markers that show the condition of fish's health both before and after trials.

Table 2. showed that the values of Mean Corpuscular Volume (115.15-112.33 fL), Hemoglobin (12.31-9.50 g/dL), Red Blood Cell (3.30-2.13 x $10^{12/L}$), White Blood Cell (15.82-10.90 x $10^{9/L}$), Packed Cell Volume (38.31%-28.11%), and Mean Cell Hemoglobin concentration (34.71-32.27 g/dL) decreased as *S. occidentalis* concentrations increased relative to the control treatment, while Heterophile (28.02%-37.04%) increased as *S. occidentalis* concentrations increased.

4 Discussion

Hematological parameters serve as standard indicators of fish health under aquaculture conditions and in ecotoxicological studies (Witeska et al., 2022). Their diagnostic value was demonstrated by Bojarski et al. (2022), who found hematological indices to be the most sensitive biomarkers of toxicity in *Cyprinus carpio*. These parameters are sensitive and are indicators of physiological alterations in fish; they provide extensive information on oxygen transport, immune status, stress, cytotoxicity, and genotoxicity (Witeska et al., 2023). In the present study, *Clarias gariepinus* exposed to *Senna occidentalis* leaf extract exhibited significant alterations in white blood cells (WBCs), red blood cells (RBCs), haemoglobin (Hb), packed cell volume (PCV), and erythrocyte indices (MCV, MCH, and MCHC), indicating that phytochemicals from *S. occidentalis* can disrupt hematological homeostasis. This supports hematology as a practical biomarker for evaluating plant-based xenobiotics in aquaculture species under laboratory conditions.

Table 2 Results of haematological parameters

Parameters	Control	Treatment A	Treatment B	Treatment C
Conc.	0 mg/25 cL	(3000 mg/25 cL)	(5000 mg/25 cL)	(7000 mg/25 cL)
PCV (%)	38.31 ^{c}	34.22 ^{b}	28.23 ^{a}	28.11 ^{a}
Hb (g/dL)	12.31 ^{d}	11.82 ^{c}	9.71 ^{a}	9.50 ^{a}
RBCs ($10^{12/L}$)	3.30 ^{b}	3.02 ^{b}	2.51 ^{a}	2.13 ^{a}
WBCs ($\times10^{9/L}$)	15.82 ^{e}	14.11 ^{d}	13.75 ^{c}	10.90 ^{a}
HET. (%)	28.02 ^{a}	34.12 ^{c}	34.02 ^{c}	37.04 ^{d}
LYM (%)	69.03 ^{c}	64.03 ^{b}	65.10 ^{b}	60.11 ^{a}
MCV (pg)	115.15 ^{c}	133.33 ^{b}	112.80 ^{a}	112.33 ^{b}
MCH (pg)	45.24 ^{d}	39.33 ^{c}	38.80 ^{b}	37.27 ^{a}
MCHC (g/dL)	34.71 ^{c}	34.64 ^{c}	33.93 ^{b}	32.27 ^{a}

Means along each row with different superscripts are significantly ($p < 0.05$) different. The values shown are the means and the standard deviations. Conc.= concentration, PCV = packed cell volume, Hb = haemoglobin, RBCs= red blood cell counts, WBCs =white blood cell count, HET = heterophil, LYM = lymphocyte, MCH = mean cell haemoglobin, MCV = mean cell volume, MCHC = mean cell haemoglobin concentration, fL = femtolitre, pg = picogram

Gebrelibanos et al. (2014), reported that Senna species had been known to cause a variety of toxicities despite their many potential medicinal benefits; this has been a major concern in aquaculture production (Idowu et al., 2017), as well as a condition wherein living organisms exhibit changes in their bodily systems and manifest symptoms due to impaired physiological functions (Adedeji et al., 2017), such as aggression, loss of balance, and erratic swimming. During this experiment, it was frequently noticed that the fish responded to environmental changes as soon as the extract was added to the water body. These responses included the morphological and behavioural indicators shown in Table 1. Fish were seen gasping for breath in the tanks containing high concentrations of *S. occidentalis* ethanol leaf extract (5000 and 7000 mg/ 25 cL), and whitening of the fins and barbels were noted in the tanks with concentrations of 3000 and 7000 mg/ 25 cL; this could be due to the potency and toxicological effectiveness of the phytochemicals (alkaloids, flavonoids, tannins, glycosides, steroids, and saponin) found in the plant extracts.

The gradual changes in the haematological parameters of *C. gariepinus* juveniles recorded in this study indicate that *S. occidentalis* ethanol leaf extract affects the blood of the exposed fish. The findings showed that the values of Mean Corpuscular Volume (115.15-112.33 fL), Hemoglobin (12.31-9.50 g/dL), Red Blood Cell (3.30-2.13 x $10^{12/L}$), White Blood Cell (15.82-10.90 x $10^{9/L}$), Packed Cell Volume (38.31%-28.11%), and Mean Cell Hemoglobin concentration (34.71-32.27 g/dL) decreased as *S. occidentalis* concentrations increased relative to the control treatment, while Heterophils (28.02%-37.04%) increased as *S. occidentalis* concentrations increased; this could be as a result of the poisonous potentials of *S. occidentalis* ethanolic extract, which rose with an increase in extract concentration in *C. gariepinus* blood; this is in line with studies by Eriegha et al., (2017); Idowu et al., (2020), who confirmed that infected fish had lower PCV values than healthy fish and observed a similar pattern in fish exposed to toxicants. Erythrocytes serve as models for assessing toxicity-induced apoptosis, oxidative stress, and cellular damage in fish (Sakuragui et al., 2019). Thus, the altered erythrocyte morphology and indices recorded here in *C. gariepinus* indicate that *S. occidentalis* phytochemicals may promote oxidative injury or apoptotic pathways in circulating red blood cells. Moreover, a decrease in RBC, Hb, and PCV typically indicates an anemic response to toxicants, resulting from direct hemolysis. A similar decrease in Ht, RBC, and Hb was reported in *Anabas testudineus* exposed to acrylamide (Ligina et al., 2022). Also, Ko et al. (2019) reported a concentration-related decrease in Ht, Hb, and RBC of *Platichthys stellatus* intoxicated with hexavalent chromium.

Leukocyte count (WBC) and differential leukocyte count (DLC) are standard indicators of immune status in fish. Toxicants commonly alter WBC, producing leukocytosis or leukopenia (Witeska et al., 2023). In the present study, *Clarias gariepinus* exposed to *Senna occidentalis* leaf extract showed a decrease in the value of WBC, suggesting that phytochemicals from *S. occidentalis* elicit a cytotoxic suppression of leukocyte homeostasis. Thus, hematology provides sensitive, practical biomarkers for assessing plant-based toxicants and indiscriminate use of *S. occidentalis* in aquaculture.

5 Conclusion

It can be concluded that haematological parameters are reliable and useful indicators of fish health status in response to environmental changes. The present study demonstrated that sub-lethal concentrations of *Senna occidentalis* leaf extract disrupt hematological homeostasis in *Clarias gariepinus.* Significant reductions in RBC, Hb, and PCV with altered MCV indicate hemolytic or hypoxic anemia. These changes are consistent with oxidative injury, impaired erythropoiesis, and cytotoxicity reported for other xenobiotics, indicating that bioactive compounds in *S. occidentalis* exert hematotoxic effects. Therefore, it can be stated that *S. occidentalis* toxicity caused consistent, gradual damage to the immune system of *C. gariepinus*. In relation to the potential risk of using *Senna occidentalis* in aquaculture environments or its relevance for toxicological assessment in fish, these data offer a reference point for assessing fish health and promoting safe application of plant-based extracts in aquaculture.

Author's Contribution

Idowu Adekunle Adedoyin conceived and designed the study, critically reviewed the manuscript, and approved the final version. Adesanya Oluwatosin Emmanuel participated in the hatchery experiment, drafted the manuscript, and contributed to funding support. Towolawi Adeleke Taofik contributed to funding and conducted the haematological analyses together with Adesanya Oluwatosin Emmanuel. Odukoya Abimbola Erastus documented the sub-acute exposure experiment and also contributed to manuscript preparation. Adekola Mukaila B. supervised the overall study, participated in the experimental design and coordination, and assisted in drafting the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The authors, in agreement, extend warm appreciation to the Head of Department (HOD) of Aquaculture and Fisheries Management for permission to experiment in the departmental hatchery. Also, to the HOD of Environmental Health Science for the facilities and support for this research.

References

Adedeji A.J., Maurice N.A., Wungak Y.S., Adole J.A., Chima N.C., Woma T.Y., Chukwuedo A.A., and Shamaki D., 2017, Diagnosis of ORF in West African dwarf goats in Uyo, Akwa Ibom State, Nigeria, African Journal of Infectious Diseases, 11(2): 90-94.
https://doi.org/10.21010/ajid.v11i2.12

Adeleye A.T., Bahar M.M., Megharaj M., Fang C., and Rahman M.M., 2024, The unseen threat of the synergistic effects of microplastics and heavy metals in aquatic environments: a critical review, Current Pollution Reports, 10: 47897.
https://doi.org/10.1007/s40726-024-00298-7

Afia O.E., and Gift S.D., 2017, Haematological profile and growth response of African sharptooth catfish (Clarias gariepinus, Burchell, 1822) fingerlings to locally formulated and commercial pelleted diets in tarpaulin tanks, American Journal of Innovative Research and Applied Sciences, 4(6): 213-222.

Ahmed I., Reshi Q.M., and Fazio F., 2020, The influence of the endogenous and exogenous factors on hematological parameters in different fish species: A review, Aquaculture International, 28: 869-899.
https://doi.org/10.1007/s10499-019-00501-3

Ali A., Devarajan S., Manickavasagan A., and Ata A., 2022, Anti-nutritional factors and biological constraints in the utilization of plant protein foods, Plant Protein Foods, Springer, pp. 407-438.
https://doi.org/10.1007/978-3-030-91206-2_14

Amoatey P., and Baawain M.S., 2019, Effects of pollution on freshwater aquatic organisms, Water Environment Research, 91: 1272-1287.
https://doi.org/10.1002/wer.1221

Belay W., and Enyew M., 2016, Major toxic plants and their effect on livestock: A review, Advances in Life Science and Technology, 45: 1-12.

Bojarski B., Osikowski A., Hofman S., Szała L., Szczygieł J., and Rombel-Bryzek A., 2022, Effects of exposure to a glyphosate-based herbicide on haematological parameters, plasma biochemical indices and the microstructure of selected organs of the common carp (Cyprinus carpio Linnaeus, 1758), Folia Biologica, 70: 213-229.
https://doi.org/10.3409/fb_70-4.24

Das S., Parida V.K., Tiwary C.S., Gupta A.K., and Chowdhury S., 2024, Emerging contaminants in the aquatic environment: fate, occurrence, impacts, and toxicity, ACS Symposium Series.
https://doi.org/10.1021/bk-2024-1475.ch001

Dey S., Saxena A., Kumar Y., Maity T., and Tarafdar A., 2022, Understanding the anti-nutritional factors and bioactive compounds of Kodo Millet (Paspalum scrobiculatum) and Little Millet (Panicum sumatrense), Journal of Food Quality, 2022: 1578448.
https://doi.org/10.1155/2022/1578448

Diouf A., Sarr F., Sene B., Ndiaye C., Fall S.M., and Ayessou N.C., 2019, Pathways for reducing anti-nutritional factors: Prospects for Vigna unguiculata, Journal of Nutritional Health and Food Science.
https://doi.org/10.15226/jnhfs.2019.001157

Egharevba H.O., Odigwe A.C., Abdullahi M.S., Okwute S.K., and Okogun J.I., 2010, Phytochemical analysis and broad-spectrum antimicrobial activity of Cassia occidentalis (L.) whole plant, New York Science Journal, 3(10): 74-81.

Erhunmwunse N.O., and Ainerua M.O., 2013, Characterisation of some blood parameters on African catfish (Clarias gariepinus), American-Eurasian Journal of Toxicological Sciences, 5(3): 72-76.

Eriegha O.J., Omitoyin B.O., and Ajani E.K., 2017, Evaluation of haematological and biochemical parameters of juvenile Oreochromis niloticus after exposure to water-soluble fractions of crude oil, Journal of Applied Sciences and Environmental Management, 21(6): 1041-1045.
https://doi.org/10.4314/jasem.v21i6.7

Gebrelibanos M., Periyasamy G., and Sintayehu B., 2014, Senna occidentalis seed: Is it a health risk or a potential medicine? International Journal of Pharmacognosy, 1(3): 161-167.

Idowu A.A., Popoola O.C., Alani J.O., Ipadeola A., and Nwekoyo V.E., 2020, Toxicity effect of Kigelia africana aqueous extract on the haematology and histopathology of juvenile Nile tilapia (Oreochromis niloticus), Agro-Science, 19(1): 37-42.
https://doi.org/10.4314/as.v19i1.6

Idowu T.A., Adedeji H.A., and Sogbesan O.A., 2017, Fish disease and health management in aquaculture production, International Journal of Environment and Agricultural Science, 1(1): 1-6.

Iheanacho S.C., Ogunji J.O., Ogueji E.O., Nwuba L.A., Nnatuanya I.O., Ochang S.N., Mbah C.E., Usman I.B., and Haruna M., 2017, Comparative assessment of ampicillin antibiotic and ginger (Zingiber officinale) effects on growth, haematology, and biochemical enzymes of Clarias gariepinus juveniles, Journal of Pharmacognosy and Phytochemistry, 6(3): 761-767.

Jun. H., Song, G., Yang, E., Youn, Y. and Kim, Y., 2012, Antioxidant activities and phenolic compounds of pigmented rice bran extracts. Journal of Food Science, vol. 77, no. 7, pp. C759-C764, 2012.
https://doi.org/10.1111/j.1750-3841.2012.02763.x

Ko H.D., Park H.J., and Kang J.C., 2019, Change of growth performance, hematological parameters, and plasma component by hexavalent chromium exposure in starry flounder, Platichthys stellatus, Fisheries and Aquatic Sciences, 22: 9.
https://doi.org/10.1186/s41240-019-0124-5

Ligina V., Martin R., Aiswarya M.V., Mashirin K.R., and Chitra K.C., 2022, Acute and sublethal effects of acrylamide on the freshwater fish Anabas testudineus (Bloch, 1792), Environmental Science and Pollution Research, 29: 90835-90851.
https://doi.org/10.1007/s11356-022-22155-0

Mustafa S.A., Al-Rudainy A.J., and Salman N.M., 2024, Effect of environmental pollutants on fish health: an overview, Egyptian Journal of Aquatic Research, 50: 225-233.
https://doi.org/10.1016/j.ejar.2024.02.006

Rocha A.C., Camacho C., Eljarrat E., Peris A., Aminot Y., and Readman J.W., 2018, Bioaccumulation of persistent and emerging pollutants in wild sea urchin Paracentrotus lividus, Environmental Research, 161: 354-363.
https://doi.org/10.1016/j.envres.2017.11.029

Sakuragui M.M., Paulino M.G., da Silva de Souza N.E., Tavares D., Terezanm A.P., Pesenti E., Giani A., Fernandes J.B., Cestari M.M., and Fernandes M.N., 2019, Crude extract of cyanobacterium Radiocystis fernandoi strain R28 induces anemia and oxidative stress in fish erythrocytes, Toxicon, 169: 18-24.
https://doi.org/10.1016/j.toxicon.2019.08.002

Samtiya M., Aluko R.E., and Dhewa T., 2020, Plant food anti-nutritional factors and their reduction strategies: An overview, Food Production, Processing and Nutrition, 2: 6.
https://doi.org/10.1186/s43014-020-0020-5

Sefali S., Ruby R., and Dimple D., 2026, Toxicological implications of emerging pollutants on aquatic organisms, Discover Environment, 4: 43.
https://doi.org/10.1007/s44274-026-00557-y

Thanigaivel S., Vickram S., Dey N., Jeyanthi P., Subbaiya R., and Kim W., 2023, Ecological disturbances and abundance of anthropogenic pollutants in the aquatic ecosystem: critical review of impact assessment on the aquatic animals, Chemosphere, 313: 137475.
https://doi.org/10.1016/j.chemosphere.2022.137475

Witeska M., Kondera E., and Bojarski B., 2023, Hematological and hematopoietic analysis in fish toxicology: A Review, Animals, 13(16): 2625.
https://doi.org/10.3390/ani13162625

Witeska M., Kondera E., Ługowska K., and Bojarski B., 2022, Haematological methods in fish: Not only for beginners, Aquaculture, 547: 737498.
https://doi.org/10.1016/j.aquaculture.2021.737498

International Journal of Aquaculture, 2026, Vol.16, No.3, 156-165
http://www.aquapublisher.com/index.php/ija

Research Article Open Access

Efficacy of a Closed-Water Depuration System in Reducing Bacterial Load in Bivalve Shellfish

K. Kavya [1], A. Raj [1], A. Suresh [1], A. A. Mohamed Hatha [1,2]

1 Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology (CUSAT), Kochi 682016, Kerala, India

2 CUSAT-NCPOR Centre for Polar Sciences, School of Marine Sciences, Cochin University of Science and Technology (CUSAT), Kochi 682016, Kerala, India

Corresponding author: mohamedhatha@gmail.com

International Journal of Aquaculture, 2026, Vol.16, No.3 doi: 10.5376/ija.2026.16.0013

Received: 23 Mar., 2026

Accepted: 25 May., 2026

Published: 15 Jun., 2026

Preferred citation for this article:

Kavya K., Raj A., Suresh A., and Hatha A.A.M., 2026, Efficacy of a closed-water depuration system in reducing bacterial load in bivalve shellfish, International Journal of Aquaculture, 16(3): 156-165 (doi: 10.5376/ija.2026.16.0013)

Abstract This study evaluated the effectiveness of a closed water depuration system equipped with a sponge filter for reducing bacterial contamination in black clams (*Villorita cyprinoides*) harvested from the Varapuzha region of the Cochin Estuary, Kerala, India. Initial bacteriological analysis revealed high levels of contamination in the clams, indicating potential food safety risks for consumers. The depuration experiment was conducted in a recirculating tank system fitted with a sponge filter, in which clams were maintained in clean water and sampled at regular time intervals up to 72 hours. Bacterial indicator reduction, including Total Coliforms (TC), Faecal Coliforms (FC), Total Heterotrophic Bacteria (THB), and *Vibrio* species were monitored to evaluate the purification efficiency of the system. Results demonstrated a marked reduction in bacterial load during depuration, with the most pronounced decrease occurring within the first 48 hours. Total coliforms and faecal coliforms were reduced by 98.54% and 98.45%, respectively, reaching substantially lower faecal indicator levels, although compliance with specific regulatory standards requires validation using the prescribed indicator organisms and units. Total heterotrophic bacteria also showed a substantial decline, while *Vibrio* species were reduced to a lesser extent. Nevertheless, a gradual increase in bacterial counts was observed on the inner walls of the depuration tank due to biofilm formation, indicating the possibility of recontamination if the system is not properly cleaned. Overall, the sponge filter-based closed water depuration system proved to be a simple, cost-effective, and water-efficient method for improving the microbiological quality of clams. This approach is suitable for small-scale depuration practices and could help to improve shellfish safety and protect public health. This study may provide practical baseline information for optimising low-cost shellfish depuration systems under tropical estuarine conditions.

Keywords Depuration; Black clam (*Villorita cyprinoides)*; Sponge filter; Bacterial reduction; Biofilm; Closed water depuration system

1 Introduction

Seafood constitutes a vital component of the global diet, particularly in developing countries where it serves as a major source of affordable animal protein. Among the various seafood categories, bivalve molluscs such as clams, mussels, oysters, and scallops are especially important due to their high nutritional value, ecological significance, and economic potential (FAO (Food and Agriculture Organization), 2022). In India, bivalves play a significant role in coastal fisheries, contributing substantially to local livelihoods and the seafood export industry (Laxmilatha, 2018; Anil et al., 2024).

Bivalves are filter-feeding organisms that obtain their nutrition by filtering large volumes of the surrounding water. This feeding mechanism allows them to accumulate suspended particulate matter, including phytoplankton, organic detritus, bacteria, and potentially harmful pathogens (Min et al., 2024; Ochoa-Esteso et al., 2024). While this ecological function contributes to water purification and nutrient cycling, it also makes bivalves susceptible to the bioaccumulation of contaminants, particularly when they inhabit or are harvested from polluted aquatic environments (Martínez-Albores et al., 2020). Consequently, bivalves can act as vectors for foodborne illnesses caused by bacterial, viral, and protozoan pathogens, especially when consumed raw or insufficiently processed (Desdouits et al., 2023).

The black clam (*Villorita cyprinoides*) is a commercially important bivalve species in India, accounting for more than 64% of clam landings in Kerala. The major harvesting grounds are located in the Vembanad and Ashtamudi lakes (Suja and Mohamed, 2010). However, important shellfish harvesting areas such as the Cochin Estuary, which forms part of the Vembanad wetland system and is recognised as a Ramsar site, are increasingly affected by anthropogenic pollution, including domestic sewage discharge, municipal runoff, and industrial effluents (Chinnadurai et al., 2020; Chinnadurai et al., 2023; Nandakrishnan and Prasad, 2024).

Several studies have reported microbial contamination in estuarine waters and shellfish from the Cochin region, raising serious public health concerns. Earlier research documented the presence of diarrheagenic strains of *Escherichia coli* and *Salmonella* spp. in the Cochin Estuary, highlighting the risks associated with untreated sewage discharge (Peralta and Andalecio, 2011). Similarly, the bacteriological quality of green mussels (*Perna viridis*) from the same estuary has revealed elevated levels of faecal indicator bacteria and *Vibrio* species (Padua et al., 2023). Clams from the adjacent Vembanad Lake have also been reported to contain faecal indicator bacteria, *Vibrio* species, and *Aeromonas spp.*, posing significant health risks to consumers and seafood handlers (Vaiyapuri et al., 2021; Silvester et al., 2022).

Apart from microbial contamination, the Cochin Estuary is increasingly affected by nutrient enrichment, chemical pollution, habitat degradation, overfishing, and unregulated coastal development, all of which have contributed to the deterioration of water quality (Thasneem et al., 2018). These environmental stressors further increase microbiological risks associated with shellfish consumption, thereby emphasising the need for stringent food safety regulations and effective sanitary measures (European Food Safety Authority [EFSA], 2010).

Globally, regulatory frameworks in regions such as the European Union, the United States, and Australia mandate routine monitoring of microbiological water quality in shellfish-growing areas to safeguard public health and ensure the safety of bivalve molluscs for consumption (European Commission, 2019; U.S. Food and Drug Administration [FDA], 2023). In the European Union, legislation requires competent authorities to classify production and relaying areas for live bivalve molluscs and to routinely monitor faecal contamination using established standards for *E. coli* levels. Harvesting is temporarily suspended when these standards are not met, until corrective actions such as relaying or depuration are carried out (Ciccarelli et al., 2022; Pinn and Le Vay, 2023).

Depuration is a widely adopted post-harvest process in which live bivalves are maintained under controlled conditions in tanks containing clean, treated water, allowing them to naturally purge accumulated contaminants (Martínez-Albores et al., 2020). The efficiency of depuration depends on several factors, including water quality, system design (flow-through or recirculating systems), duration of depuration, and the use of disinfection methods such as ultraviolet irradiation or biofiltration (Oliveira et al., 2011; Küniļi, 2024).

Among the various depuration methods, closed water depuration systems have emerged as a promising alternative to conventional open-flow systems. These systems utilise recirculating water coupled with biological filtration to remove microorganisms while conserving water and allowing greater control over environmental conditions (Campbell et al., 2022; Chinnadurai et al., 2023). Such systems are particularly beneficial in areas where access to clean water is limited or unreliable. During depuration, clams expel ingested microorganisms and contaminants in mucus-coated faecal pellets, which may lead to recontamination if not effectively removed from the system. Therefore, an efficient biofilter is essential for trapping and reducing expelled microorganisms in closed-water depuration systems.

Despite these advantages, closed water depuration systems face challenges such as the formation of biofilms on tank surfaces and filtration units. Biofilms consist of complex microbial communities embedded within an extracellular polymeric substance (EPS) matrix, which provides protection against environmental stressors such as ultraviolet radiation, salinity fluctuations, and antimicrobial agents (Flemming et al., 2016). Biofilm formation begins with bacterial attachment to submerged surfaces, followed by colonisation, EPS secretion, and maturation.

Once established, biofilms can harbour pathogenic and antibiotic-resistant bacteria, potentially turning depuration systems into sources of recontamination if not properly maintained (Azeredo et al., 2017). In aquaculture systems, such biofilms may contribute to water quality deterioration and increased microbial loads. Therefore, regular sanitisation of depuration tanks after each cycle is essential to maintain system efficiency and biosecurity (Canadian Food Inspection Agency (CFIA)., 2017).

The present study aimed to evaluate the effectiveness of a sponge filter-based closed-water depuration system in reducing bacterial contamination in black clams collected from the Varapuzha region of the Cochin Estuary. Changes in THB, TC, FC, and *Vibrio* spp. during depuration were monitored, and the microbial load on tank-wall biofilms was furthermore assessed to evaluate the potential of recontamination.

2 Results

2.1 Bacterial load in initial shellfish samples

The initial bacteriological load of *Villorita cyprinoides* (black clam) was determined prior to depuration. The bacterial parameters assessed included Total Coliforms (TC), Faecal Coliforms (FC), Total Heterotrophic Bacteria (THB), and *Vibrio* spp. (Table 1).

Table 1 Initial bacteriological load in raw shellfish samples

Bacteriological Parameter	Load
Total Heterotrophic Bacteria (THB)	1.68×10^7 CFU/g
Total Coliforms (TC)	2.4×10^4 MPN/100 g tissue
Faecal Coliforms (FC)	1.5×10^4 MPN/100 g tissue
Vibrio spp.	5.1×10^5 CFU/g

2.2 Bacteriological changes in clams during depuration

Bacterial load in the *Villorita cyprinoides* (black clam) samples at different depuration time intervals (0 h, 6 h, 12 h, 24 h, 48h and 72 h) was recorded for all parameters (Table 2).

Table 2 Bacteriological load in shellfish samples at different depuration time intervals

Sample No	Depuration time intervals (Hours)	Total Heterotrophic Bacteria load (CFU/g)	Total Coliforms load (MPN index/100mL)	Faecal Coliforms load (MPN index/100mL)	*Vibrio* spp. load (CFU/g)
1	0.00	1.68×10^7	2.4×10^4	1.5×10^4	5.1×10^5
2	6.00	1.29×10^7	1.5×10^4	1.1×10^4	4.4×10^5
3	12.00	9×10^6	1.1×10^4	4.6×10^3	3×10^5
4	24.00	1.54×10^6	4.6×10^3	2.1×10^3	1.5×10^5
5	48.00	1.03×10^6	3.5×10^2	2.3×10^2	1.2×10^5
6	72.00	1.22×10^6	3.8×10^2	2.3×10^2	1.4×10^5

2.2.1 Reduction in THB (Total Heterotrophic Bacteria) count in *Villorita cyprinoides*

The initial THB count was found to be 1.68×10^7 cfu/g. A 1.21 logs reduction was obtained within the first 48 hours of depuration to a THB count of 1.03×10^6 cfu/g. After 48 hours of depuration around 93.86% (1.21 logs) of THB reduction was observed (Table 2 and Figure 1). However, complete depuration of total heterotrophic bacteria (THB) was not achieved. Total Heterotrophic Bacteria showed a moderate but statistically significant reduction ($F = 18.6$, $p < 0.05$).

2.2.2 Reduction in Total Coliform (TC) Count in *Villorita cyprinoides*

The initial TC count was found to be 2.4×10^4 MPN/100 mL (Table 2). Reduction of 0.72 log was obtained within the 24 hours of depuration, and a further 1.84 logs (98.54%) reduction was observed after 48 hours of depuration to a final TC count of 2.54 logs (Figure 2). Thus, during the entire 72 h depuration process using a sponge filter, a total reduction of nearly 1.8 logs to a final count of 3.8×10^2 MPN/100 mL could be accomplished. However, complete depuration of TC could not be attained, even after 72 h of depuration. TC load of 2.57 logs remained in shellfish.

The reductions in Total Coliform counts were highly significant across depuration intervals ($F = 27.3$, $p < 0.01$), with the most pronounced effects observed within the first 48 hours.

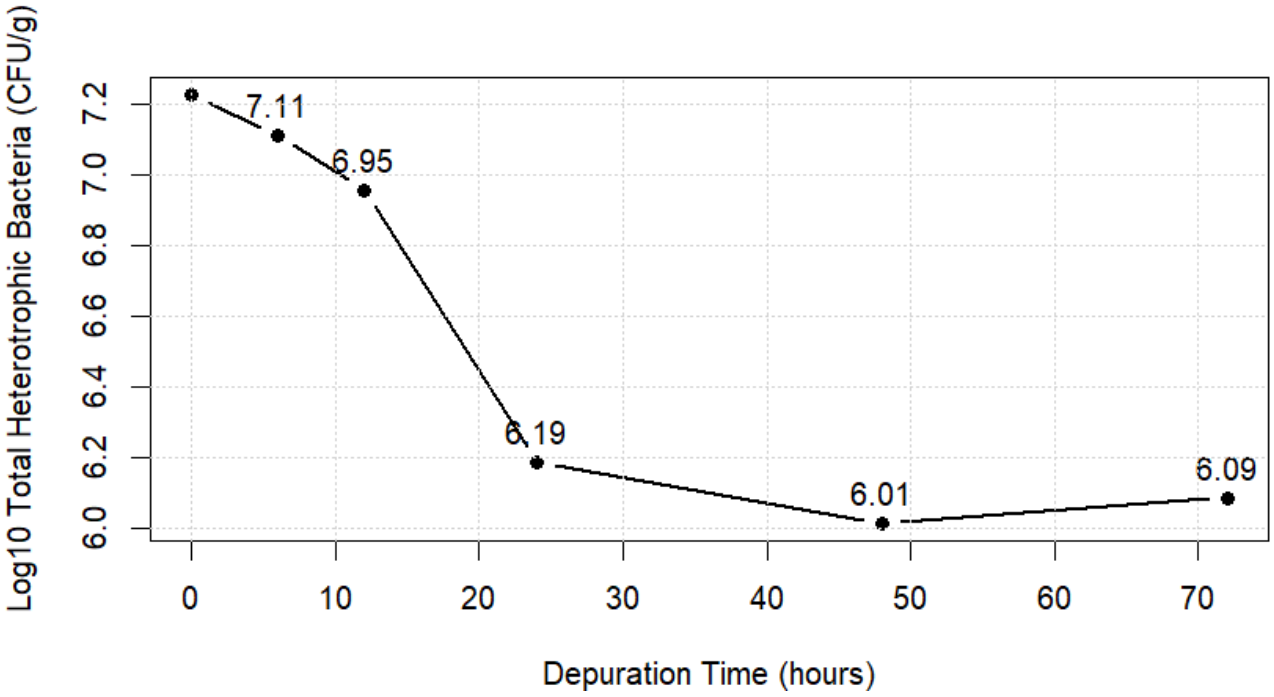

Figure 1 THB reduction in *Villorita cyprinoides* during closed water depuration with a sponge filter

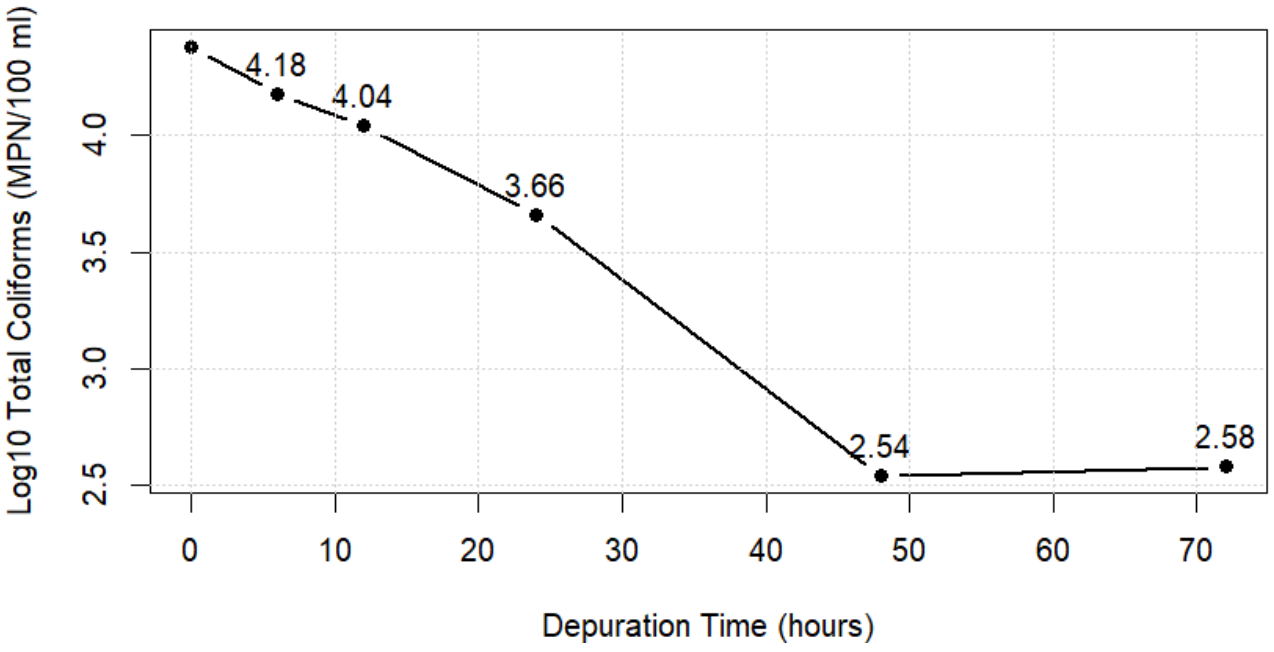

Figure 2 TC reduction in *V. cyprinoides* during closed water depuration with a sponge filter

2.2.3 Reduction in Faecal Coliform (FC) count in *Villorita cyprinoides*

The initial Faecal Coliform (FC) concentration in naturally contaminated *Villorita cyprinoides* from the Cochin Estuary was recorded at 1.5×10^4 MPN/100 mL (4.17 logs), aligning only with Class C shellfish growing area standards under EU regulations. After 48 hours, FC levels decreased to 230 MPN/100 mL (2.36 logs), and by 72 hours, a total removal rate of 98.45% (1.82 logs) was attained, which falls in the acceptable FC regulatory limits of

depurated shellfish (Table 2 and Figure 3). The reductions in Faecal Coliform counts were highly significant across depuration intervals ($F = 25.8$, $p < 0.01$), with the most pronounced effects observed within the first 48 hours.

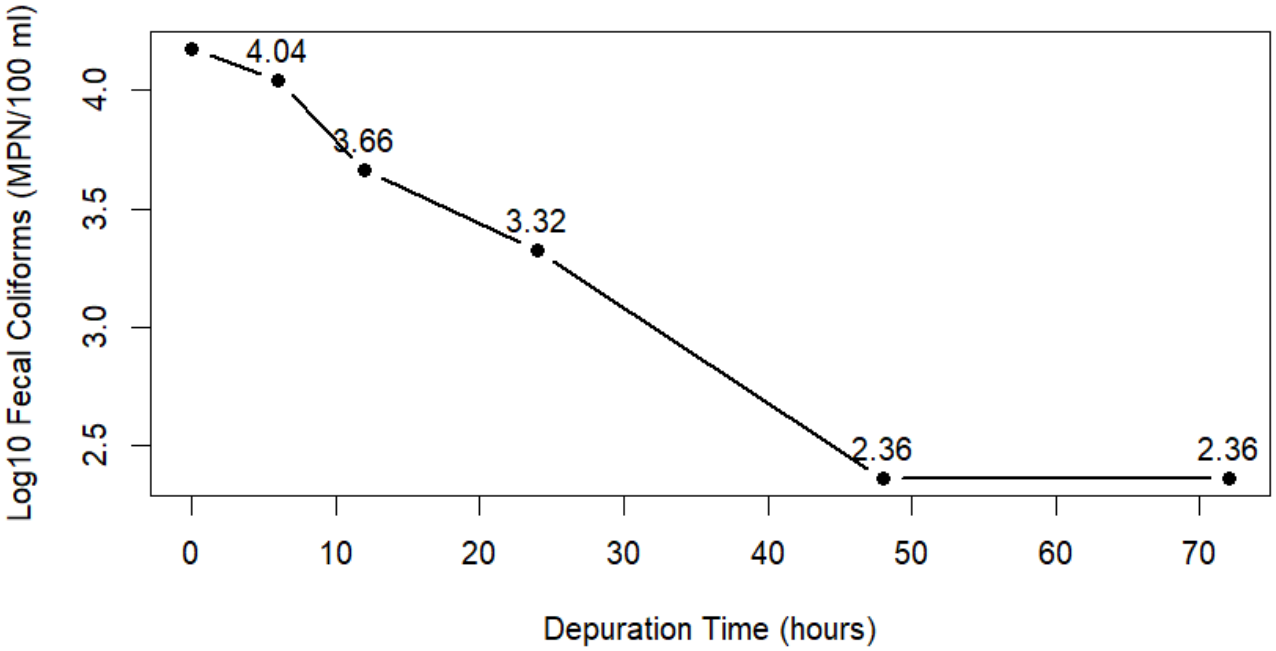

Figure 3 FC reduction in *V. cyprinoides* during closed water depuration with a sponge filter

2.2.4 Reduction in *Vibrio* spp. count in the black clam *Villorita cyprinoides*

The initial *Vibrio* spp. count was found to be $5.1x10^5$ cfu/g. After 48 hours of depuration the *Vibrio* spp. count becomes $1.2x10^5$ cfu/g (reduction around 76.47 %) (Table 2 and Figure 4). Complete depuration of *Vibrio* spp was not achieved within the 72 hours of depuration.

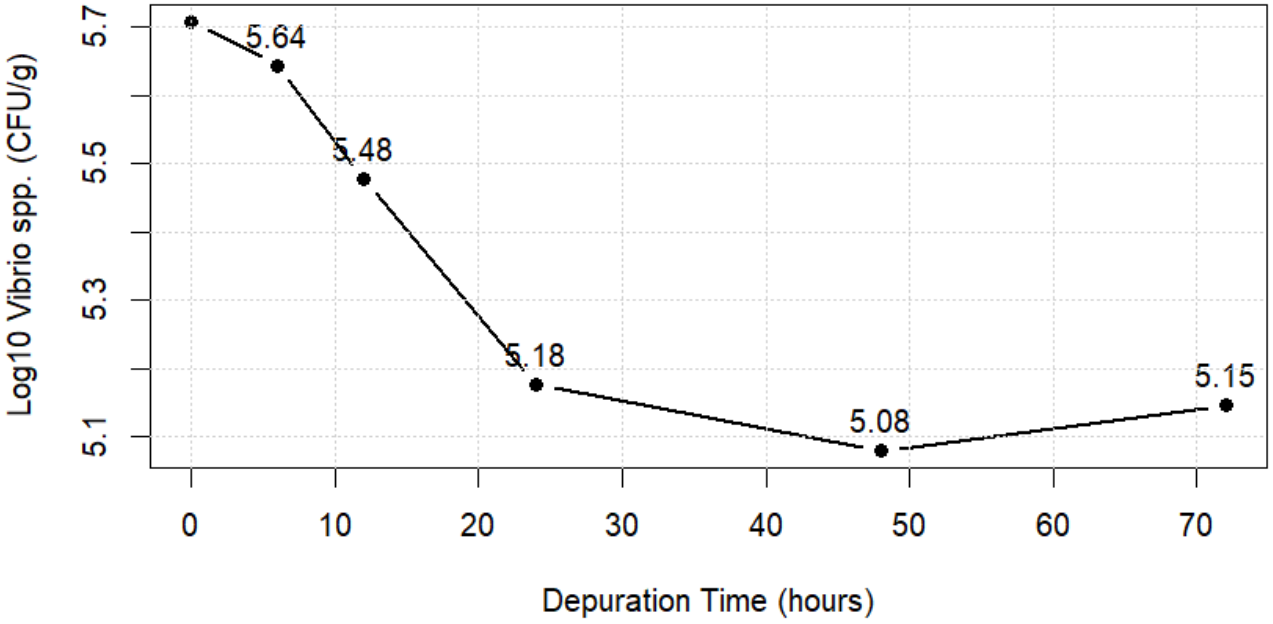

Figure 4 *Vibrio* spp. reduction in *V. cyprinoides* during closed water depuration with a sponge filter

2.3 Bacteriological changes in biofilm-associated microbes on the depuration tank walls during depuration

Bacterial load in the biofilm samples of the depuration tank wall at different depuration time intervals (0 h, 24 h, 48h, 72 h) was recorded for all parameters, including TC, FC, THB and *Vibrio* spp. Count (Table 3). The results showed

a progressive increase in microbial load on the tank walls over time, indicating the formation and accumulation of biofilm-associated bacteria during depuration.

Table 3 Microbial load in biofilm on depuration tank walls at different time intervals

Sample No	Depuration time intervals (Hours)	Total Heterotrophic Bacteria (CFU/g)	Total Coliforms load (MPN index/100mL)	Faecal Coliforms load (MPN index/100mL)	*Vibrio* spp. Load (CFU/g)
1	0.00	1.4×10^3	<3	<3	0
2	24.00	7.5×10^3	21	210	2.9×10^4
3	48.00	1.36×10^5	1100	>1100	2.48×10^5
4	72.00	2.83×10^5	>1100	>1100	2.85×10^6

3 Discussion

The high levels of faecal indicator bacteria detected in raw black clams collected from the Cochin Estuary indicate poor sanitary conditions in the harvesting environment and suggest a potential risk of enteric pathogen contamination. This is consistent with the filter-feeding habit of bivalves, which enables them to accumulate microorganisms from the surrounding water at levels often higher than those in the environment. Similar observations have been reported for shellfish harvested from polluted estuarine systems, where bacterial contamination has been associated with untreated sewage discharge, surface runoff, and other anthropogenic inputs. In the present study, the high initial counts of total coliforms, faecal coliforms, total heterotrophic bacteria, and *Vibrio* spp. confirm that clams collected from the study area may pose a food safety risk if consumed without adequate post-harvest treatment.

The results of the depuration trial demonstrated that the closed-water depuration system equipped with a sponge filter was effective in reducing bacterial contamination in *Villorita cyprinoides.* The most pronounced reduction occurred during the first 48 h of depuration, during which total coliforms, faecal coliforms, and total heterotrophic bacteria declined substantially. This pattern suggests that the early phase of depuration is the most efficient period for bacterial elimination under the present system conditions. Similar findings have been reported in previous studies, where the majority of bacterial reduction in bivalves occurred within the first 24~48 h of depuration. The marked decline observed in the present study indicates that the recirculating sponge filter system provided suitable conditions for effective microbial purging while maintaining clam survival.

Among the microbial indicators examined, faecal coliforms showed one of the highest rates of reduction and reached levels close to accepted regulatory limits after 48 h, further supporting the effectiveness of the system for improving the sanitary quality of the shellfish. In contrast, *Vibrio* spp. showed comparatively lower removal efficiency and persisted throughout the depuration period. This lower reduction may reflect the greater environmental resilience of *Vibrio* spp. and their ability to survive under depuration conditions more effectively than faecal indicator bacteria. The persistence of *Vibrio* spp. observed in this study is important from a food safety perspective, as it indicates that while the system is effective for reducing indicator bacteria, it may be less efficient in eliminating more robust or potentially pathogenic bacterial groups. Therefore, depuration alone may not always be sufficient to completely remove microbiological hazards from shellfish harvested from contaminated environments.

An additional practical finding of this study was the progressive increase in bacterial load on the inner walls of the depuration tank during the later stages of depuration. This trend suggests the formation of biofilms, which may act as reservoirs of microorganisms and contribute to secondary contamination of the system. The slight increase in bacterial counts observed after prolonged depuration may therefore be related to biofilm-associated recontamination. This finding highlights an important operational limitation of closed-water depuration systems: although they are simple, water-efficient, and suitable for small-scale use, their effectiveness depends strongly on proper cleaning and maintenance between depuration cycles. Overall, the present study demonstrates that a sponge filter-based closed-water depuration system can significantly improve the microbiological quality of black clams within a

relatively short period, particularly within the first 48 h. However, regular sanitation and system management are essential to minimize biofilm formation and maintain long-term depuration efficiency.

4 Materials and Methods

4.1 Study area

Live black clams (*Villorita cyprinoides*) were collected from Varapuzha region (10°04′34.85″ N, 76°16′00.33″ E) of Ernakulam District, Kerala, India (Figure 5). This site is an oligohaline zone of the Cochin backwater estuary with salinity recorded at 0.03‰ during the wet season and 0.05‰ during the dry season (Abhilash et al., 2012).

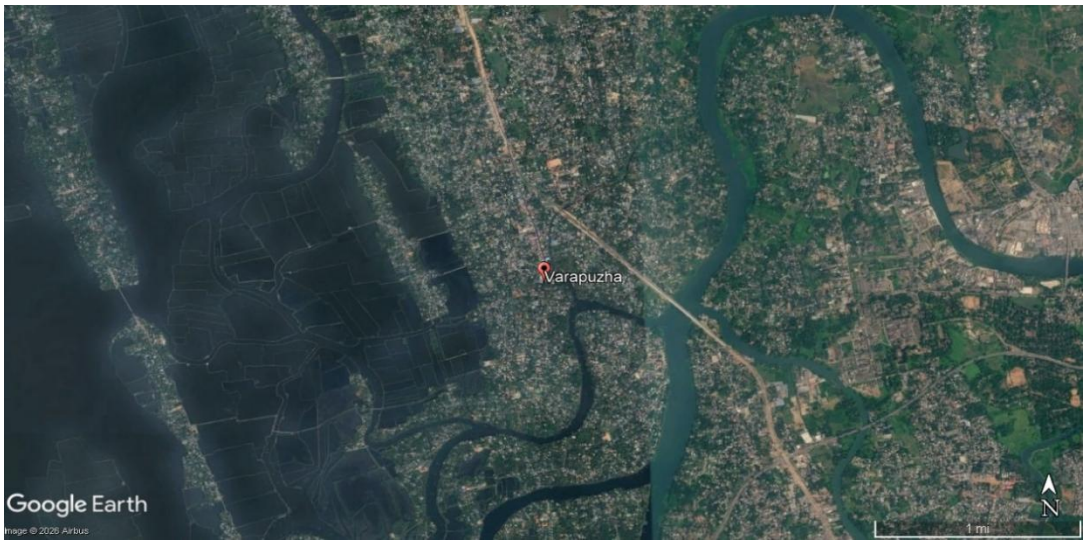

Figure 5 Map showing the sampling site at Varapuzha, Kerala, India

4.2 Collection of shellfish samples

The samples were used for bacteriological analysis and depuration experiments.

Bacteriological analyses were performed on shellfish samples to determine the initial microbial load and to monitor changes during the depuration process. Samples were collected at different depuration intervals (0, 6, 12, 24, 48, and 72 h). Total Coliforms (TC) and Faecal Coliforms (FC) were enumerated using the Most Probable Number (MPN) method, while Total Heterotrophic Bacteria (THB) and *Vibrio* spp. were determined using the total plate count method following standard microbiological procedures (Cappuccino and Sherman, 2014; APHA, 2017). Biofilm samples were also collected from the inner walls of the depuration tank at 0, 24, 48, and 72 h using sterile swabs and analyzed for TC, FC, THB, and *Vibrio* spp. using the same procedures.

4.3.1 Enumeration of Total Coliforms (TC)

Most Probable Number (MPN) method using lactose broth as the medium was used to enumerate total coliform load in the shellfish sample. Ten grams of bivalve tissue is aseptically weighed and homogenized in 90mL of sterile distilled water using tissue homogenizer (Masticator, Spain). From this homogenate 3×10 mL samples were transferred to 10 mL double strength lactose broth. 3×1 mL samples were transferred to 9 mL single strength lactose broth and 3×0.1 mL into 9.9 single strength lactose broth. Tubes were incubated at 37 ºC and observed for growth and gas production in the Durham tubes kept inverted in the test tubes. Gas production after 24 to 48 hours is considered positive for the presence of coliforms. The number of positive tubes in each dilution (10 mL, 1 mL, and 0.1 mL) was recorded and referred to *McCardy's* MPN table to estimate the total coliform load.

4.3.2 Enumeration of Faecal Coliforms (FC)

The tissue was processed as mentioned in the above section 4.3.1, and incubated at 44.5 °C to enumerate faecal coliform in the sample. After the incubation (growth and gas production) positive tubes in each dilution were recorded and referred to McCardy's MPN table to estimate the faecal coliform load.

4.3.3 Enumeration of Total Heterotrophic Bacteria (THB)

Ten grams of tissue was aseptically weighed and transferred into a tissue homogeniser (Masticator, Spain) and homogenised in 90 mL sterile distilled water for one minute. Serial dilutions were made from this homogenised sample by transferring 1 mL to 9 mL sterile distilled normal saline blank. Serial dilutions were made up to 10^{-5}. For enumeration of THB, 0.1 mL of appropriate dilutions (10^{-3} to 10^{-5}) were spread plated on sterile nutrient agar plates. The plates were then incubated at room temperature for 24 to 48 hours. Plates with colony number ranging from 25 - 250 were taken for counting and THB load is expressed as the number of colony forming units per gram of bivalve tissue (cfu/g).

4.3.4 Enumeration of *Vibrio* spp.

For enumeration of *Vibrio* spp., tissue samples were processed as described for THB analysis. Appropriate serial dilutions (10^{-3}~10^{-5}) were spread-plated onto Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar plates. The plates were incubated at room temperature for 24~48 h, and plates containing 25~250 colonies were counted. The results were expressed as CFU g^{-1} of bivalve tissue.

4.3.5 Biofilm sample analysis

Biofilm samples were collected from the inner walls of the depuration tank at 0, 24, 48, and 72 h using sterile cotton swabs. The swabs were transferred into sterile conical flasks containing 90 mL sterile distilled water and vortexed to ensure uniform suspension of biofilm-associated bacteria. The resulting suspension was analyzed for TC, FC, THB, and *Vibrio* spp. using the same procedures, media, and incubation conditions described for shellfish samples (Sections 4.3.1~4.3.4).

4.4 Depuration experiment

Prior to the experiment, the collected clams (*Villorita cyprinoides*) were examined to ensure that they were alive and actively filtering. The sampling site, characterized by fine silty-clay substrate rich in suspended particulate organic matter, provided favorable conditions for active siphonal filtration, which was further confirmed by visual observation of siphon extension, valve gaping, and rapid valve closure upon tactile stimulation. Clams showing no response, persistent shell opening, or foul odour were discarded prior to analysis.

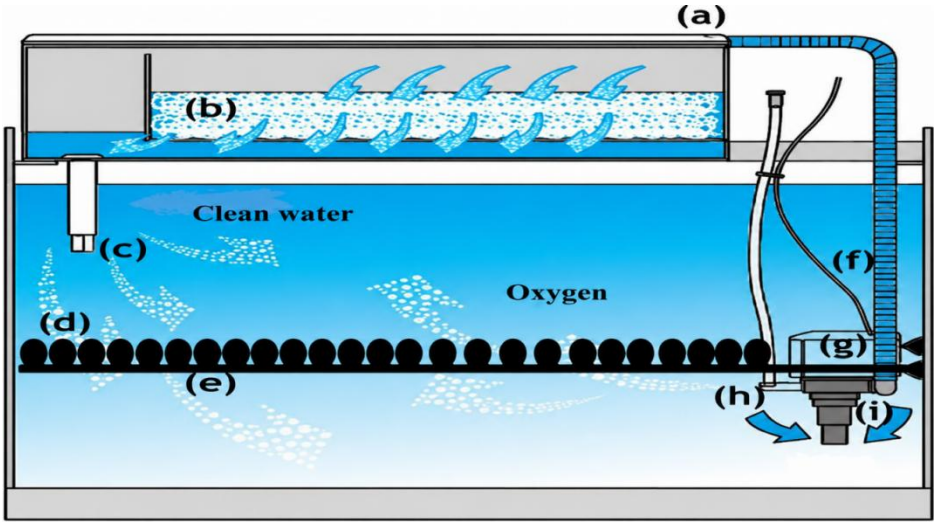

Figure 6 Schematic representation of the depuration tank

Figure caption: (a) Water outlet to bio filter (b) Sponge layer (c) Water outlet to tank after passing through the biofilter (d) Clam (e) Wire mesh (f) Water transporting pipe (g) Water pump (h) Air inlet (i) Water inlet

4.4.1 Design of depuration tank and depuration process

The depuration system consisted of a closed water holding glass tank with a capacity of 55 litres and dimensions 70 x 30 x 30 cm. A wall hung immersion water pump (Dophin P-708, China), placed 10 cm above the bottom of the tank, re-circulated (15 litres/min) the water in the depuration tank which was then passed through sponge filter held

within a holder placed above the tank. For depuration, 45 litres of tap water were used. The experiment was carried out at ambient temperature (29℃~30℃). Approximately, 100 medium sized clams were arranged in monolayer on a plastic mesh rack which was suspended 15 cm above, from the tank bottom to prevent re-contamination from the faecal material settled at the bottom. About 10~15 shellfish were taken out at various intervals of 0 h, 6 h, 12 h, 24 h, 48 h and 72 h using a sterile spatula. Then total coliforms, faecal coliforms, total heterotrophic bacteria and *Vibrio* spp count were enumerated as described above Sections 4.3.1 to 4.3.4. Clams survived well throughout the experiment, however, any dead ones if found were removed from the system immediately. Clams were not fed during the entire period of depuration process.

Author's contributions

Mohamed Hatha conceptualised the work plan, designed the depuration system, reviewed the results and reviewed the manuscript. Kavya, Raj and Suresh carried out the lab work. Kavya also involved in developing the manuscript.

Acknowledgments

The authors also express their sincere thanks to the Head, Department of Marine Biology, Microbiology and Biochemistry, Cochin University of Science and Technology (CUSAT) for providing laboratory facilities to carry out this study. We extend our gratitude to all the staff and colleagues who assisted during sample collection, laboratory analysis, and data processing.

References

Abhilash K.R., Raveendran T.V., Mol V.L., and Deepak M.P., 2012, Sediment oxygen demand in Cochin backwaters, a tropical estuarine system in the south-west coast of India, Marine Environmental Research, 79: 160-166.
https://doi.org/10.1016/j.marenvres.2012.05.005

Anil M.K., Gomathi P., Kavitha M., Raju B., Shalini O., Krishna Priya P.M., and Laxmilatha P., 2024, Recent advances in bivalve seed production in India: salient research findings, technologies and their social impact, Marine Fisheries Information Service; Technical and Extension Series, 261: 7-14.

Azeredo J., Azevedo N.F., Briandet R., Cerca N., Coenye T., Costa A.R., and Sternberg C., 2017, Critical review on biofilm methods, Critical Reviews in Microbiology, 43(3): 313-351.
https://doi.org/10.1080/1040841X.2016.1208146

Campbell V.M., Chouljenko A., and Hall S.G., 2022, Depuration of live oysters to reduce Vibrio parahaemolyticus and Vibrio vulnificus: a review of ecology and processing parameters, Comprehensive Reviews in Food Science and Food Safety, 21(4): 3480-3506.
https://doi.org/10.1111/1541-4337.12969

Canadian Food Inspection Agency (CFIA), Fisheries and Oceans Canada (DFO), and Environment and Climate Change Canada (ECCC), 2017, Canadian Shellfish Sanitation Program (CSSP) Manual, Government of Canada, Ottawa, Canada.

Chinnadurai S., Campos C.J., Geethalakshmi V., Sharma J., Kripa V., and Mohamed K.S., 2020, Microbiological quality of shellfish harvesting areas in the Ashtamudi and Vembanad estuaries (India): environmental influences and compliance with international standards, Marine Pollution Bulletin, 156: 111255.
https://doi.org/10.1016/j.marpolbul.2020.111255

Chinnadurai S., Elavarasan K., Geethalakshmi V., Kripa V., and Mohamed K.S., 2023, Development of a depuration protocol for commercially important edible bivalve molluscs of India: ensuring microbiological safety, Food Microbiology, 110: 104172.
https://doi.org/10.1016/j.fm.2022.104172

Ciccarelli C., Leinoudi M., Semeraro A.M., Di Trani V., Ciccarelli E., and Consorti G., 2022, European legislation and live bivalve molluscs: are the criteria for microbiological safety matching with the criteria for sanitary classification of harvesting areas?, Italian Journal of Food Safety, 11(1): 9956.
https://doi.org/10.4081/ijfs.2022.9956

Desdouits M., Reynaud Y., Philippe C., and Guyader F.S.L., 2023, A comprehensive review for the surveillance of human pathogenic microorganisms in shellfish, Microorganisms, 11(9): 2218.
https://doi.org/10.3390/microorganisms11092218

European Commission, 2019, Commission Implementing Regulation (EU) 2019/627 of 15 March 2019 laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption, European Commission, Brussels, Belgium.

European Food Safety Authority (EFSA), 2010, Scientific opinion on marine biotoxins in shellfish - emerging toxins: brevetoxin group, EFSA Journal, 8(7): 1677
https://doi.org/10.2903/j.efsa.2010.1677

Flemming H.C., Wingender J., Szewzyk U., Steinberg P., Rice S.A., and Kjelleberg S., 2016, Biofilms: an emergent form of bacterial life, Nature Reviews Microbiology, 14(9): 563-575.
https://doi.org/10.1038/nrmicro.2016.94

Food and Agriculture Organization (FAO), 2022, The state of world fisheries and aquaculture 2022, Food and Agriculture Organization of the United Nations, Rome, Italy.

Künili İ.E., 2024, Comparative analysis of the efficiency of different commercial depuration systems and the evaluation of species-specific depuration conditions in bivalve mollusc production, Aquacultural Engineering, 107: 102468.
https://doi.org/10.1016/j.aquaeng.2024.102468

Lalitha K.V., and Surendran P.K., 2005, Microbial profile of black clam (Villorita cyprinoides), Fishery Technology, 42: 69-74.

Laxmilatha P., 2018, Sustainable bivalve farming: alternate livelihood options for empowerment of women, In: Aquaculture for nutritional and livelihood security, Narendra Publishing House, New Delhi, India, pp.109-134.

Malarvannan G., and Edward J.K.P., 2015, Bacterial quality and depuration of the green mussel Perna viridis from natural beds, Journal of Indian Fisheries Association, 29.

Martinez-Albores A., Lopez-Santamarina A., Rodriguez J.A., Ibarra I.S., Mondragon A.D.C., Miranda J.M., and Cepeda A., 2020, Complementary methods to improve the depuration of bivalves: a review, Foods, 9(2): 129.
https://doi.org/10.3390/foods9020129

Min J.G., Kim Y.C., and Kim K.I., 2024, Role of filter-feeding bivalves in the bioaccumulation and transmission of white spot syndrome virus (WSSV) in shrimp aquaculture systems, Pathogens, 13(12): 1103.
https://doi.org/10.3390/pathogens13121103

Nandakrishnan, and Prasad P.R.C., 2024, Geoscape characterization of Ashtamudi, Sasthamkotta, and Vembanad Ramsar sites in Kerala, India, Water Resources, 51(4): 462-474.
https://doi.org/10.1134/S009780782360273X

Ochoa-Esteso C., Roselló-Carrió A., Carrasco-Correa E.J., and Lerma-García M.J., 2024, Bioaccumulation of environmental pollutants and marine toxins in bivalve molluscs: a review, Exploration of Foods and Foodomics, 2(6): 788-809.
https://doi.org/10.37349/eff.2024.00062

Oliveira J., Cunha A., Castilho F., Romalde J.L., and Pereira M.J., 2011, Microbial contamination and purification of bivalve shellfish: crucial aspects in monitoring and future perspectives - a mini-review, Food Control, 22(6): 805-816.
https://doi.org/10.1016/j.foodcont.2010.11.032

Padua S., Kripa V., Prema D., Mohamed K.S., Jeyabaskaran R., Kaladharan P., and Babu A., 2023, Assessment of ecosystem health of a micro-level Ramsar coastal zone in the Vembanad Lake, Kerala, India, Environmental Monitoring and Assessment, 195(1): 95.
https://doi.org/10.1007/s10661-022-10692-7

Peralta E.M., and Andalecio M.N., 2011, Microbiological quality of oyster (Crassostrea sp.) and mussel (Perna viridis) in selected growing areas in Western Visayas, Philippines, Philippine Journal of Natural Science, 16: 1-8.

Pinn E.H., and Le Vay L., 2023, Interpretation of the European legal framework for the microbiological classification of bivalve mollusc production areas, Marine Policy, 148: 105479.
https://doi.org/10.1016/j.marpol.2023.105479

Silvester R., Saji A., Divakaran A.R., Dilshana P.M., Nair R., Hatha M., and Harikrishnan M., 2022, Increased incidence and antimicrobial resistance among Vibrio parahaemolyticus in shellfishes from major fish markets in Cochin, South India: seafood risk assessment, Annals of Animal Science, 22(3): 1105-1114.
https://doi.org/10.2478/aoas-2021-0077

Suja N., and Mohamed K.S., 2010, The black clam, Villorita cyprinoides, fishery in the State of Kerala, India, Marine Fisheries Review, 72(3): 48-61.

Thasneem S., Salim S.S., and Nandan S.B., 2018, Anthropogenic impacts on Cochin Estuary, Indian Journal of Geo-Marine Sciences, 47: 1625-1632.

U.S. Food and Drug Administration (FDA), 2023, National Shellfish Sanitation Program (NSSP) guide for the control of molluscan shellfish: 2023 revision, U.S. Department of Health and Human Services, Washington, D.C., USA.

Vaiyapuri M., Sebastian A.S., George I., Variem S.S., Vasudevan R.N., George J.C., and Mothadaka M.P., 2021, Predominance of genetically diverse ESBL Escherichia coli identified in resistance mapping of Vembanad Lake, Environmental Science and Pollution Research, 28(46): 66206-66222.
https://doi.org/10.1007/s11356-021-15110-y

International Journal of Aquaculture, 2026, Vol.16, No.3, 166-183
http://www.aquapublisher.com/index.php/ija

Case study Open Access

Assessment of Genetic Diversity in Germplasm Resources of Cultured Marine Groupers

Hejun Peng [1]
Yuanhe Street Office, Yunhe County, Lishui City, Yuanhe 323600, Zhejiang, China
Corresponding author: 276529718@qq.com
International Journal of Aquaculture, 2026, Vol.16, No.3 doi: 10.5376/ija.2026.16.0014
Received: 18 Apr., 2026
Accepted: 30 May., 2026
Published: 21 Jun., 2026

Preferred citation for this article:
Peng H.J., 2026, Assessment of genetic diversity in germplasm resources of cultured marine groupers, International Journal of Aquaculture, 16(3): 166-183 (doi: 10.5376/ija.2026.16.0014)

Abstract This study explores the classification characteristics, geographic distribution, and utilization status of grouper germplasm resources, and elaborates on the theoretical basis and major evaluation indices of genetic diversity. It focuses on summarizing the application progress of molecular techniques—such as microsatellite markers (SSR), single nucleotide polymorphisms (SNP), mitochondrial DNA, and high-throughput sequencing—in population genetic analyses, and analyzes the genetic structural differences between cultured and wild populations through representative case studies. The results indicate that wild grouper populations generally maintain relatively high genetic diversity, whereas cultured populations commonly exhibit reduced allelic richness, decreased heterozygosity, and increased genetic differentiation, mainly due to founder effects, genetic drift, and artificial selection. Meanwhile, genetic bottlenecks and inbreeding effects have gradually emerged in some cultured populations. Current research still faces limitations, including insufficient sample coverage, lack of a unified evaluation system, and inadequate integration of multi-omics approaches. Based on these findings, this study proposes strengthening genetic monitoring, optimizing broodstock management, establishing germplasm conservation systems, and promoting the application of high-throughput genomic technologies in genetic evaluation and molecular breeding. The results provide a theoretical basis for the conservation of grouper germplasm resources, genetic improvement, and the high-quality development of the aquaculture industry.
Keywords Grouper; Germplasm resources; Genetic diversity; Molecular markers; Population genetic structure

1 Introduction

Groupers (*Epinephelus* spp.), as one of the most important marine economic fish species, occupy a significant position in global mariculture due to their rapid growth, high flesh quality, and high market value. This group is mainly distributed and farmed in tropical and subtropical regions, with Asia—particularly China, Taiwan, and Indonesia—contributing over 90% of global production. Driven by market demand, the grouper industry has expanded rapidly, with global production increasing from approximately 60,000 tons in 1990 to nearly 200,000 tons in 2007. In China, coastal regions in the south have developed grouper aquaculture into a dominant specialty industry, supported by continuous advancements in farming technologies. Artificial breeding techniques have gradually matured, and superior varieties such as orange-spotted grouper, giant grouper, and their hybrids have been widely applied. However, the rapid expansion of the industry has, to some extent, outpaced the development of technology and management. Current farming systems still face challenges such as insufficient seed supply, high dependence on wild-caught juveniles, unbalanced feed structures, and frequent disease outbreaks (Ybanez and Gonzales, 2023). Meanwhile, long-term artificial selection and closed breeding practices have led to issues such as germplasm degradation, reduced growth performance, and weakened disease resistance, posing significant constraints on the sustainable development of the industry.

Germplasm resources constitute the fundamental basis for the sustainable development of aquaculture, and their level of genetic diversity directly determines a population's adaptability to environmental changes and resistance to pathogenic stress (Yang et al., 2024). High genetic diversity not only helps maintain population vitality but also provides potential resources for the genetic improvement of desirable traits. However, under the combined pressures of overfishing, habitat destruction, and aquaculture practices reliant on wild resources, the genetic diversity of wild grouper populations is declining. At the same time, non-standardized seed production processes

can induce genetic bottlenecks, inbreeding accumulation, and allele loss, further weakening the genetic foundation of cultured populations (Ai et al., 2025). Therefore, protecting wild populations, optimizing broodstock management, and conducting continuous genetic monitoring are of great importance for maintaining germplasm stability and promoting selective breeding programs (Nousias et al., 2021; Li, 2022).

With the development of molecular biology technologies, research on genetic diversity in groupers has continued to deepen. Commonly used approaches include microsatellite markers (SSR), ISSR, mitochondrial DNA (mtDNA), DNA barcoding, as well as more recent high-throughput sequencing and environmental DNA (eDNA) technologies (Hassanien and Al-Rashada, 2020; Ai et al., 2025). Studies have shown that cultured populations generally exhibit lower genetic diversity compared to wild populations and often display significant genetic differentiation, mainly due to genetic mechanisms such as founder effects, genetic drift, and inbreeding. In addition, parent-offspring comparative studies based on microsatellite markers have revealed evident allele loss and genetic bottleneck signals during artificial breeding, highlighting the importance of genetic quality control in breeding programs. Molecular surveys at regional scales have also demonstrated significant genetic structure differences among geographically distinct populations, providing important evidence for resource conservation and stock enhancement programs (Tavakoli-Kolour et al., 2022; Nurdin et al., 2025).

This study aims to explore the research progress and existing issues in the genetic diversity of grouper germplasm resources. Although extensive studies have been conducted both domestically and internationally, limitations remain, including restricted sample coverage, the lack of a unified evaluation index system, and insufficient integration of multi-omics technologies, making it difficult to comprehensively characterize the overall genetic landscape of grouper germplasm resources. Taking marine-cultured grouper germplasm as the research subject, this study systematically reviews the theoretical foundations and technical systems for genetic diversity assessment and, combined with representative case studies, conducts a comprehensive analysis of current genetic diversity levels and existing challenges. The findings are expected to provide a theoretical basis for the scientific conservation and efficient utilization of grouper germplasm resources, offer references for optimizing molecular breeding strategies and developing improved varieties, and ultimately promote the high-quality and sustainable development of the grouper aquaculture industry.

2 Overview of Grouper Germplasm Resources

2.1 Classification of groupers and major cultured species

Groupers, in a broad sense, belong to the order Perciformes. Traditionally, they were classified under the family *Serranidae*, subfamily Epinephelinae. However, with advances in molecular phylogenetics, their classification has been progressively revised, and they are now recognized as an important group within the family *Epinephelidae*. This group is species-rich, with more than 160 species recorded worldwide, primarily distributed in tropical and subtropical waters, especially in the Indo-Pacific region (Ybanez and Gonzales, 2023). Nevertheless, due to both significant differences and certain convergent characteristics in body coloration, morphology, and ecological habits among species, traditional morphological methods have limitations in identifying closely related species, thereby affecting the accuracy of germplasm resource surveys and breeding practices.

With the development of molecular marker technologies, phylogenetic analyses based on mitochondrial genes such as Cyt b and COI have become important tools for grouper classification. Studies have shown that some morphologically distinct groups are not genetically independent; for example, Cromileptes is phylogenetically nested within the genus *Epinephelus*, revealing inconsistencies between traditional morphological classification and genetic relationships (Hassanien and Al-Rashada, 2020). Therefore, establishing a phylogeny-based classification system not only improves species identification accuracy but also provides a theoretical basis for pedigree management, hybrid design, and germplasm standardization.

In aquaculture applications, groupers have developed into high-value marine farming species. Globally, commercial aquaculture involves at least 47~48 grouper species and 15~16 hybrid combinations, with Asia playing a dominant role (Ybanez and Gonzales, 2023). In China, the main cultured species include orange-spotted

grouper (*E. coioides*), Malabar grouper (*E. malabaricus*), giant grouper (*E. lanceolatus*), and yellow grouper (*E. awoara*). Additionally, species such as brown-marbled grouper, camouflage grouper, and coral trout also hold high economic value in regional aquaculture (Das et al., 2021). In recent years, hybrid breeding has developed rapidly; for instance, hybrids between giant grouper and orange-spotted grouper exhibit significant advantages in growth and stress resistance. However, differences in genetic background and pedigree stability among species and hybrids may lead to germplasm admixture and distortion of resource information if standardized classification and genetic monitoring are lacking. Therefore, systematic classification and phylogenetic organization of germplasm resources form the foundation for genetic evaluation and breeding utilization.

2.2 Distribution characteristics of different geographic populations

Groupers are widely distributed in tropical and subtropical seas, and in China they are mainly found in the South China Sea, East China Sea, and Taiwan Strait. Although their distribution range is broad, genetic studies indicate that natural populations are not randomly mixed but generally exhibit significant geographic structure (Chen et al., 2025). This differentiation is mainly influenced by ocean current systems, strait barriers, island distribution, and historical sea-level fluctuations during the Pleistocene, as well as species-specific dispersal capacity and reproductive behavior (Yang et al., 2022; Fadli et al., 2023).

Taking orange-spotted grouper (*E. coioides*) as an example, microsatellite analyses have revealed significant genetic differentiation among populations from China, Malaysia, and Indonesia, while populations within China's coastal waters show relatively low differentiation. This suggests the formation of regional genetic structure at large spatial scales, with some degree of gene flow at local levels. Further studies indicate that this species may comprise two major evolutionary lineages, likely shaped by marginal sea isolation and Pleistocene sea-level changes (Chen et al., 2025). Similarly, yellow grouper (*E. awoara*) exhibits relatively high genetic diversity, but the Beibu Gulf population shows distinct characteristics, with the Qiongzhou Strait and Taiwan Strait acting as important barriers in its genetic differentiation (Yang et al., 2022).

At broader spatial scales, different grouper species exhibit diverse patterns of genetic structure. Studies in the Indo-Malay Archipelago show that some species display clear geographic differentiation, whereas others exhibit genetic homogeneity, closely related to larval dispersal capacity and habitat dependence (Fadli et al., 2023). In addition, regional genetic structure and low effective population sizes have been observed in species such as Nassau grouper and brown grouper in the Atlantic (Vaini et al., 2021). Notably, in aquaculture systems, frequent translocation of juveniles and artificial breeding have led to increasing mixing of geographic populations, gradually weakening original genetic structures. While this improves resource utilization efficiency, it also introduces risks of genetic contamination and loss of local adaptation. Therefore, both natural geographic structure and human intervention must be considered in germplasm utilization to ensure genetic compatibility and resource security.

2.3 Current status and challenges of germplasm resources

At present, grouper germplasm resources are characterized by "rich diversity but uneven utilization." Wild populations are highly diverse with complex genetic backgrounds, providing important genetic sources for breeding; however, aquaculture mainly relies on a limited number of dominant species and their hybrids (Ybanez and Gonzales, 2023). Although Asia contributes over 90% of global production, germplasm utilization is relatively concentrated. While this improves production efficiency, it also exacerbates issues such as a narrow genetic base and germplasm homogenization. Under long-term artificial selection and closed breeding systems, some cultured populations have exhibited genetic degeneration, including reduced growth performance, weakened stress resistance, and increased disease susceptibility (Li, 2022; Sonesson et al., 2023).

From a genetic perspective, these problems mainly arise from founder effects, genetic drift, and the accumulation of inbreeding. Studies have shown that in species such as orange-spotted grouper, kelp grouper, and sevenband grouper, cultured populations often experience the loss of rare alleles and reduced allelic richness. Even when heterozygosity does not change significantly, their genetic potential may still decline. In addition, insufficient

broodstock numbers and inadequate pedigree management can lead to excessive contributions from certain families, accelerating the loss of genetic diversity (Sonesson et al., 2023). Meanwhile, although hybrid breeding can enhance growth performance, the absence of molecular marker-assisted management may result in genetic background confusion and biased evaluation.

On the other hand, wild germplasm resources are also under continuous pressure. Overfishing, habitat degradation, and environmental disturbances have led to population declines and reduced genetic diversity in some species (Fadli et al., 2023; Ybanez and Gonzales, 2023; Nurdin et al., 2025). Some populations have shown signals of historical contraction and even low levels of genetic variation (Vaini et al., 2021; Chen et al., 2025). Additionally, management challenges remain, including insufficient genetic assessment, incomplete broodstock renewal mechanisms, lack of germplasm databases, and inadequate coordination of conservation policies (Li, 2022; Sonesson et al., 2023). Therefore, it is necessary to establish a full-chain management system covering "wild resources—conservation populations—breeding populations—commercial seed," integrating molecular monitoring, broodstock management, and ecological conservation to ensure the long-term stability and sustainable utilization of grouper germplasm resources (Li, 2022).

3 Theoretical Basis for Genetic Diversity Assessment in Groupers

3.1 Concept and evaluation indices of genetic diversity

Genetic diversity refers to the quantity and distribution patterns of genetic variation within a species and among different populations. It is an important component of biodiversity and forms the basis for aquatic organisms to adapt to environmental changes, maintain population stability, and support genetic improvement (Hassanien and Al-Rashada, 2020; Yang et al., 2022). For marine aquaculture species such as groupers, genetic diversity is primarily reflected at the DNA level, including the number and frequency distribution of alleles, haplotype composition, and variation at polymorphic loci across the genome. These genetic differences may further manifest as phenotypic variations in traits such as growth rate, body shape, disease resistance, and environmental adaptability. Therefore, a high level of genetic diversity generally indicates stronger environmental adaptability, greater evolutionary potential, and lower risk of population decline, making it an important criterion for evaluating the quality and breeding potential of grouper germplasm resources (Chen et al., 2025).

Compared with traditional morphological indicators, molecular marker technologies can more directly reflect genetic variation and are less affected by environmental factors; thus, they have become core tools in studies of genetic diversity in groupers. Currently, commonly used methods include microsatellite markers (SSR), mitochondrial DNA (mtDNA) haplotype analysis, single nucleotide polymorphisms (SNPs), ISSR, as well as rapidly developing high-throughput genotyping and whole-genome resequencing technologies (Hassanien and Al-Rashada, 2020; Hsu et al., 2023; Wu et al., 2024). These techniques have enabled genetic diversity assessment to evolve from early low-resolution morphological or limited-locus analyses to comprehensive multi-locus, genome-wide, and functionally associated analyses, improving both the accuracy of population comparisons and the interpretation of the genetic basis of germplasm resources (Houston et al., 2020).

In practice, genetic diversity assessment typically relies on a set of quantitative indices to form a comprehensive analytical framework. Common within-population diversity indices include the number of alleles (Na), effective number of alleles (Ne), allelic richness, observed heterozygosity (Ho), and expected heterozygosity (He), which reflect variation at the allele and genotype levels (Hassanien and Al-Rashada, 2020). Among these, Na represents the total number of alleles detected at a locus, whereas Ne emphasizes the contribution of allele frequency distribution to effective variation. Ho and He represent the actual proportion of heterozygous individuals and the theoretically expected heterozygosity, respectively. In addition, polymorphism information content (PIC), nucleotide diversity (π), and haplotype diversity (Hd) are commonly used to evaluate marker polymorphism and sequence-level variation (Chen et al., 2025). For comparisons among populations, indices and methods such as FST, ΦST, GST, DEST, genetic distance, principal component analysis (PCA), and Bayesian clustering are used to

reveal genetic structure differences among geographic populations, between wild and cultured populations, and between parents and offspring (Figure 1) (Vaini et al., 2021; Yang et al., 2022).

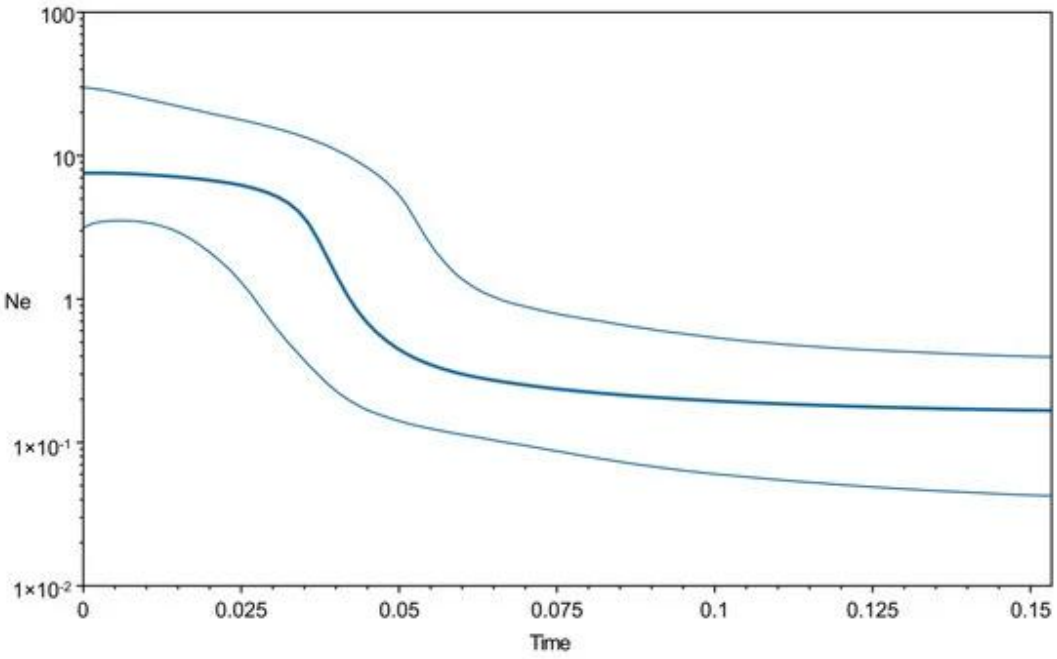

Figure 1 Bayesian skyline plot of the effective population sizes through time for *Epinephelus awoara* (Adopted from Yang et al., 2022)

Image caption: The y-axis is the product of effective population size (Ne) and generation length in a log scale while the x-axis is the time scale before present in units of million years ago (Adopted from Yang et al., 2022)

3.2 Population genetic structure and evolutionary mechanisms

Population genetic structure refers to the distribution patterns of genotypes and allele frequencies within and among populations and is a key aspect of genetic diversity research. In groupers, varying degrees of genetic differentiation may exist among geographic populations, among cultured populations from different hatcheries, and even among different families within the same farming system. Such differentiation can be quantified and visualized using methods such as FST, genetic distance, analysis of molecular variance (AMOVA), PCA, and clustering analysis, thereby revealing phylogenetic relationships, levels of gene flow, and potential boundaries of genetic units (Vaini et al., 2021; Yang et al., 2022). Therefore, population genetic structure analysis is not only fundamental for understanding patterns of population differentiation but also provides an important basis for germplasm conservation, broodstock management, and breeding utilization.

From the perspective of natural populations, the genetic structure of groupers results from the combined effects of gene flow, genetic drift, natural selection, and historical population dynamics. Traditional views suggest that marine fishes, due to their pelagic larval stages, should exhibit high connectivity and genetic homogeneity. However, extensive empirical studies have demonstrated that grouper populations are not completely randomly mixed. Studies on Nassau grouper, yellow grouper, orange-spotted grouper, and brown grouper indicate that their population structures are often influenced by straits, marginal seas, island barriers, ocean current systems, and dependence on specific spawning grounds, leading to significant regional differentiation (Vaini et al., 2021; Yang et al., 2022; Chen et al., 2025). In addition, Pleistocene glaciations, sea-level fluctuations, and repeated isolation-connection events in marginal seas have shaped the present-day genetic patterns of groupers over longer timescales. In other words, the observed population genetic structure reflects not only contemporary ecological processes but also the combined effects of historical geological events and modern ecological dynamics.

In aquaculture systems, anthropogenic factors play an even more significant role in shaping population genetic structure. Artificial breeding typically relies on a limited number of broodstock, and the effective population size

is often smaller than the apparent number of parents. As a result, offspring populations are prone to founder effects and genetic drift, leading to rapid loss of rare alleles. Notably, such genetic erosion may not immediately manifest as a significant decline in heterozygosity but is often first reflected in reduced allelic richness and imbalanced family contributions. Therefore, relying on a single indicator to evaluate the genetic quality of cultured populations may underestimate potential risks. Moreover, directional selection for traits such as growth rate, feed efficiency, and disease resistance may lead to selective sweeps in specific genomic regions, forming distinct patterns of population differentiation (Wu et al., 2024). Without proper pedigree records and parentage verification, repeated use of closely related broodstock can exacerbate inbreeding and relatedness accumulation. Thus, molecular marker-based parentage analysis, pedigree reconstruction, and family contribution assessment are crucial for maintaining stable genetic structure in cultured populations (Weng et al., 2021).

3.3 Relationship between genetic diversity and germplasm improvement

Genetic diversity is the foundation of germplasm improvement and genetic breeding. Abundant genetic variation provides the necessary basis for selecting desirable traits, estimating genetic parameters, and improving breeding values. In grouper breeding, whether through traditional mass selection, family-based selection, marker-assisted selection, or genomic selection, all approaches fundamentally rely on the available genetic variation within populations. If genetic diversity is insufficient, the reservoir of favorable alleles and opportunities for recombination are limited, leading to reduced breeding response and even stagnation in long-term improvement (Houston et al., 2020; Hsu et al., 2023; Wu et al., 2024). This is particularly important for complex economic traits such as growth rate, feed efficiency, disease resistance, and environmental tolerance, which are typically controlled by multiple genes. Sustained genetic gain can only be achieved when the base population maintains sufficient genetic variation.

On the other hand, genetic diversity assessment provides direct guidance for broodstock management and breeding population design. Using microsatellite and SNP markers for pedigree reconstruction, parentage identification, and kinship analysis enables accurate evaluation of parental contributions, control of inbreeding accumulation, balancing of family representation, and optimization of hybrid combinations (Weng et al., 2021). For aquaculture species like groupers, which rely heavily on artificial propagation and hatchery expansion, the absence of molecular-level pedigree management can result in repeated use of a few core broodstock, reducing effective population size and weakening the breeding foundation. In hybrid breeding, an appropriate level of genetic divergence is also a prerequisite for heterosis. If parental genetic backgrounds are too similar, heterosis may be limited; if the genetic distance is too large, risks such as unstable combining ability, developmental abnormalities, and increased segregation in offspring may arise (Xu et al., 2025). Therefore, genetic diversity analysis not only aids in broodstock selection but also helps establish theoretical relationships between genetic divergence and hybrid performance.

Furthermore, genetic diversity assessment provides important support for wild resource conservation and the renewal of cultured germplasm. By comparing genetic differences between wild and cultured populations, it is possible to identify whether genetic diversity loss, pedigree admixture, or localized genetic degeneration has occurred in aquaculture systems, thereby informing decisions on introducing wild germplasm or restoring local populations (Weng et al., 2021; Yang et al., 2022; Chen et al., 2025). At a broader scale, studies of population structure and historical dynamics help define management units, protect key spawning populations, and conserve unique genetic lineages—factors that are critical not only for natural resource conservation but also for maintaining a sufficiently broad genetic base for future breeding systems (Vaini et al., 2021). Therefore, grouper germplasm improvement must adhere to the principle of balancing “conservation and utilization”: on the one hand, maintaining sources of variation by increasing effective population size, introducing new germplasm, and preserving clear genetic structure; on the other hand, improving selection efficiency through molecular markers, whole-genome information, and trait association analyses. Only by advancing germplasm improvement on the premise of preserving genetic diversity can the grouper aquaculture industry achieve sustainable, stable, and high-quality development.

4 Genetic Diversity Assessment of Grouper Populations

4.1 Sample sources and experimental design

To evaluate the genetic diversity of typical marine-cultured grouper populations and their differences from adjacent wild populations, this study selected grouper populations from a representative aquaculture area along the South China Sea coast of China. Both cultured populations and nearby wild populations were included. Such a design is highly representative in grouper genetics, as key cultured species such as orange-spotted grouper (*Epinephelus coioides*) and giant grouper *(E. lanceolatus*) often coexist as both hatchery-produced populations and regional wild stocks, providing ideal materials for comparing genetic differences between "cultured domestication" and "wild retention" (Figure 2) (Tavakoli-Kolour et al., 2022; Chen et al., 2025). For sampling, cultured populations were collected from three large-scale aquaculture farms, with 30 individuals randomly sampled from each farm to capture genetic variation within different production units. Wild populations were obtained through nearshore fishing or traceability from regional fish markets, with approximately 40 individuals collected to represent the genetic background of adjacent natural populations.

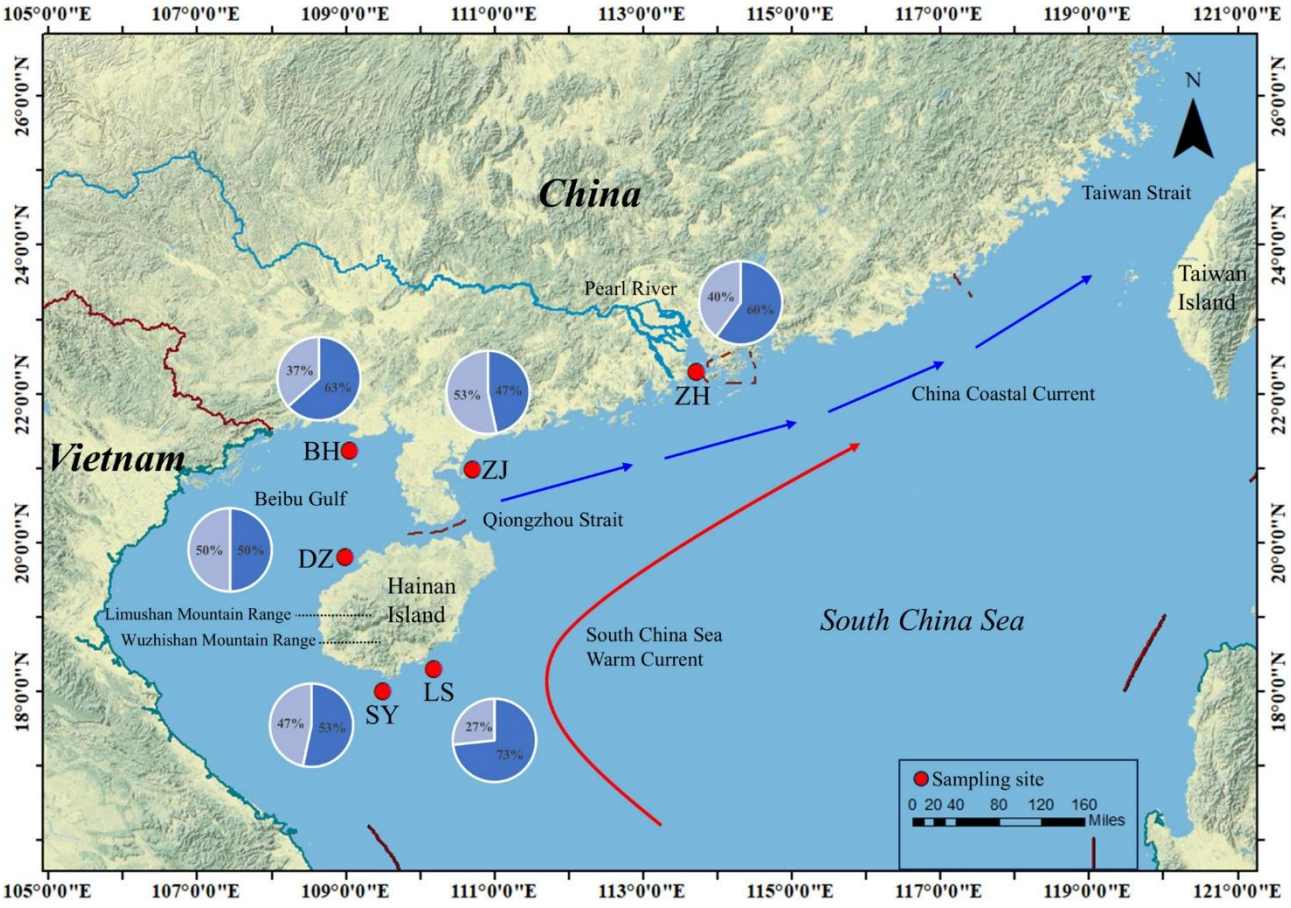

Figure 2 Map of the coast of Hainan Island and mainland China along the South China Sea illustrating the sampling locations of *Epinephelus* coioides (Adopted from Chen et al., 2025)

Image caption: Each color in the pie charts represents the frequencies of the Lineage A (orange) and Lineage B (gray) haplotypes in each population (Adopted from Chen et al., 2025)

All samples consisted of muscle or fin tissue, preserved in liquid nitrogen or absolute ethanol in the field and transported to the laboratory for DNA extraction and molecular analysis. Sampling procedures aimed to avoid repeated collection from the same families or closely related individuals. Information such as sample origin, collection site, farming history, broodstock source, and generation data was recorded to minimize sampling bias and improve interpretability. This design balanced spatial representativeness and genetic heterogeneity within aquaculture systems, providing a solid basis for comparing genetic differentiation between wild and cultured populations.

In terms of experimental design, a combined analysis using microsatellite markers (SSR) and selected SNP loci was adopted to achieve both high polymorphism detection and high-resolution population structure analysis. Previous studies indicate that grouper genetic analyses typically use 8-20 SSR loci supplemented by mitochondrial genes or SNP data derived from reduced-representation sequencing, enhancing the detection of within-population diversity, kinship, and differentiation patterns (Weng et al., 2021; Yang et al., 2022; Wu et al., 2024). In this study, DNA was extracted using the standard phenol-chloroform method, followed by PCR amplification of target fragments. SSR genotyping was performed using capillary electrophoresis platforms, while SNP loci were detected using high-throughput sequencing. Data analysis was conducted using software such as PopGen, Arlequin, and STRUCTURE to calculate indices including allele number, heterozygosity, polymorphism information content, inbreeding coefficient, and Hardy-Weinberg equilibrium. Further analyses such as FST, AMOVA, PCA, and clustering were used to characterize population differentiation. If known parent-offspring relationships were included, kinship and parentage analyses were also performed to estimate effective breeding population size and identify unequal reproductive contributions (Hassanien and Al-Rashada, 2020; Weng et al., 2021; Yang et al., 2022).

4.2 Molecular marker results and population structure analysis

The molecular marker results indicated clear differences in genetic diversity among grouper populations from different sources. Overall, wild populations exhibited higher allele numbers, allelic richness, and heterozygosity than cultured populations, suggesting greater genetic variation. In contrast, cultured populations showed allele loss, reduction of rare alleles, and imbalanced allele frequencies at certain loci, indicating genetic variation loss during artificial breeding and seed propagation. This pattern is consistent with previous studies showing that cultured populations are more susceptible to founder effects and genetic drift under domestication and limited broodstock conditions, resulting in a narrower genetic base.

From the perspective of heterozygosity and inbreeding parameters, wild populations generally showed higher expected heterozygosity (He) than cultured populations. In cultured populations, observed heterozygosity (Ho) at some loci was lower than expected, leading to elevated FIS values and suggesting heterozygote deficiency (Yang et al., 2022). This pattern may result from inbreeding, family structure, Wahlund effects, or the presence of mixed subpopulations. Notably, some hatchery studies have shown that even when offspring heterozygosity is similar to that of parents, rare alleles are significantly reduced and genetic bottleneck signals are detectable. This suggests that genetic drift may first manifest as a reduction in allelic richness rather than an immediate decline in heterozygosity (Wenne, 2023). Therefore, interpreting genetic status in cultured populations requires a multi-indicator approach rather than reliance on a single heterozygosity metric.

Population structure analyses further revealed that FST values among cultured populations were generally low, indicating frequent germplasm exchange or shared broodstock sources among farms. In contrast, FST values between cultured and wild populations were relatively high, indicating a certain degree of genetic differentiation. AMOVA results typically supported this pattern, showing that a significant proportion of genetic variation was attributable to differences among populations, particularly between “wild” and “cultured” groups. STRUCTURE, PCA, and PCoA analyses commonly separated samples into two main genetic clusters corresponding to wild and cultured origins, although some individuals exhibited admixture (Chen et al., 2025). Such admixture may reflect gene flow caused by stock enhancement, cross-regional seed transfer, introduction of wild broodstock, or historical germplasm exchange. Additionally, selection signals detected at certain SNP loci suggest that artificial selection has left functional genetic footprints in genomic regions associated with growth, stress resistance, or disease resistance (Wu et al., 2024).

4.3 Genetic diversity evaluation and germplasm conservation recommendations

Based on the comprehensive analysis of genetic diversity indices in this case, wild grouper populations in the study area still maintain relatively high genetic diversity, whereas cultured populations have shown varying degrees of genetic variation decline, reduced allelic richness, and increased differentiation from wild populations.

This pattern—"high variation in wild populations and gradual drift in cultured populations"—is consistent with general trends observed in multiple grouper studies, indicating that long-term artificial selection, limited broodstock usage, and patterns of germplasm exchange among hatcheries have significantly influenced the genetic structure of cultured populations (Chen et al., 2025). Without timely intervention, this trend may lead to further inbreeding accumulation, narrowing of the genetic base, and germplasm degradation, ultimately reducing production performance and future breeding potential.

Based on these findings, several measures should be implemented in aquaculture practice to protect and enhance genetic diversity. First, broodstock sources should be expanded by establishing base breeding populations from multiple genetically diverse and well-documented populations, while avoiding indiscriminate mixing of highly divergent management units to prevent disruption of local adaptation or new genetic instability (Yang et al., 2022). Second, standardized breeding management systems should be established, including pedigree recording, controlled reuse of broodstock, balanced family contributions, and rotational mating strategies to reduce inbreeding and maintain effective population size (Weng et al., 2021). In addition, molecular marker monitoring should be incorporated into routine germplasm management to continuously track key parameters such as allelic richness, He, FIS, and effective population size, and to detect genetic bottlenecks and germplasm degradation signals in a timely manner (Wenne, 2023).

Conservation of wild grouper resources is essential for maintaining overall germplasm quality. Studies have shown that although many wild populations still retain relatively high genetic diversity, some regional populations exhibit signs of historical contraction, low effective population size, and significant geographic differentiation (Vaini et al., 2021; Yang et al., 2022; Chen et al., 2025). Therefore, it is necessary to strengthen the protection of key spawning grounds, juvenile habitats, and locally unique populations, while restricting intensive fishing and habitat destruction. In stock enhancement programs, genetic data should be used to select appropriate source populations to avoid genetic homogenization or dilution of local genotypes in wild populations. Where feasible, long-term conservation strategies such as germplasm reserves, live conservation populations, cryopreserved sperm banks, and DNA repositories should be established to preserve important genetic resources. Ultimately, a dynamic management framework integrating "wild resource conservation—cultured germplasm optimization—genetic monitoring feedback—re-conservation" should be developed. Through this integrated cycle of conservation, utilization, and re-conservation, it is possible to maintain evolutionary potential and future breeding resources while ensuring production performance, thereby supporting the high-quality and sustainable development of the marine aquaculture industry (Hassanien and Al-Rashada, 2020; Weng et al., 2021).

5 Methods for Genetic Diversity Analysis in Groupers

5.1 Molecular marker technologies

Molecular marker technologies based on DNA variation are among the most widely used and informative approaches in grouper genetic diversity studies. Their core principle is to detect genetic variation in the nuclear or mitochondrial genome to reveal population genetic structure, diversity levels, kinship relationships, and germplasm origins (Hassanien and Al-Rashada, 2020). Compared with traditional phenotypic traits, molecular markers directly target genetic material and are less influenced by environmental factors, thus offering clear advantages in population identification, broodstock management, inbreeding monitoring, population differentiation analysis, and molecular breeding. Commonly used techniques include microsatellite markers (SSR), ISSR, single nucleotide polymorphisms (SNPs), and mitochondrial DNA markers. Each type differs in resolution, cost, and application scenarios, collectively forming the technical foundation for genetic diversity analysis in groupers.

Microsatellite markers (simple sequence repeats, SSR) are typical codominant markers characterized by high polymorphism, abundant information content, high resolution, and well-established protocols, and they have long been core tools in grouper population genetic studies (Hassanien and Al-Rashada, 2020). By analyzing allele number, frequency distribution, heterozygosity, and polymorphism information content at SSR loci, researchers

can effectively assess genetic variation within populations, genetic differentiation among populations, and kinship among individuals. In important cultured species such as *Epinephelus* spp. and giant grouper, relatively stable SSR marker systems have been established and are widely used for population structure analysis, molecular fingerprinting, parentage identification, and broodstock management, particularly for detecting inbreeding accumulation and unequal reproductive contributions in cultured populations. In addition to SSR, ISSR and its derivative technologies are also applied in grouper genetic diversity analysis. ISSRseq combined with high-throughput sequencing can simultaneously generate large amounts of SNP data, making it especially suitable for materials lacking complete pedigree information or reference genomes (Hsu et al., 2023).

In recent years, SNPs have gradually become mainstream tools for grouper genetic diversity studies and precision breeding (Sherman et al., 2020; Hsu et al., 2023). As the most common type of genomic variation, SNPs are widely distributed, genetically stable, and suitable for automated and high-throughput detection. Using approaches such as RAD-seq, ISSRseq, genotyping-by-sequencing (GBS), or whole-genome resequencing, large numbers of SNP loci can be identified at the genome-wide scale, enabling fine-resolution analysis of population structure and further applications such as selection signal detection, genome-wide association studies, and candidate gene identification. For example, in leopard coral grouper, whole-genome resequencing identified more than 8.7 million SNPs, enabling high-resolution population clustering and identification of candidate genomic regions associated with growth traits (Wu et al., 2024). In addition, mitochondrial DNA markers, due to their maternal inheritance, lack of recombination, and relatively rapid evolutionary rate, have unique advantages in phylogenetics, phylogeography, DNA barcoding, and historical population dynamics analysis in groupers (Mainna et al., 2025). Therefore, combining nuclear and mitochondrial markers allows a more comprehensive characterization of genetic diversity patterns across different genetic levels.

5.2 Phenotypic and morphological analysis methods

Phenotypic and morphological analyses are traditional approaches in genetic diversity research, primarily based on measuring and comparing external morphological traits, growth characteristics, and color patterns to indirectly reflect genetic differences among populations. In grouper studies, commonly used indicators include body length, body height, body weight, head length, eye diameter, fin ray counts, scale counts, and body coloration patterns (Figure 3) (Hassanien and Al-Rashada, 2020; Mainna et al., 2025). These traits have a genetic basis but are also closely influenced by environmental conditions, nutritional status, developmental stage, and culture practices. Therefore, they remain valuable in germplasm description, preliminary species identification, and evaluation of production traits. Particularly in baseline resource surveys and germplasm inventories, morphological methods are indispensable as initial screening tools due to their simplicity, low cost, and intuitive results.

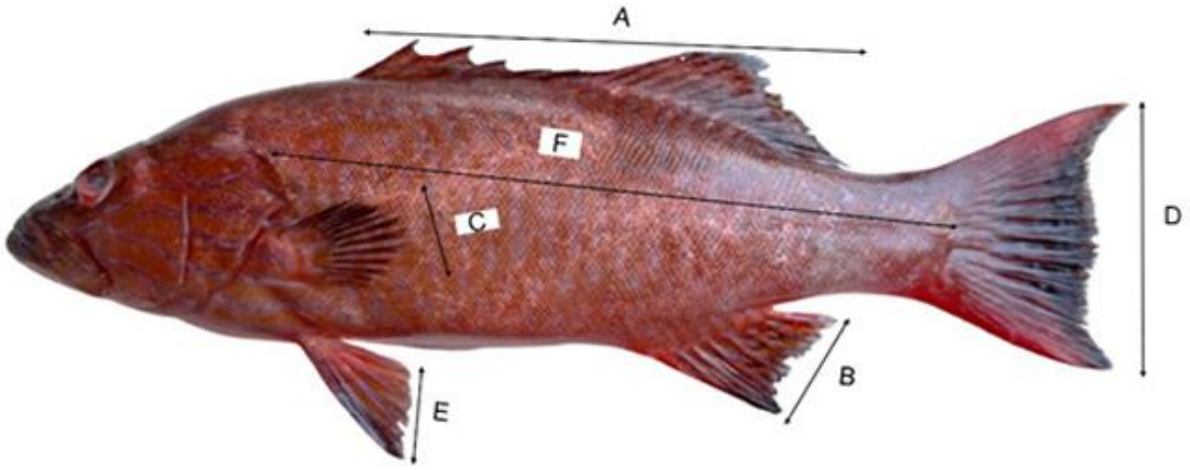

Figure 3 Meristic counts used in this study (Adopted from Mainna et al., 2025)

Image caption: Spines and rays: (A) Dorsal fin, (B) Anal fin, (C) Pectoral fin, (D) Caudal fin, (E) Pelvic fin; Scale counts: (F) Lateral line (Adopted from Mainna et al., 2025)

In practical applications, phenotypic and morphological analyses are usually combined with multivariate statistical methods. By standardizing multiple morphological traits, methods such as principal component analysis (PCA), discriminant analysis (DA), cluster analysis, and diversity indices can be applied to reveal phenotypic differences and classification relationships among populations (Sarif et al., 2020; Tian et al., 2024; Verma et al., 2024). These approaches not only provide preliminary grouping for molecular analyses but also help identify candidate germplasm with desirable traits such as superior growth, body shape, or coloration from a production perspective. Thus, phenotypic and morphological analyses have strong practical significance in grouper germplasm evaluation, particularly when aligned with breeding objectives and production needs.

However, morphological traits in groupers also have notable limitations. First, groupers exhibit strong morphological plasticity at different developmental stages, especially during the juvenile phase, when body coloration and patterns are highly variable and often similar among species, leading to potential misidentification and taxonomic confusion (Mainna et al., 2025). Second, environmental factors such as water temperature, salinity, feeding conditions, stocking density, and habitat can significantly influence morphological and growth traits, reducing the reliability of genetic inference based solely on phenotype (Hassanien and Al-Rashada, 2020). As a result, integrated "morphology-molecular" approaches have been increasingly developed, combining morphometric measurements, color phenotypes, or growth indicators with DNA barcoding, SSR, or SNP data. These combined approaches show higher reliability in species identification, germplasm evaluation, and cryptic species detection (Mainna et al., 2025). This trend indicates that although phenotypic and morphological analyses alone are insufficient for precise genetic evaluation, they remain important complementary tools in grouper germplasm research, supporting validation and interpretation of molecular results.

5.3 High-throughput sequencing and genomic technologies

With rapid advances in sequencing technologies and bioinformatics, high-throughput sequencing (next-generation sequencing, NGS) has become one of the most advanced and scalable approaches in grouper genetic diversity research. Compared with traditional molecular markers based on a limited number of loci, NGS enables the acquisition of large-scale genome-wide genetic data within a relatively short time, allowing high-resolution analysis of population genetic structure, selection signals, population history, effective population size, and genotype-phenotype relationships (Wu et al., 2024; Lu et al., 2025). This technological advancement has shifted grouper genetic diversity analysis from "marker-based" to "genome-wide" approaches, significantly improving the depth and accuracy of germplasm evaluation.

Whole-genome resequencing (WGR) is currently one of the most information-rich methods in population genomics. By sequencing multiple individuals and aligning them to a reference genome, millions of SNPs can be identified, enabling analyses of population differentiation, linkage disequilibrium, selective sweeps, runs of homozygosity, and candidate functional genes. For example, in a study of 326 leopard grouper individuals, WGR identified eight genetic groups, characterized growth-related selection regions, and established a haplotype reference database to support low-depth sequencing and genotype imputation, thereby reducing costs while maintaining high resolution (Wu et al., 2024). In addition to WGR, reduced-representation sequencing methods such as RAD-seq and GBS are widely used in grouper population genetics. These approaches can generate thousands to tens of thousands of SNPs without requiring a complete reference genome, making them suitable for non-model species and for analyzing population structure and adaptive variation (Sherman et al., 2020; Martchenko and Shafer, 2023).

High-throughput sequencing is also widely used for developing molecular marker resources and supporting breeding tools. For example, pyrosequencing has been used to develop numerous polymorphic SSR loci in giant grouper, facilitating parentage analysis, individual identification, and population genetic studies. ISSRseq has been applied in tomato grouper to generate genome-wide SNP data for analyzing genetic diversity, constructing kinship networks, and identifying high-growth populations (Hsu et al., 2023). In addition, transcriptome sequencing (RNA-seq), although primarily used for gene expression analysis, can complement population

genomic data in elucidating the mechanisms of economically important traits, identifying candidate functional genes, and interpreting selection signals.

6 Current Status of Genetic Diversity Research in Groupers

6.1 Comparison of genetic diversity among different grouper species

At present, based on microsatellite markers, mitochondrial DNA, and other molecular marker technologies, researchers have systematically evaluated the genetic diversity of multiple grouper species (*Epinephelus* spp. and related taxa). Overall, most grouper species exhibit moderate to high levels of genetic variation; however, significant differences exist among species. These differences are closely related to their evolutionary history, distribution range, ecological niches, and population dynamics (Hassanien and Al-Rashada, 2020). Therefore, comparative studies of genetic diversity among different grouper species not only help to elucidate their evolutionary divergence but also provide important references for germplasm conservation and the selection of superior breeding stocks. Existing studies suggest that species with wider distribution ranges and more complex ecological environments generally possess higher genetic diversity.

For example, yellow grouper (*Epinephelus awoara*) exhibits high haplotype diversity (h = 0.968) and a rich number of microsatellite alleles (13-20 alleles per locus), indicating strong genetic variation potential (Yang et al., 2022). Giant grouper (*E. lanceolatus*) shows an average of approximately 5.9 alleles per locus and expected heterozygosity (He) ranging from 0.62 to 0.80, suggesting a relatively high level of nuclear genetic diversity suitable for fine-scale population analysis and molecular breeding studies. Although brown grouper (E. marginatus) maintains relatively high heterozygosity across multiple regions, its effective population size is relatively low, indicating potential risks to its genetic stability (Vaini et al., 2021).

In contrast, some grouper species with narrower ecological niches or more restricted distributions tend to exhibit lower levels of genetic diversity and are more prone to pronounced population differentiation. In certain island or localized populations, limited gene flow can lead to the accumulation of genetic drift and the formation of unique lineages (Hassanien and Al-Rashada, 2020; Yang et al., 2022). In addition, different marker systems may vary in their ability to detect genetic variation; for example, studies on Plectropomus species have reported discrepancies in diversity estimates across different technical platforms. With the increasing study of hybrid groupers, their offspring often show relatively high diversity in some indices; however, their long-term stability and genetic security still require further evaluation.

6.2 Genetic differences between wild and cultured populations

Wild grouper populations generally exhibit higher genetic diversity, as they have long been subjected to natural environmental conditions, including ocean currents, habitat heterogeneity, and natural selection (Yang et al., 2022). Gene flow among wild populations helps maintain genetic connectivity, and although geographic differentiation may occur at large spatial scales, they collectively form a relatively stable genetic resource pool. Therefore, wild populations are not only critical for conservation but also serve as key genetic sources for artificial breeding and broodstock renewal.

In contrast, cultured populations typically show lower genetic diversity, a conclusion consistently supported by multiple studies. Research on giant grouper in the South China Sea indicates that cultured populations have significantly lower allelic richness and heterozygosity compared to wild populations. At the same time, analyses such as FST, AMOVA, and STRUCTURE reveal significant genetic differentiation between the two groups. Similarly, in orange-spotted grouper, clear genetic differences exist between cultured populations and multiple wild populations, while variation among wild populations is relatively small (Wang et al., 2011), indicating that genetic changes occur more rapidly within aquaculture systems.

The decline in genetic diversity in cultured populations is mainly attributed to founder effects, genetic drift, and the accumulation of inbreeding. In aquaculture, limited broodstock numbers and repeated use reduce effective population size and lead to unequal family contributions (Hsu et al., 2023). In addition, artificial selection for

economic traits can impose directional selection on specific genomic regions, thereby altering population structure. Although introducing new broodstock or hybrid breeding may partially increase genetic diversity, these practices may also blur the boundaries between wild and cultured populations and increase the risk of genetic contamination. Therefore, enhanced genetic monitoring and structural assessment are necessary.

6.3 Analysis of genetic bottlenecks and inbreeding effects

A genetic bottleneck refers to the loss of genetic diversity resulting from a sharp reduction in population size, while inbreeding effects arise under conditions of small population size or closed breeding systems. In grouper aquaculture, these issues are relatively common, particularly during artificial seed production and juvenile propagation (Hsu et al., 2023). Without effective management, genetic bottlenecks and inbreeding can reduce population adaptability and breeding potential, negatively impacting industry development.

Under aquaculture conditions, genetic bottlenecks often occur during the initial stages of artificial breeding. When broodstock numbers are limited or derived from a single source, offspring inherit only a restricted portion of genetic variation, leading to rapid allele loss. Studies have shown that offspring populations of kelp grouper and tomato grouper exhibit bottleneck signals, closely associated with the contributions of a small number of broodstock (Hsu et al., 2023). Inbreeding effects manifest as an increased probability of homozygosity for deleterious recessive alleles, often resulting in reduced growth, lower survival rates, and decreased disease resistance in production systems.

It is noteworthy that genetic bottlenecks and inbreeding are not limited to cultured populations but are also observed in some wild populations. For example, yellow grouper shows high haplotype diversity but low nucleotide diversity, indicating historical population contraction (Yang et al., 2022), while brown grouper exhibits low effective population sizes globally (Vaini et al., 2021). To mitigate these issues, it is necessary to expand broodstock sources, optimize mating designs, and establish pedigree management systems, combined with molecular marker-based kinship analysis and population renewal strategies. Furthermore, strengthening habitat protection and resource management for wild populations is essential for maintaining the long-term genetic stability of grouper germplasm resources.

7 Existing Issues and Future Development Trends

7.1 Current limitations in research

Although significant progress has been made in recent years in grouper genetic diversity research—particularly in molecular marker development, population structure analysis, and germplasm evaluation—there are still notable limitations overall. First, in terms of sampling and study design, most research focuses on a limited number of economically important species or specific key aquaculture regions. Sample sizes are often relatively small and geographic coverage is insufficient, making it difficult to comprehensively reveal large-scale spatial genetic patterns and their temporal dynamics in grouper germplasm resources (Hassanien and Al-Rashada, 2020; Tavakoli-Kolour et al., 2022; Yang et al., 2022). This is particularly problematic for grouper species with strong connectivity across marine regions and complex historical population dynamics, where limited sampling may fail to accurately identify true genetic boundaries and evolutionary units.

In terms of methodology and analytical frameworks, there is a lack of standardization across studies regarding marker types, number of loci, and evaluation indices, resulting in limited comparability among findings. Some studies rely on a single marker system, such as mtDNA or a small number of SSR loci, which can provide preliminary insights but are insufficient for integrating multiple layers of information, including neutral variation, adaptive variation, and selection signals. This limitation reduces the ability to resolve fine-scale population structure and local adaptive differentiation (Hassanien and Al-Rashada, 2020; Saha et al., 2021; Tavakoli-Kolour et al., 2022). In addition, inconsistencies in statistical models, parameter settings, and quality control procedures further affect the robustness and reproducibility of results.

Species misidentification and unclear sample origins remain significant issues. Groupers are highly diverse and morphologically similar, and samples from markets, fisheries, or hatcheries often involve mixed species or inconsistent naming. Without molecular-level species identification, genetic structure and diversity assessments may be based on incorrect taxonomic assumptions, leading to biased conclusions (Hassanien and Al-Rashada, 2020; Tavakoli-Kolour et al., 2022). Meanwhile, genetic monitoring of cultured populations lacks systematic and continuous implementation. Most studies are based on single-time sampling and static analyses, making it difficult to detect ongoing processes such as inbreeding accumulation, allele loss, and genetic drift in a timely manner (Sonesson et al., 2023; Ybanez and Gonzales, 2023). Overall, current research on grouper genetic diversity remains largely descriptive, with insufficient integration of dynamic processes, environmental drivers, and anthropogenic effects.

7.2 Challenges in germplasm conservation and utilization

Grouper germplasm conservation and utilization are currently facing dual pressures from the decline of wild resources and the rapid expansion of aquaculture. Declines in wild populations have become a major issue in many regions. In areas such as the South China Sea and the Persian Gulf, overfishing, habitat degradation, and marine environmental changes have led to reduced population sizes and habitat deterioration. Additionally, groupers generally have relatively slow growth rates, late sexual maturity, and, in some species, spawning aggregation behavior. These life-history traits increase their vulnerability to genetic diversity loss and resource depletion (Tavakoli-Kolour et al., 2022; Yang et al., 2022; Ai et al., 2025). The decline of wild populations not only weakens the stability of natural germplasm reservoirs but also limits access to valuable genetic resources and locally adapted genetic units.

Within aquaculture systems, germplasm management remains insufficiently standardized, and genetic risk management often lags behind breeding and industry expansion. Many cultured strains and hybrid combinations are developed primarily for growth performance, stress resistance, and economic returns, while systematic evaluations of their genetic background, long-term stability, and potential impacts on wild populations are still lacking (Yang et al., 2021; Yang et al., 2023; Wu et al., 2024). Particularly with the widespread use of hybrid groupers, the absence of germplasm purity identification, pedigree management, and risk warning systems may lead to disturbances in wild population genetic structures through introduction, stock enhancement, or escape. At the same time, stock enhancement programs lacking proper genetic planning may increase genetic homogenization due to limited source populations or mismatches with local wild populations.

From a management perspective, national-level systems, information platforms, and commercial frameworks for aquatic genetic resources (AqGR) remain relatively underdeveloped. Many regions lack clear regulations for the introduction of cultured species, the spread of exotic species, hybrid management, and genetic risk assessment, making it difficult to achieve a balance between utilization and conservation (Sonesson et al., 2023). Furthermore, ex situ conservation systems—such as germplasm banks, live conservation populations, and cryopreservation platforms—are still in early stages of development, facing challenges in scale, quality control, and integration into long-term conservation strategies (Li, 2022). Therefore, achieving efficient utilization while maintaining genetic diversity remains a key issue in both research and industry practice.

7.3 Prospects for emerging technologies in genetic diversity assessment

With the rapid advancement of biotechnology, sequencing technologies, and data science, new technologies offer unprecedented opportunities for grouper genetic diversity assessment and germplasm management. First, continuous improvements in whole-genome resequencing, chromosome-level genome assembly, and high-density SNP genotyping enable researchers to analyze genetic variation, selection signals, population structure, and quantitative trait loci (QTLs) at the genome-wide level, significantly enhancing the resolution and functional interpretation of genetic diversity assessments (Yang et al., 2021; Yang et al., 2023; Wu et al., 2024). Meanwhile, SNP genotyping platforms based on multiplex PCR capture or targeted enrichment provide cost-effective, high-throughput solutions for breeding and genetic monitoring (Shan et al., 2022; Wu et al., 2024).

Beyond individual-level genotyping, the development of environmental DNA (eDNA) and metabarcoding technologies offers efficient and non-invasive approaches for wild resource surveys and population monitoring. Compared with traditional capture-based methods, eDNA can identify target species and haplotype information from DNA traces in water samples, making it particularly suitable for continuous monitoring in restoration areas, protected zones, and critical habitats of wild populations (Li, 2022; Ai et al., 2025). In terms of germplasm conservation, the establishment of standardized germplasm banks and high-throughput cryopreservation systems also holds great promise. By developing live conservation populations, cryopreserved sperm banks, DNA repositories, and integrated database platforms, it is possible to systematically preserve allelic diversity and provide long-term support for resource restoration, broodstock renewal, and breeding innovation (Li, 2022).

In the future, grouper genetic resource management will increasingly rely on bioinformatics, big data analytics, artificial intelligence-assisted phenotyping, and genome-based decision support systems. By integrating genomic, transcriptomic, phenotypic, and environmental data, researchers can more comprehensively elucidate the genetic mechanisms underlying important economic traits, thereby supporting molecular-assisted breeding and genomic selection (Yang et al., 2021; Wu et al., 2024). At the same time, the development of national or regional aquatic genetic resource information systems that integrate genetic monitoring, germplasm conservation, breeding records, and risk warning mechanisms is expected to significantly enhance the standardization and intelligence of germplasm management (Sonesson et al., 2023; Wenne, 2023). Overall, future grouper genetic diversity assessment will move toward "genome-wide analysis, dynamic monitoring, data platform integration, and intelligent management."

8 Conclusions and Recommendations

Studies on the genetic diversity of groupers indicate that most wild populations still maintain moderate to high levels of genetic variation, although significant differences exist among species and regions. For example, yellow grouper and brown grouper exhibit high haplotype or microsatellite diversity, while also showing clear population structure and, in some cases, historical declines in effective population size. These patterns are largely influenced by marine environmental barriers, hermaphroditic reproductive traits, and historical population fluctuations. Research on orange-spotted grouper and giant grouper consistently shows that cultured populations exhibit reduced allelic richness, lower heterozygosity, and significant genetic differentiation compared to wild populations, mainly due to founder effects, genetic drift, and artificial selection. Overall, in the aquaculture sector, the management of aquatic genetic resources (AqGR) has lagged behind production development. Many cultured populations lack systematic genetic evaluation, and genetic monitoring has not yet been routinely implemented. Meanwhile, rapid advances in molecular and genomic technologies—such as microsatellites, SNPs, reduced-representation sequencing, and whole-genome resequencing—have made it possible to assess genetic diversity, monitor inbreeding, and conduct molecular-assisted breeding even in non-model aquaculture species. Existing evidence suggests that cultured grouper germplasm is facing ongoing risks of genetic deterioration, but also has the potential for sustainable management through the application of advanced technologies.

The conservation of grouper germplasm resources should be based on systematic genetic evaluation, with genetic management incorporated into seed production systems. For major cultured species such as orange-spotted grouper and giant grouper, molecular markers (e.g., microsatellites or SNPs) should be regularly used at broodstock, seed, and grow-out stages to monitor allelic richness, heterozygosity, FST, and effective population size, with wild populations serving as references. Broodstock populations should be maintained at sufficiently large sizes with high genetic diversity and, where feasible, derived from multiple genetically compatible wild populations, while avoiding indiscriminate mixing of highly divergent management units to preserve local adaptation. In seed production, molecular marker-based parentage and kinship analyses should be applied to balance family contributions, reduce inbreeding, and prevent unintended domestication effects in stock enhancement programs. At the policy and management level, it is necessary to strengthen capacity for aquatic genetic resource management, promote cost-effective genotyping technologies, incorporate genetic risk assessment into species introduction and stock enhancement planning, and integrate these efforts with marine

protected area development and fisheries management measures. Incorporating these strategies into national aquaculture development plans will help maintain genetic diversity in both wild and cultured populations and provide a solid foundation for future breeding improvement.

Future research on grouper genetic diversity should increasingly rely on high-throughput genomic technologies and integrate genetic variation with production traits and adaptive potential. Whole-genome resequencing and reduced-representation methods such as RAD-seq and ddRAD can provide high-density SNP data for fine-scale population structure analysis, detection of selection signals, and genomic selection of traits such as growth, disease resistance, and environmental adaptability—approaches that have already proven successful in species such as salmonids. At the same time, low-cost, species-specific genotyping tools (e.g., targeted SNP panels) suitable for small and medium-sized aquaculture enterprises should be developed, and the effects of different detection strategies (e.g., sequencing depth and marker density) on genetic diversity and kinship assessment should be systematically evaluated. At the ecological level, integrating population genomics with marine environmental factors and tagging technologies can further optimize the delineation of management units, particularly for heavily exploited species such as Nassau grouper and brown grouper. In addition, incorporating genetic data into stock enhancement evaluation, studies of interactions between cultured and wild populations, and emerging biotechnologies (e.g., genomic selection, surrogate broodstock technology, and gene editing) will help develop breeding and conservation strategies that balance production efficiency with genetic security. Ultimately, such integrated approaches will ensure the long-term health and sustainable utilization of grouper germplasm resources.

Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Ai C.H., Lin Y.L., Chen M.Z., and Xia J.H., 2025, eDNA-based evaluation of the haplotypic diversity of orange-spotted grouper (*Epinephelus* coioides) in stock enhancement areas of Wanshan Archipelago, Marine Biotechnology, 27(4): 98.
https://doi.org/10.1007/s10126-025-10471-8

Chen Y., Luo Z., Zhang Z., Luo Z., Ren M., He X., Lin H., and Yan Y., 2025, Genetic pattern and demographic history of orange-spotted grouper (*Epinephelus* coioides) in the South China Sea by the influence of Pleistocene climatic oscillations, Ecology and Evolution, 15(2): e70967.
https://doi.org/10.1002/ece3.70967

Das S.K., Xiang T.W., Noor N.M., De M., Mazumder S.K., and Goutham-Bharathi M.P., 2021, Temperature physiology in grouper (Epinephelinae: Serranidae) aquaculture: A brief review, Aquaculture Reports, 20: 100682.
https://doi.org/10.1016/j.aqrep.2021.100682

Fadli N., Damora A., Muchlisin Z., Dewiyanti I., Ramadhaniaty M., Razi N., Jamaluddin, Macusi E.D., and Siti-Azizah M.N., 2023, Phylogeographic insights of five co-habiting grouper species in the Indo-Malaya Archipelago, HAYATI Journal of Biosciences, 30(4): 743-756.
https://doi.org/10.4308/hjb.30.4.743-756

González-Salas C., Villegas-Hernández H., Poot-López G., Pech-Puch D., Guillén-Hernández S., and Barrera-Guzmán A., 2020, Genetic population structure of black grouper (*Mycteroperca bonaci*) in the northern coast of Yucatan, Regional Studies in Marine Science, 37: 101327.
https://doi.org/10.1016/j.rsma.2020.101327

Hassanien H., and Al-Rashada Y., 2020, Assessment of genetic diversity and phylogenetic relationship among grouper species *Epinephelus* spp. from the Saudi waters of the Arabian Gulf, Saudi Journal of Biological Sciences, 28: 1779-1786.
https://doi.org/10.1016/j.sjbs.2020.12.020

He H., Tian H., Wang X., Peng L., Zhong L., Qi P., and Ma B., 2025, Genetic diversity of Gymnocypris firmispinatus: Insights from microsatellites and mitochondrial DNA among wild, broodstock source, and broodstock populations, Aquaculture Reports, 42: 102767.
https://doi.org/10.1016/j.aqrep.2025.102767

Houston R.D., Bean T.P., Macqueen D.J., Gundappa M.K., Jin Y.H., Jenkins T.L., Selly S.L.C., Martin S.A.M., Stevens J.R., Santos E.M., Davie A., and Robledo D., 2020, Harnessing genomics to fast-track genetic improvement in aquaculture, Nature Reviews Genetics, 21: 389-409.
https://doi.org/10.1038/s41576-020-0227-y

Hsu T.H., Chu P.Y., Gong H.Y., Nan F.H., and Huang C.W., 2024, Utilizing ISSRseq genotyping to assist growth selection in tomato grouper (*Cephalopholis sonnerati*) without broodstock information, Journal of the World Aquaculture Society, 55(2): e13035.
https://doi.org/10.1111/jwas.13035

Li C., 2022, Conservation of genetic resources for sustainable aquaculture, Journal of the World Aquaculture Society, 53(1): 4-7.
https://doi.org/10.1111/jwas.12875

Lou R.N., Jacobs A., Wilder A.P., and Therkildsen N.O., 2020, A beginner's guide to low-coverage whole genome sequencing for population genomics, Molecular Ecology, 30: 5966-5993.
https://doi.org/10.1111/mec.16077

Lu Y., Li M., Gao Z., He H., Chong Y., Hong J., Wu J., Wu D., Xi D., and Deng W., 2025, Advances in whole genome sequencing: Methods, tools, and applications in population genomics, International Journal of Molecular Sciences, 26(1): 372.
https://doi.org/10.3390/ijms26010372

Mainna M., Kadir N.N., Husain A.A.A., Hidayani A.A., Umar W., and Moore A.M., 2025, DNA barcoding and morphological characters of juvenile Plectropomus (Perciformes: *Epinephelidae*) caught in Makassar Strait, Jurnal Ilmiah Perikanan dan Kelautan, 17(3): 577.
https://doi.org/10.20473/jipk.v17i3.72091

Martchenko D., and Shafer A.B.A., 2023, Contrasting whole-genome and reduced representation sequencing for population demographic and adaptive inference: An alpine mammal case study, Heredity, 131: 273-281.
https://doi.org/10.1038/s41437-023-00643-4

Nousias O., Tzokas K., Papaharisis L., Ekonomaki K., Chatziplis D., Batargias C., and Tsigenopoulos C.S., 2021, Genetic variability, population structure, and relatedness analysis of meagre stocks as an informative basis for new breeding schemes, Fishes, 6(4): 78.
https://doi.org/10.3390/fishes6040078

Nurdin M.S., Hasanah N., Serdiati N., Putra A.E., Mansyur K., and Rizal A., 2025, Genetic conservation of two grouper species (*Epinephelus*) through DNA barcoding in Palu Bay, Indonesia, Arwana: Jurnal Ilmiah Program Studi Perairan, 7(1): 1-7.
https://doi.org/10.51179/jipsbp.v7i1.2986

Saha A., Kent M.P., Hauser L., Drinan D.P., Nielsen E.E., Westgaard J.I., Lien S., and Johansen T., 2021, Hierarchical genetic structure in an evolving species complex: Insights from genome-wide ddRAD data in Sebastes mentella, PLoS ONE, 16(5): e0251976.
https://doi.org/10.1371/journal.pone.0251976

Shan X., Zhang X., Ruan Z., Chen J., Shi Q., Xu J., and You X., 2023, Genomic selection of orange-spotted grouper (*Epinephelus coioides*) based on multiplex PCR enrichment capture sequencing, Aquaculture and Fisheries, 8(6): 681-688.
https://doi.org/10.1016/j.aaf.2022.08.006

Sherman K.D., Paris J.R., King R.A., Moore K.A., Dahlgren C.P., Knowles L.C., Stump K.L., Tyler C.R., and Stevens J.R., 2020, RAD-seq analysis and in situ monitoring of Nassau grouper reveal fine-scale population structure and origins of aggregating fish, Frontiers in Marine Science, 7: 157.
https://doi.org/10.3389/fmars.2020.00157

Sonesson A.K., Hallerman E.M., Humphries F., Hilsdorf A.W.S., Leskien D., Rosendal G.K., Bartley D.M., Hu X., Gomez G., and Mair G.C., 2023, Sustainable management and improvement of genetic resources for aquaculture, Journal of the World Aquaculture Society, 54(2): 364-396.
https://doi.org/10.1111/jwas.12968

Tavakoli-Kolour P., Farhadi A., Ajdari A., Bagheri D., Hazraty-Kari S., Ghasemi A., and Vazirzadeh A., 2022, Genetic species identification and population structure of grouper *Epinephelus coioides* collected from fish markets along the Persian Gulf and the Oman Sea, PeerJ, 10: e14179.
https://doi.org/10.7717/peerj.14179

Vaini J.O., Domingues R.R., Ferrette B.L.S., Hallerman E.M., Mota K.G., Barreiros J.P., and Hilsdorf A.W.S., 2021, Global population genetic structure of the sequential hermaphrodite, dusky grouper (*Epinephelus marginatus*), Aquatic Conservation: Marine and Freshwater Ecosystems, 31: 2119-2130.
https://doi.org/10.1002/aqc.3608

Wenne R., 2023, Microsatellites as molecular markers with applications in exploitation and conservation of aquatic animal populations, Genes, 14(4): 808.
https://doi.org/10.3390/genes14040808

Wu S., Zeng Q., Han W., Wang M., Ding H., Teng M., Wang M., Li P., Gao X., Bao Z., Wang B., and Hu J., 2024, Deciphering the population structure and genetic basis of growth traits from whole-genome resequencing of the leopard coral grouper (*Plectropomus leopardus*), Zoological Research, 45: 329-340.

Xu Y., Tian Y., Wang S., Ding T., Wang L., Li Z., Li L., and Liu Y., 2025, Embryonic and larval development of an interspecific grouper backcross between the kelp grouper (*Epinephelus* moara) ♀ and KGGG (*E. moara* ♀ × *E. lanceolatus* ♂) ♂, Fishes, 10(10): 488.
https://doi.org/10.3390/fishes10100488

Yang K., Lin H., Liu R., and Ding S., 2022, Genetic structure and demographic history of yellow grouper (*Epinephelus awoara*) from the coast of southeastern mainland China, inferred by mitochondrial, nuclear and microsatellite DNA markers, Diversity, 14(6): 439.
https://doi.org/10.3390/d14060439

Yang Y., Chu P., Chen C., Yang W., Hsu T., Gong H., Liao I., and Huang C., 2024, Transcriptomic insights and the development of microsatellite markers to assess genetic diversity in the broodstock management of Litopenaeus stylirostris, Animals, 14(11): 1685.
https://doi.org/10.3390/ani14111685

Yang Y., Wang T., Chen J., Wu L., Wu X., Zhang W., Luo J., Xia J., Meng Z., and Liu X., 2021, Whole-genome sequencing of brown-marbled grouper (*Epine*phelus fuscoguttatus) provides insights into adaptive evolution and growth differences, Molecular Ecology Resources, 22: 711-723.
https://doi.org/10.1111/1755-0998.13494

Yang Y., Zeng L., Wang T., Wu L., Wu X., Xia J., Meng Z., and Liu X., 2023, Assembly of genome and resequencing provide insights into genetic differentiation between parents of Hulong hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *E. lanceolatus* ♂), International Journal of Molecular Sciences, 24(15): 12007.
https://doi.org/10.3390/ijms241512007

Ybanez C.J.E., and Gonzales R.C., 2023, Challenges and progress of grouper aquaculture in Asia: A review, Davao Research Journal, 14(2): 6-29.
https://doi.org/10.59120/drj.v14i2.109

International Journal of Aquaculture, 2026, Vol.16, No.3, 184-195
http://www.aquapublisher.com/index.php/ija

Original Research Article Open Access

Antimicrobial Susceptibility, Microbial Loads and Isolation of *Plesiomonas shigelloides* from African Sharptooth Catfish (*Clarias gariepinus*) Juveniles and Experimental Pond Water

Sunday Emmanuel Olusola [1], Temitope Deborah Agboola [2], Tolulope Martins Ogunmakinwa [2], Folasade Damilola Amulejoye [1], Mariam Iyabo Adeoba [3], Omotola Victoria Ayebidun [1]

1 Department of Fisheries and Aquaculture Technology, School of Agriculture, Food and Natural Resources, Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria

2 Department of Biological Sciences (Microbiology Programme), School of Science, Olusegun Agagu University of Science and Technology, Okitipupa, Nigeria

3 Unisa Biomechanics Research Group, Department of Mechanical, Bioresources and Biomedical Engineering, College of Science Engineering and Technology (CSET) University of South Africa, Pretoria, South Africa

Corresponding author: belloolus@yahoo.com

International Journal of Aquaculture, 2026, Vol.16, No.3 doi: 10.5376/ija.2026.16.0015

Received: 3 Apr., 2026

Accepted: 31 May., 2026

Published: 25 Jun., 2026

Preferred citation for this article:

Olusola S.E., Agboola T.D., Ogunmakinwa T.M., Amulejoye F.D., Adeoba M.I., and Ayebidun O.V., 2026, Antimicrobial susceptibility, microbial loads and isolation of *Plesiomonas shigelloides* from African sharptooth catfish (*Clarias gariepinus*) juveniles and experimental pond water, International Journal of Aquaculture, 16(3): 184-195 (doi: 10.5376/ija.2026.16.0015)

Abstract The aim of this study was to investigate the antimicrobial susceptibility, microbial loads and isolation of *Plesiomonas shigelloides* isolated from African catfish, *Clarias gariepinus* juveniles and experimental pond water. Microbial loads of pond water and fish tissues (gill, liver, and intestine) were evaluated using standard methods. Isolation and antibiotic susceptibility of the bacterial species were carried out using standard microbiological techniques. Antibiotic susceptibility of the isolates was assessed using a panel of 12 antibiotics by disc diffusion method and standard guidelines. The microbial loads in water from the experimental ponds ranged from 5.60 to 7.00 $\log_{10}$ CFU/mL, while those in gill, liver, and intestine samples ranged from 6.40 to 7.00 $\log_{10}$ CFU/g. The microbial loads were higher than the permissible limits for wastewater and fish tissues. The microscopic cell morphology analysis of presumptive *P. shigelloides* revealed 40 isolates of round-ended, straight rod shape, which were motile, positive to oxidase, catalase, mannitol, and citrate biochemical test, negative to urease, methyl red, and glucose biochemical test. Antibiotic susceptibility results showed that the presumptive *P. shigelloides* were 100% resistant to cefuroxime and cefotaxime, 87.5% to meropenem, and 77.5% to ceftazidime. However, the isolates were 0% resistant to gentamicin and amikacin of aminoglycoside derivatives, suggesting that these might be only two out of the 12 panels of antibiotics used that presumptive *P. shigelloides* might have responded to. The findings highlight the need for routine microbial monitoring, improved pond hygiene, and responsible antimicrobial use in catfish aquaculture. The observed *in vitro* susceptibility to gentamicin and amikacin may provide useful baseline information for future risk assessment and antimicrobial stewardship.

Keywords Antibiotics; Biochemical test; *Clarias gariepinus*; Microbial loads; *Plesiomonas shigelloides*

1 Introduction

Fisheries and aquaculture products worldwide are important sources of high-quality aquatic animal proteins and good sources of income, foreign exchange, and employment. About 950 million people worldwide rely on fisheries and aquaculture directly or indirectly for their livelihoods. Globally, the consumption of fish and fishery products as a protein source has increased considerably over the years, constituting about 20% of total protein (FAO, 2020). The aquaculture industry grows at a fast rate when compared to all other animal food-producing sectors worldwide, with the world average annual growth rate of 8.8% /yr since 1970, compared with only 1.2% for capture fisheries and 2.8% for land-farmed animal production systems (FAO, 2020). However, as aquaculture production increases, aquaculture waste also increases, and this aquaculture wastewater harbours diverse pollutants, including pathogenic organisms, which are detrimental to public health when released into the environment, and this makes fish farmers treat aquaculture effluent with pesticides and antibiotics.

The use of antibiotics can result in drug-resistant strains of diseases, causing bacteria that can harm aquatic animal populations and consumers of aquaculture fish. There are also increasing concerns about foodborne hazards, such as chemicals and microbial contaminants, that might be present in fish. These concerns can also result in decreasing demand for farmed fish (Smallwood and Blaylock, 1991). In an approach to satisfy the growing demand for fish, food safety is a major factor to be considered since these animals can be routes for the transmission of various pathogens such as *Salmonella* spp., *Vibrio* spp., *Aeromonas* spp., *Campylobacter* spp., *Shigella* spp., *Yersinia* spp., *Clostridium* spp., *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Plesiomonas shigelloides* which have been responsible for numerous cases and outbreaks of food-borne diseases in humans worldwide (Cortes-Sanchez et al., 2021).

Plesiomonas shigelloides is an oxidase-positive, facultatively anaerobic, Gram-negative, motile, rod-shaped bacterium commonly found in aquatic environments (Jagger, 2010; Janda et al., 2016). Aquaculture-raised fish for commercial purposes appear to be strongly associated with the presence of P. shigelloides (Janda et al., 2016). Plesiomonas was a common pathogen in the gills, muscles and intestines of fish as well as in rearing waters and pond sediment (Pakingking et al., 2015). Many works of literature reported that *Plesiomonas shigelloides* caused diarrhoea/gastroenteritis in humans via water or fish contaminated with this pathogen (Gonzalez - Rey et al., 2000). Plesiomonads are mesophiles with growth temperatures ranging between 8 ℃~45 ℃ with (a) pH ranging between 4.5 and 9.0 (Janda et al., 2016). Several factors that encourage the growth of *Plesiomonas shigelloides* include(s) overcrowding, oxygen levels, temperature, and climatic conditions, as well as food sources (Jun et al., 2011). However, little or no information is documented on *Plesiomonas shigelloides* as a causal agent of enteritis in pond water and African catfish (*C. gariepinus*). Hence, this study aimed at evaluating the microbial loads, isolation and antimicrobial susceptibility of *Plesiomonas shigelloides* from experimental pond water and *C. gariepinus*.

2 Materials and Methods

2.1 Study area

This study was conducted at the Department of Fisheries and Aquaculture Technology's Teaching and Research Farm, Olusegun Agagu University of Science and Technology, Okitipupa. Okitipupa is located in the Ondo State of Nigeria, and it is reported to have a geographical coordinate of 6º 27' 25" N,4º 46' 00" E.

2.2 Media preparation and sterilization of materials

All media (Inositol brilliant green agar, nutrient agar, Mueller Hinton agar, nutrient broth, blood agar and alkaline peptone water) were prepared according to the manufacturer's instruction; the media were weighed out accurately and dissolved in an appropriate volume of water. The prepared mixture was homogenized and sterilized by autoclaving at 121 ℃ for 15 min. All these media were allowed to cool after sterilization to about 45 ℃ before pouring into Petri dishes. Alkaline peptone water was used as an enrichment medium.

2.3 Sample collection and design

Experimental ponds (6) were randomly selected and used for this study. The experimental ponds were replicated twice and the pond water (aquaculture water) was collected from each experimental pond at 0, 2, 4, 6 and 8 weeks while fish tissues (gill, liver and intestine) were collected from each experimental pond at 0, 4 and 8 weeks. Three fish were sampled from each experimental pond every 4 weeks. The experimental design was completely randomized block design.

2.4 Water quality analysis

A water sample (50 mL) was taken at 25 cm below the water surface by using a Van Dom water sampler (Denmark) from each experimental pond. The water quality parameters such as pH, temperature and total dissolved solids were taken at 0, 2, 4, 6 and 8 weeks as described by Olaifa and Bello (2011).

2.5 Isolation of bacteria/ bacterial counts

One gram (1 g) of gills, intestine and liver sample of C. gariepinus were separately macerated and put into a sterile capped test tube containing 9 mL of sterilized alkaline peptone water and 1 ml of experimental pond water was dispensed into 9 mL of sterilized peptone water (Bello et al., 2012; Bello, 2014). The pond water was also enriched in alkaline peptone water for the isolation of the presumptive *Plesiomonas shigelloides*. Serial dilution was carried out and 0.1 mL each from 10^{-4} and 10^{-5} dilution factor was dispensed into Petri dishes that were appropriately labelled and the molten sterilized medium was poured aseptically into a Petri dish. The plates were swirled gently for even distribution of inoculums and allowed to set/gel and then incubated at 37 ℃ for 24 h. The organism grew into visible different colonies after 24 h. Total viable counts were determined and the results were expressed in $\log_{10}$ CFU/mL for pond water and $\log_{10}$ CFU/g for fish tissues. Also, 3~5 colonies of presumptive *P. shigelloides* were picked, purified and stocked on nutrient agar slant for further study.

2.6 Identification of isolates

Identification of the isolates was based on the procedures described by Mohammed et al. (2026). After observing cultural growth indices, the positive culture was subjected to Gram staining to study staining properties and cellular morphology under a 100X objective of a light microscope. Mixed colonies and Gram-negative bacteria were subcultured on both broth and nutrient agar (Oxoid, UK) and further incubated aerobically for 24 h. Pure culture of single colony type from (both broth and) nutrient agar were transferred onto nutrient slant for a biochemical test including catalase, oxidase, urease test, motility test, indole reaction test and fermentative/oxidative tests, hemolysis on blood agar and Gram staining techniques as described by Quinn et al. (2002) and Medical Research Council (MRC, 2017).

2.7 Gram staining technique

Young growing cultures of 18~24 h of the test isolates were used to prepare smears on clean grease-free microscopic slides. This was done by first cleaning the glass slides with cotton wool soaked with ethanol. A distinct colony of the isolates was picked with a sterile wire loop and emulsified with distilled water to form a smear and fixed. The smear was then stained with aqueous crystal violet for 1 min and was rinsed off gently with water, 95% Lugol iodine was added. The smear was decolourized with acetone until there was no violet colour on the slide. This was then rinsed off gently with water again, counter-stained with safranin for about 30 seconds, and then rinsed with water. The slide was carefully dried and examined under an oil immersion microscope with a 100x objective (MRC, 2017).

2.8 Antibiotic susceptibility

The antibiotic susceptibility profile of presumptive *P. shigelloides* was determined by using the disc diffusion technique as described by Kirby-Bauer with some modified disc diffusion techniques using 12 antibiotic discs (Biotec Lab. the United Kingdom) corresponding to the drugs containing most used in the treatment of human and animal infections caused by bacteria. The antibiotic sensitivity results for presumptive P. shigelloides were interpreted using the recommended guidelines by the Clinical Laboratory Standard Institute (CLSI, 2020). The antibiotics include; Cotrimoxazole (COT) 25 µg, Cefuroxime (CRX) 30 µg, Tetracycline (TET) 10 µg, Gentamicin (GEN) 10 µg, Ceftazidime (CPZ) 30 µg, Chloramphenicol (CHL) 10 µg, Ceftriaxone (CTR) 30 µg, Ciprofloxacin (CPR) 5 µg, Cefotaxime (CTX) 30 µg, Vancomycin (VAN) 30 µg, Amikacin (AMK) 30 µg and Meropenem (MEM) 10 µg. An 18~24 h old culture of all the isolates was prepared, after which the standardized broth culture of the inoculum was used to inoculate solidified pre-sterilized Mueller Hinton Agar plates. The antibiotics disc containing a specific concentration of antibiotics was placed on the Mueller Hinton Agar using sterile forceps and incubated at 32 oC for 24 h. The diameter of zones of inhibition was measured in millimetres and interpreted using CLSI (2020) standard and classified as sensitive, intermediate sensitive and resistant (MRC, 2017).

2.9 Statistical analysis

Bacteriological characteristics and physiochemical analysis resulting from the experiment were subjected to one-way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences 2006 version 15.0). Duncan's multiple range test was used to compare differences among individual means.

3 Results

3.1 Water quality parameters of the experimental pond water

The physicochemical properties of the experimental pond water showed a pH range of 7.0~8.5, Temperature 21.3 ℃ ~33.0 ℃, and Total suspended solids 25.0~41.0 (mg/L), and there were significant differences ($p < 0.05$) among the treatments in pH, temperature and total dissolved solids (Table 1).

Table 1 Water quality parameters of the experimental pond water

1	0	2	4	6	8
Ph					
Pond 1	7.10±0.01^{a}	8.00±0.06^{b}	7.50±0.01^{ab}	7.20±0.00^{a}	8.50±0.06^{c}
Pond 2	7.40±0.03^{a}	7.20±0.00^{a}	8.00±0.04^{bc}	7.50±0.04^{ab}	7.50±0.04^{ab}
Pond 3	7.10±0.04^{a}	7.50±0.01^{ab}	8.50±0.03^{c}	7.30±0.06^{b}	8.00±0.02^{bc}
Pond 4	7.20±0.02^{a}	7.70±0.03^{ab}	7.70±0.02^{ab}	8.50±0.08^{a}	7.20±0.01^{a}
Pond 5	7.00±0.05^{a}	8.20±0.05^{b}	8.00±0.05^{bc}	7.20±0.03^{ab}	7.60±0.03^{ab}
Pond 6	7.30±0.07^{a}	8.00±0.00^{b}	7.20±0.02^{a}	7.00±0.01^{ab}	7.40±0.04^{ab}
Temperature (℃)					
Pond 1	21.30±0.02^{a}	26.60±0.00^{c}	27.90±0.03^{c}	29.70±0.08^{e}	33.00±0.09^{d}
Pond 2	25.60±0.08^{c}	27.00±0.01^{c}	25.00±0.02^{b}	26.70±0.01^{c}	27.30±0.02^{b}
Pond 3	23.70±0.04^{b}	22.00±0.04^{a}	21.60±0.01^{a}	27.20±0.07^{c}	25.20±0.03^{a}
Pond 4	30.40±0.09^{e}	28.60±0.06^{e}	22.70±0.95^{a}	24.30±0.03^{a}	29.00±0.07^{c}
Pond 5	28.50±0.06^{d}	24.90±0.05^{b}	31.20±0.09^{d}	28.20±0.06^{d}	25.00±0.05^{a}
Pond 6	25.80±0.07^{c}	27.90±0.03^{d}	22.00±0.06^{b}	25.00±0.04^{b}	27.00±0.03^{b}
Total dissolved solid (TDS) (mg/L)					
Pond 1	39.40±0.07^{e}	29.20±0.03^{c}	27.50±0.05^{b}	36.50±0.02^{f}	38.00±0.05^{c}
Pond 2	26.20±0.02^{b}	25.50±0.00^{a}	30.50±0.07^{d}	31.60±0.04^{d}	40.20±0.09^{d}
Pond 3	41.00±0.09^{e}	36.20±0.05^{f}	31.20 ±0.01^{e}	29.60±0.06^{c}	29.80± 0.01^{b}
Pond 4	30.20±0.08^{c}	27.40±0.04^{b}	28.50±0.02^{c}	24.20±0.00^{a}	30.00±0.03^{b}
Pond 5	25.00±0.05^{a}	30.00±0.01^{d}	25.60±0.04^{a}	33.00±0.03^{e}	27.50± 0.02^{a}
Pond 6	33.40±0.03^{d}	31.00±0.2 ^{e}	30.00 ± 0.06^{d}	26.20±0.01^{b}	28.00± 0.04^{a}

Means (n =2) in the same column with similar superscripts are not significantly different ($p > 0.05$)

3.2 Bacteria counts of experimental pond water and fish tissues (gill, liver and intestine)

A total of 30 water samples from the experimental fish ponds were analyzed for total bacteria counts at 0, 2, 4, 6 and 8th week and 54 fish tissues (gills, intestine, liver) from *C. gariepinus* juveniles in all the experimental ponds were analyzed for total bacteria counts at 0, 4 and 8th week. Test for the presence of presumptive *P. shigelloides* revealed that the bacterium was present in *C. gariepinus* tissues and experimental pond water. The Plesiomonas count on *C. gariepinus* tissues (gills, liver and intestine) ranges between 6.4 to 7.0 $\log_{10}$ CFU/g while the Plesiomonas count on experimental pond water ranges between 5.6 to 7.0 $\log_{10}$ CFU/mL. There was no significant difference ($p > 0.05$) in the total bacteria observed in the gill, liver and intestine and experimental pond water among the experimental groups except for experimental pond water at the 6th week who recorded significant differences ($p < 0.05$) among the groups (Table 2).

3.3 Isolation of *Plesiomonas shigelloides*

A total of 250 isolates were obtained from both the aquaculture effluent (pond water) and fish tissues (gill, liver and intestine). Morphological identification was analyzed based on the shape, texture and colour of bacteria colonies on inositol brilliant bile green agar. The microscopic cell morphology analysis of the presumptive *P.*

shigelloides however shows only 40 isolates obtained from the selective agar were round-ended, straight rod shapes which are motile (15 isolates from experimental pond water and 25 from fish tissues (Table 3 and Table 4).

3.4 Morphological and biochemical characteristics of isolates of experimental pond water and fish tissues

The 40 isolates further tested positive for oxidase, catalase, mannitol and citrate biochemical tests, they also tested negative for urease, methyl red and glucose biochemical tests (Table 5 and Table 6).

Table 2 Total bacteria counts of experimental pond water and fish tissues (gill, liver and intestine)

Weeks	Pond 1	Pond 2	Pond 3	Pond 4	Pond 5	Pond 6
Water sample						
0	5.70±0.00^{a}	6.00±0.01^{a}	5.60±0.02^{a}	6.10±0.03^{a}	6.00±0.02^{a}	6.00±0.01^{a}
2	7.00±0.02^{a}	7.00±0.03^{a}	7.00±0.01^{a}	7.00±0.06^{a}	6.90±0.09^{a}	7.00±0.03^{a}
4	6.40±0.05^{a}	6.80±0.05^{a}	6.70±0.03^{a}	6.80±0.08^{a}	6.60±0.07^{a}	6.70±0.02^{a}
6	6.20±0.03^{ab}	6.60±0.03^{b}	6.50±0.04^{ab}	6.00±0.03^{ab}	5.80±0.01^{a}	6.50±0.05^{ab}
8	6.50±0.07^{a}	6.70±0.06^{a}	6.30±0.02^{a}	6.60±0.05^{a}	6.30±0.03^{a}	6.60±0.07^{a}
Gill						
0	6.60±0.03^{a}	6.90±0.02^{a}	6.70±0.00^{a}	6.80±0.01^{a}	6.80±0.07^{a}	6.50±0.04^{a}
4	7.00±0.05^{a}	6.80±0.05^{a}	6.90±0.05^{a}	6.80±0.06^{a}	6.60±0.04^{a}	6.60±0.06^{a}
8	6.50±0.02^{a}	6.40±0.00^{a}	6.80±0.03^{a}	6.60±0.02^{a}	6.50±0.01^{a}	6.50±0.02^{a}
Liver						
0	6.90±0.04^{a}	6.70±0.03^{a}	6.80±0.05^{a}	6.80±0.2^{a}	6.70±0.05^{a}	6.60±0.01^{a}
4	6.80±0.02^{a}	6.90±0.07^{a}	7.00±0.08^{a}	6.70±0.2^{a}	6.60±0.03^{a}	6.50±0.04^{a}
8	6.50±0.00^{a}	6.90±0.09^{a}	6.90±0.04^{a}	6.70±0.2^{a}	6.60±0.06^{a}	6.40±0.05^{a}
Intestine						
0	6.50±0.01^{a}	6.90±0.03^{a}	6.80±0.07^{a}	6.60±0.2^{a}	6.50±0.00^{a}	6.50±0.01^{a}
4	6.80±0.02^{a}	6.70±0.01^{a}	7.00±0.09^{a}	6.80±0.2^{a}	6.70±0.04^{a}	6.60±0.02^{a}
8	6.80±0.04^{a}	6.80±0.00^{a}	6.80±0.03^{a}	6.80±0.2^{a}	6.70±0.03^{a}	6.80±0.05^{a}

Means (n =2) in the same row with similar superscripts are not significantly different ($p > 0.05$)

Table 3 Colony characteristics of isolates from experimental pond water

Weeks	Isolate code	Colony shape	Elevation	Edge	Surface	Pigmentation	Opacity
4	Dctr	Short rod	Raised	Regular	Smooth	Pink	Opaque
	Dctr	Short rod	Raised	Regular	Dull	Pink	Opaque
	DTii	Short rod	Raised	Regular	Smooth	Pink	Opaque
	DTii	Short rod	Raised	Entire	Smooth	Pink	Opaque
	DTiii	Short rod	Raised	Regular	Smooth	Pink	Opaque
	DTiv	Short rod	Raised	Entire	Smooth	Pink	Opaque
	DTv	Short rod	Raised	Regular	Smooth	Pink	Opaque
	DTvi	Short rod	Raised	Regular	Dull	Pink	Opaque
	DTvi	Short rod	Raised	Irregular	Rough	Pink	Opaque
8	Ectr	Short rod	Raised	Regular	Smooth	Pink	Opaque
	ETii	Short rod	Raised	Regular	Smooth	Pink	Opaque
	ETiii	Short rod	Raised	Regular	Smooth	Pink	Opaque
	ETiv	Short rod	Raised	Regular	Smooth	Pink	Opaque
	ETv	Short rod	Raised	Entire	Smooth	Pink	Opaque
	ETvi	Short rod	Raised	Regular	Dull	Pink	Opaque

Keys: D, water sample at week 4; E, water sample at week 8; Ctr, experimental pond 1; Tii, experimental pond 2; Tiii, experimental pond 3; Tiv, experimental pond 4; Tv, experimental pond 5; Tvi, experimental pond 6; SR, Short rod

Table 4 Colony characteristics of isolates from gill, liver and intestine

Weeks	Isolate code	Colony shape	Elevation	Edge	Surface	Pigmentation	Opacity
0	FGctr	Short rod	Raised	Regular	Dull	Pink	Opaque
	FGTii	Short rod	Raised	Entire	Smooth	Pink	Opaque
	FITv	Short rod	Raised	Regular	Smooth	Pink	Opaque
	Fiiv	Short rod	Raised	Regular	Smooth	Pink	Opaque
	FLTiii	Short rod	Raised	Irregular	Rough	Pink	Opaque
4	GGTii	Short rod	Raised	Entire	Smooth	Pink	Opaque
	GGTiii	Short rod	Raised	Regular	Smooth	Pink	Opaque
	GGTv	Short rod	Raised	Regular	Smooth	Pink	Opaque
	GGTiv	Short rod	Raised	Entire	Smooth	Pink	Opaque
	GIctr	Short rod	Raised	Regular	Smooth	Pink	Opaque
	GITiv	Short rod	Raised	Regular	Dull	Pink	Opaque
	GITv	Short rod	Raised	Regular	Dull	Pink	Opaque
	GLctr	Short rod	Raised	Regular	Smooth	Pink	Opaque
	GLTii	Short rod	Raised	Irregular	Rough	Pink	Opaque
	GLTv	Short rod	Raised	Regular	Smooth	Pink	Opaque
8	HGTii	Short rod	Raised	Regular	Smooth	Pink	Opaque
	HGTv	Short rod	Raised	Entire	Smooth	Pink	Opaque
	HGTvi	Short rod	Raised	Irregular	Rough	Pink	Opaque
	HITiii	Short rod	Raised	Regular	Smooth	Pink	Opaque
	HITiv	Short rod	Raised	Regular	Smooth	Pink	Opaque
	HLctr	Short rod	Raised	Entire	Smooth	Pink	Opaque
	HLctr	Short rod	Raised	Regular	Dull	Pink	Opaque
	HLTii	Short rod	Raised	Regular	Smooth	Pink	Opaque
	HLTiii	Short rod	Raised	Regular	Dull	Pink	Opaque
	HLTvi	Short rod	Raised	Regular	Smooth	Pink	Opaque

FG, fish gills at 0 weeks; FI, fish intestine at 0 weeks; FL, fish liver at 0 weeks; GG, fish gill at 4 weeks; GI, fish intestine at 4 weeks; GL, fish liver at 4 weeks; HG, fish gill at 8 weeks; HI, fish intestine at 8 weeks; HL, fish liver at 8 weeks; Ctr, experimental pond 1; Tii, experimental pond 2; Tiii, experimental pond 3; Tiv, experimental pond 4; Tv, experimental pond 5; Tvi, experimental pond 6

3.5 Antibiotic sensitivity test

The antibiotic sensitivity test for presumptive *Plesiomonas shigelloides* was interpreted using the recommended guidelines by the Clinical Laboratory Standard Institute (CLSI, 2020) and is shown in table 5. Presumptive *Plesiomonas shigelloides* that were observed showed 100% resistance to cefotaxime, and cefuroxime, which belongs to the antibiotic class of cephems, followed by meropenem 87.5%, which belong to the antibiotic class of Carbapenems, Ceftazidime 77.5%, which belong to the antibiotic class of cephems, Vancomycin 70% which belong to the antibiotic class of glycopeptides tetracycline 40% which belong to the antibiotic class of tetracycline, ceftriaxone 37.5% which belong to the antibiotic class of cephems, chloramphenicol 20% which belong to the antibiotic class of phenicols, ciprofloxacin 20% which belong to a class of fluoroquinolones, cotrimoxazole 17.5% which belong to a class of sulfonamides, gentamicin and amikacin 0% which belong to a class of aminoglycosides (Table 7).

3.6 Multiple antibiotic resistance phenotypes of *Plesiomonas shigelloides*

All presumptive *P. shigelloides* obtained from this study exhibited resistance to at least one antibiotic. Meanwhile, most of the isolates (85%) showed resistance to three (3) or more classes of antibiotics. Resistant to four (4) classes of antibiotics had the highest frequency of occurrence. Out of the seventeen isolates resisting the effect of four (4) classes of antibiotics, resistance to Tetracycline, Cephalosporins, Carbapenem and Glycopeptides (76.5%) was seen to be the highest compared to other phenotypes (Table 8).

Table 5 Morphological and biochemical characteristics of isolates of gill, liver and intestine

Weeks	Isolate code	Cell shape	Gram reaction	Catalase	TSI reaction	Urease	Motility test	H_2S test	Citrate reaction	Methyl red	Hemolysis	Glucose	Mannitol	Oxidase reaction	Possible organisms
0	FGctr	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	FGTii	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	FITv	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	FITiv	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	FLTiii	SR	-	-	-	-	-	-	-	-	B	+	+	+	presumptive Plesiomonas shigelloides
4	GGTii	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	GGTiii	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	GGTiv	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	GGTv	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	GIctr	SR	-	-	-	-	-	-	-	-	B	+	+	+	presumptive Plesiomonas shigelloides
	GITiv	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	GITv	SR	-	-	-	-	-	-	-	-	B	+	+	+	presumptive Plesiomonas shigelloides
	GLctr	SR	-	-	-	-	-	-	-	-	B	+	+	+	presumptive Plesiomonas shigelloides
	GLTii	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	GLTv	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
8	HGTii	SR	-	-	-	-	-	-	-	-	B	+	+	+	presumptive Plesiomonas shigelloides
	HGTv	SR	-	-	-	-	-	-	-	-	B	+	+	+	presumptive Plesiomonas shigelloides
	HGTvi	SR	-	-	-	-	-	-	-	-	B	+	+	+	presumptive Plesiomonas shigelloides
	HITiii	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	HLTii	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	HITiv	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	HLctr	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	HLctr	SR	-	-	-	-	-	-	-	-	B	+	+	+	presumptive Plesiomonas shigelloides
	HLTii	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	HLTvi	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides

FG, fish gills at 0 weeks; FI, fish intestine at 0 weeks; FL; fish liver at 0 weeks; GG, fish gill at 4 weeks; GI, fish intestine at 4 weeks, GL, fish liver at 4 weeks, HG, fish gill at 8 weeks, HI, fish intestine at 8 weeks; HL, fish liver at 8 weeks; Ctr, experimental pond 1; Tii, experimental pond 2; Tiii, experimental pond 3; Tiv, experimental pond 4; Tv, experimental pond 5; Tvi, experimental pond 6; SR, Short rod; +, Positive; β, Beta; -, Negative

International Journal of Aquaculture, 2026, Vol.16, No.3, 184-195
http://www.aquapublisher.com/index.php/ija

Table 6 Morphological and biochemical characteristics of isolates of experimental pond water

Weeks	Isolate code	Cell shape	Gram reaction	Catalase	TSI reaction	Urease	Motility test	H_2S test	Citrate reaction	Methyl red	Hemolnysis	Glucose	Mannitol	Oxidase reaction	Possible organisms
4	Dctr	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	Dctr	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	DTii	SR	-	-	-	-	-	-	-	-	B	+	+	+	presumptive Plesiomonas shigelloides
	DTii	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	DTiii	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	Dtiv	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	DTv	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	Dtvi	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	Dtvi	SR	-	-	-	-	-	-	-	-	B	+	+	+	presumptive Plesiomonas shigelloides
8	Ectr	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	Etii	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	Etiii	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	Etiv	SR	-	+	-	-	+	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides
	Etv	SR	-	+	-	-	+	-	+	-	B	-	+	+	presumptive Plesiomonas shigelloides
	ETvi	SR	-	+	-	-	-	-	+	-	B	+	+	+	presumptive Plesiomonas shigelloides

D-, water sample at week 4; E, water sample at week 8; Ctr, experimental pond 1, Tii, experimental pond 2; Tiii, experimental pond 3; Tiv, experimental pond 4; Tv, experimental pond 5; Tvi, experimental pond 6; SR, Short rod; +, Positive; β, Beta; -. Negative

Table 7 Antimicrobial susceptibility profile for *Plesiomonas* isolate

Antibiotic class	Antibiotic tested	Susceptibility pattern							
		Disc code	P (μg)	Sensitive		Intermediate		Resistant	
				N	%	N	%	N	%
Fluoroquinolones	Ciprofloxacin	CIP	5	24	60	8	20	8	20
Tetracyclines	Tetracycline	TET	10	23	57.5	0	0	17	42.5
Sulfonamides	Cotrimoxazole	COT	25	28	70	5	12.5	7	17.5
Aminoglycosides	Gentamicin	GEN	10	40	100	0	0	0	0
	Amikacin	AMK	30	40	100	0	0	0	0
Cephems	Ceftazidime	CPZ	30	4	10	5	12.5	31	77.5
	Cefotaxime	CTX	30	0	0	0	0	40	100
	Cefuroxime	CRX	30	0	0	0	0	40	100
	Ceftriaxone	CTR	30	14	35	11	27.5	15	37.5
Phenicols	Chloramphenicol	CHR	10	26	65	5	12.5	9	22.5
Glycopeptides	Vancomycin	VAN	30	8	20	4	10	28	70
Carbapenems	Meropenem	MEM	10	2	5	3	7.5	35	87.5

Keys: N – number of isolates

Table 8 Multiple antibiotic-resistant phenotypes of *Plesiomonas shigelloides*

No of antibiotics	Resistance Pattern	Frequency
2	Ceph- carb	4
3	Ceph- Carb- phe	1
	Tet- Ceph- Carb	1
	Ceph- Gly- Carb	8
4	Sul- Ceph- Phe- Carb	1
	Flu- Phe- Gly- Carb	2
	Ceph- Gly- Carb- Phe	1
	Tet- Ceph- Carb- Gly	13
5	Tet- Ceph- Sul - Gly- Carb	4
	Tet- Ceph- Phe- Gly- Carb	2
6	Flu- Tet- Ceph- Phe- Gly- Carb	1

KEY: Ceph, Cephems; Gly, Glycopeptides; Carb, Carbapenems; Tet, Tetracyclines; Sul, Sulfonamides; Phe, Phenicols; Ami, Aminoglycosides; Flu, Fluoroquinolones

5 Discussion

The result of the study revealed that water quality parameters such as pH, temperature and total dissolved solids measured every two weeks show variation in the values obtained among these parameters and there was a significant difference ($p< 0.05$) among the experimental ponds. The water quality values observed in this study are comparable to those reported by Olaifa and Bello (2011) who reported a temperature of 25℃~28℃ and pH of 6-8.5 for *C. gariepinus* on feed supplemented with walnut leaves and onion bulb-based diet. This finding supports the report of Olusola and Olorunfemi (2017) who observed a temperature range of 28 ℃~30 ℃ and pH of 5.70 - 6.19 for *C. gariepinus* fed guava *(Psidium guajava)* leaves and drumstick (*Moringa oleifera)* leaves extracts supplemented diet. This finding also aligns with the report of Omotayo et al. (2006). The value of total dissolved solid obtained was within the acceptable limit (2000 mg/L) by Food and Agriculture Organization, FAO (2013) for culture water.

Most microbes are transients in aquatic animals and may change rapidly with the intrusion of microbes coming from water and food. The growing demand for fish, and food safety is an essential element to consider since these animals can be vehicles for the transmission of various pathogens (Cortes-Sanchez et al., 2021). The result of this

study revealed that the value of bacteria counts in experimental pond water was higher in 2 weeks in all the experimental ponds and the values decreased in 4, 6, and 8 weeks. Pond 2 recorded higher values of bacteria counts at 8 weeks when compared to other experimental ponds and there was no significant difference ($p > 0.05$) among the experimental ponds except 6 weeks that recorded a significant difference ($p < 0.05$) among the experimental ponds. The value of bacteria counts in the gill, liver and intestine was higher in pond 3 at 4 and 8 weeks when compared to other experimental ponds and there was no significant difference ($p > 0.05$) among the experimental ponds. The value of microbial loads obtained in the gill, liver and intestine were higher than the world health organization (WHO) acceptable limits 3.0 $\log_{10}$ CFU/g for fish and 6.2~6.5 $\log_{10}$ CFU/mL for wastewater.

Morphological identification was analyzed based on the shape, texture and colour of bacteria colonies on inositol brilliant bile agar. Forty (40) isolates tested positive for oxidase, catalase, mannitol and citrate biochemical tests, they also tested negative for urease, methyl red and glucose biochemical tests. This study supports the report of Wang et al. (2020) who observed a similar trend in the morphology and biochemical test of *P. shigelloides* during isolation and characterization from wastewater and tissues of *Ictalurus punctatus*. The identification of presumptive *P. shigelloides* in the pond water and fish tissues (gill, liver and intestine) is aligned with the observation of Krovacek et al. (2000) that fish and shellfish are the natural habitats of *P. shigelloides*. Jon et al. (2013) also isolated *P. shigelloides* from farm-cultured eels (*Anguilla japonica*) and their environmental waters in Korean eel farms. This study also agrees with Adesiyan et al. (2019) who stated that *P. shigelloides* is one of the indigenous bacteria of an aquatic environment.

This study was evaluated to look at the pattern of resistance or susceptibility to some of the commonly used 12 antibiotics in Nigeria and the forty presumptive *P. shigelloides* isolates were obtained from the pond water and gill, liver and intestine of *C. gariepinus*. This study revealed that cefuroxime and cefotaxime, a derivative of cephems is the least effective because all the 40 presumptive *P. shigelloides* were (100%) resistant to this class of antibiotics. The resistance of the isolates is also high in meropenem (87.5%) and ceftazidime (77.5%), which are the derivatives of carbapenems and cephems, respectively. Vancomycin (70%) a derivative of glycopeptides, tetracycline (42.5%), a derivative of tetracycline, ceftriaxone (37.5%), a derivative of cephems, chloramphenicol (22.5%) a derivative of phenols, ciprofloxacin (20%), a derivative of fluoroquinolones and cotrimoxazole (17.5%), a derivative of sulfonamides. However, presumptive *P. shigelloides* exhibited 100 % susceptibility to gentamicin and amikacin, a derivative of aminoglycosides. Gentamicin and amikacin proved to be excellent options for the treatment of infection associated with this organism. This study supports the report of Wang et al. (2020) who reported a high resistance value of *Plesiomonas shigelloides* in *Ictalurus punctatus* against cefotaxime, ciprofloxacin, ceftazidime and chloramphenicol.

The continuous use of antibiotics in veterinary medicine have resulted in a prompt selective potency in the emergence of drug resistance among several Gram-negative bacteria. However, the presence of antibiotic resistance mediated by extrachromosomal elements or R-plasmid is common among the members of Enterobacteriaceae (Some et al., 2021). This study revealed a high occurrence/ frequency of antibiotic resistance in experimental pond water and tissue of *C. gariepinus*. It was found from this study that presumptive *P. shigelloides* were resistant to multiple antibiotics which suggests consumption of such fishes could be detrimental to human health and wastewater from the pond could serve as a means of transmitting antibiotic-resistant bacteria such as *P. shigelloides* which are of public health importance into the environment. Multiple antibiotic-resistant phenotypes with tetracycline, cephems, carbapenems and glycosides (13) were observed to have the highest frequency of occurrence and this aligned with the report of Cooke (1976) and Reinthaler et al. (2003) who stated that natural water sample, effluents and aquatic organisms were more resistant to multiple antibiotics. Multiple antibiotics resistant phenotypes exhibited by the larger percentage of presumptive *P. shigelloides* obtained from this study in an indication of abuse of antibiotics in aquaculture settings.

6 Conclusion

This study corroborates other studies that showed *P. shigelloides* is indigenous to the aquatic environment. Meanwhile, gentamicin and amikacin, a derivative of aminoglycosides, are shown to be more promising antibiotics that can be employed in aquaculture. The study provides baseline information for microbial risk assessment, antimicrobial resistance monitoring, and pond hygiene management in catfish aquaculture. It is therefore recommended that future studies should include molecular identification, resistance gene detection, and broader field sampling.

Acknowledgments

We wish to appreciate the efforts of Mr. T. A. Fatokun for his technical assistance during the study.

Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Study limitations

Lack of molecular confirmation, and absence of resistance gene testing for the study were observed.

References

Adesiyan I.M., Bisi-Johnson M.A., Ogunfowokan A.O., and Okoh A.I., 2019, Incidence and antimicrobial susceptibility fingerprints of *Plesiomonas shigelloides* s isolate in water samples collected from some freshwater resources in Southwest Nigeria, Science of the Total Environment, 665: 632-640. https://doi.org/10.1016/j.scitotenv.2019.02.062

Bello O.S., Olaifa F.E., Emikpe B.O., and Ogunbanwo S.T., 2012, The effect of walnut (*Tetracarpidium conophorum*) leaf and onion (*Allium cepa*) bulb residues on the tissue bacteriological changes of *Clarias gariepinus* juveniles, Bulletin of Animal Health and Production in Africa, 60(2): 205-212.

Clinical and Laboratory Standards Institute (CLSI), 2020, Performance standards for antimicrobial susceptibility testing, Twenty-fifth informational supplement, Clinical and Laboratory Standards Institute, Wayne, PA, USA.

Cooke M.D., 1976, Antibiotic resistance in coliform and faecal coliform bacteria from natural waters and effluents, New Zealand Journal of Marine and Freshwater Research, 10: 391-397. https://doi.org/10.1080/00288330.1976.9515625

Cortes-Sanchez A.D.J., Espinosa-Chaurand L.D., Díaz-Ramirez M., and Torres-Ochoa E., 2021, Plesiomonas: A review on food safety, fish-borne diseases and tilapia, The Scientific World Journal, Article ID 3119958: 10 pages. https://doi.org/10.1155/2021/3119958

Food and Agriculture Organization (FAO), 2013, FAO Fisheries and Aquaculture Department has published the Global Aquaculture Production Statistics for the year 2011.

Food and Agriculture Organization (FAO), 2020, The state of world fisheries and aquaculture 2020: Sustainability in action.

Jagger T.D., 2010, *Plesiomonas shigelloides*: A veterinary perspective, Infectious Disease Review, 2: 199-210.

Janda J.M., Abbott S.L., and McIver C.J., 2016, *Plesiomonas shigelloides* revisited, Clinical Microbiology Reviews, 29: 349. https://doi.org/10.1128/CMR.00103-15

Jun J.W., Kim J.H., Choresca C.H., and Shin S.P., 2011, Isolation and molecular detection of *Plesiomonas shigelloides* containing tetA gene from Asian Arowana (Scleropages formosus) in a Korean aquarium, African Journal of Microbiology, 5: 5019-5021.

Krovacek K., Eriksson L.M., González-Rey C., Rosinsky J., and Ciznar I., 2000, Isolation, biochemical and serological characterization of *Plesiomonas shigelloides* from freshwater in Northern Europe, Comparative Immunology, Microbiology and Infectious Diseases, 23: 45-51. https://doi.org/10.1016/S0147-9571(99)00058-2

Mohammed H.H., ElBanna N.I., Erdogan O., Aarattuthodi S., Tekedar H.C., Abdelhamed H., and Diaz-Delgado J., 2026, *Plesiomonas shigelloides* as an emerging pathogen in catfish aquaculture: A case from a South Texas commercial farm, Microorganisms, 14(1): 144. https://doi.org/10.3390/microorganisms14010144

Olusola S.E., and Olorunfemi B.V., 2017, Bioproductive effects of *Clarias gariepinus* fingerlings fed guava (*Psidium guajava*) leaves and drumstick (*Moringa oleifera*) leaves extracts supplemented diet, Journal of Applied and Tropical Agriculture, 22(2): 156-165.

Omotayo A.M., Akegbejo-Samson Y., and Olaoye O.J., 2006, Fish production, preservation, processing and storage, Training Manual of the 2006 Joint Training of Fish Farmers in Epe, Lagos State Agricultural Media Resources and Extension Centre (AMREC), Federal University of Agriculture Abeokuta, Ogun State and BATN Foundation, Victoria Island, Lagos, pp. 19-20.

Pakingking R., Palma P., and Usero R., 2015, Quantitative and qualitative analyses of the bacterial microbiota of tilapia (*Oreochromis niloticus*) cultured in earthen ponds in the Philippines, World Journal of Microbiology and Biotechnology, 31: 265-275. https://doi.org/10.1007/s11274-014-1758-1

Quinn P.J., Markey B.K., Carter M.E., Donnelly W.J., and Leonard F.C., 2002, Veterinary microbiology and microbial diseases, 4th edition, Blackwell Science, Oxford, Chapter 6: 33-34.

Reinthaler F.F., Posch J., Feierl G., Wüst G., Haas D., Ruckenbauer G., Mascher F., and Marth E., 2003, Antibiotic resistance of *E. coli* in sewage and sludge, Water Resources, 37: 1685-1690.
https://doi.org/10.1016/S0043-1354(02)00569-9

Smallwood D.M., and Blaylock J.R., 1991, Food safety and the demand for food: Methodological models and applications, In Caswell J.A. (ed.), Economics of Food Safety, Elsevier Science Publishing Co., Amsterdam, The Netherlands, pp. 3-27.
https://doi.org/10.1007/978-94-011-7076-5_1

Wang H., Gu Y., Zhou H., Chen J., Wang M., Jiang H., and Cao H., 2020, *Plesiomonas shigelloides*, a potential pathogen of enteritis in Ictalurus punctatus, The Israeli Journal of Aquaculture-Bamidgeh, 72: 1119149.
https://doi.org/10.46989/001c.21407

Olaifa F.E., and Bello O.S., 2011, Effect of differently processed African yam beans (*Sphenostylis stenocarpa* Harms) on performance of African catfish (*Clarias gariepinus*) juveniles, Israeli Journal of Aquaculture-Bamidgeh, 63: 595-602.

Bello O.S., 2014, Performance and antimicrobial potentials of onion (*Allium cepa* Linn) bulb and walnut (*Tetracarpidium conophorum* Mull. Arg) leaves in the diet of *Clarias gariepinus*, Ph.D. Thesis, University of Ibadan, Ibadan, pp.187.

Medical Research Council (MRC), 2017, 4th International Clinical Trials Methodology Conference and Society for Clinical Trials, 38th Annual Meeting, 40 pp.

Some S., Mondal R., Mitra D., Jain D., Verma D., and Das S., 2021, Microbial pollution of water with special reference to coliform bacteria and their nexus with the environment, Energy Nexus, 1: 100008.
https://doi.org/10.1016/j.nexus.2021.100008

International Journal of Aquaculture, 2026, Vol.16, No.3, 196-202
http://www.aquapublisher.com/index.php/ija

Research Article Open Access

Effects of *Saccharomyces cerevisiae* Supplementation on Growth Performance and Nutrient Utilization of African Catfish *Clarias gariepinus* (Burchell, 1822)

Abdurrazzaq Ibrahim Abdullahi [1], Maryam Abdullahi Sanda [1], Tukur Mohammed [2], Abubakar Mohammed Mohammed [3], Yakubu Ibrahim [4], Sani Maiwada [5], Goni Bukar Lawan [6]

1 Department of Fisheries, University of Maiduguri, Borno State, Nigeria
2 Department of Fisheries and Aquaculture, Ahmadu Bello University, Zaria-Nigeria
3 Department of Fisheries and Aquaculture, Federal University, Wukari-Nigeria
4 Department of Fisheries Technology, Federal Polytechnic, Bauchi-Nigeria
5 Department of Fisheries Technology, College of Agriculture and Animal Science Mando, Kaduna-Nigeria
6 Department of Agricultural Technology, Federal Polytechnic Monguno-Nigeria

Corresponding author: aiabdullahi@unimaid.edu.ng

International Journal of Aquaculture, 2026, Vol.16, No.3 doi: 10.5376/ija.2026.16.0016
Received: 15 May, 2026
Accepted: 16 Jun., 2026
Published: 30 Jun., 2026

Preferred citation for this article:
Abdullahi A.I., Sanda M.A., Mohammed T., Mohammed A.M., Ibrahim Y., Maiwada S., and Lawan G.B., 2026, Effects of *Saccharomyces cerevisiae* supplementation on growth performance and nutrient utilization of African catfish *Clarias gariepinus* (Burchell, 1822), International Journal of Aquaculture, 16(3): 196-202 (doi: 10.5376/ija.2026.16.0016)

Abstract This study evaluated the effects of *Saccharomyces cerevisiae* on the growth performance and nutrient utilization of *Clarias gariepinus* fingerlings. Five isoproteinous diets were formulated with *Saccharomyces cerevisiae* at inclusion levels of 0.0%, 0.5%, 1.0%, 1.5%, and 2.0%, representing one control and four treatment diets. Completely randomized design was employed. One hundred and fifty (150) *Clarias gariepinus* fingerlings were used for the experiment. Ten fish were randomly assigned to 1 m^2 Hapa net. A total of 15 Hapa nets were used in polythene-lined pond of 10 m × 7 m (l×b) and depth of 1.5 m, the five formulated diets were fed to the experimental fish at 5% body weight for a period of 8 weeks. Highest mean weight gain of 68.65±8.49 g was obtained in fish fed 0.5% followed by 54.37±8.49 g obtained in the fish fed 1.5%. The least mean weight gain of 37.66±8.49 g was recorded in fish fed 2.0%. The highest FCR value of 1.51 was recorded in fish fed the 1.5% diet, while the lowest and best FCR value of 0.52 was recorded in fish fed the 0.5% *S. cerevisiae* diet, no significant difference (P >0.05) was observed in the feed conversion ratio of the fish fed 0% and 1% inclusion level of *S. cerevisiae.* This study revealed that the fish fed 0.5% *Saccharomyces cerevisiae* had the best growth performance and nutrient utilization, unveiling the positive effect of *Saccharomyces cerevisiae* on the culture of *Clarias gariepinus*. Therefore, dietary supplementation with 0.5% *S. cerevisiae* may be considered a natural feed additive for improving growth performance and nutrient utilization in *C. gariepinus*. This study is expected to provide baseline information for the practical use of yeast-based probiotics in African catfish culture.

Keywords *Saccharomyces cerevisiae*; African catfish; Yeast; Natural growth promoter; *Clarias gariepinus*

1 Introduction

African catfish, *Clarias gariepinus* is of great economic importance to aquaculture in Nigeria because of its high market price, fast growth rate, ability to withstand adverse conditions especially low dissolved oxygen content, ability to practice aquatic and aerial respiration and resistance to parasites and diseases. African catfish production accounts for 85% total aquaculture production in Nigeria (Bolorunduro, 2016).

Dietary requirements are among the most important factors influencing the success of fish farming. Over the past three decades, fish nutrition research has expanded to include functional ingredients, feed additives, and probiotics that may improve growth, feed utilization, and fish health. In recent years, the role of probiotics in nutrition and health of certain aquaculture species have been investigated (Ringo et al., 2010). To improve aquaculture, the use of several types of feed additives known as growth promoters are increasingly used by fish farmers to improve growth performance and feed efficiency. These growth promoters enhance fish growth by increasing digestibility, immune stimulation, nutrient assimilation, and supplying essential micro-nutrients in the diet or combining these functions (Anwar, 2018).

Saccharomyces cerevisiae (Louis Pasteur (1857 or 1858 contextually, but name is Louis)) is a naturally occurring yeast (probiotics), is used in aquaculture as a dietary supplement. It contains functional components such as β-glucans and oligosaccharides, which may enhance immune responses and support fish growth. This fermented product includes various beneficial components but rarely contains living cells (El-Nobi, 2021). Probiotics are sometimes expected to have direct growth promoting effects on fish, either by directly involving nutrient uptake or by providing nutrients or vitamins. Using yeast as a probiotic was studied by Andlid et al. (1995), The supplementation of yeast (*Saccharomyces cerevisiae*) has significantly improved aquatic animals' health, physiological status, and productivity (Gonçalves and Gallardo-Escárate, 2017; Zaineldin et al., 2021). Yeast cell walls are known for their protective role against mycotoxin contamination by reducing aflatoxin B1 absorption in the GIT of fish (Pinheiro et al., 2020). Chemical product such as mycotoxins and Nitrates used as an additive cause undesirable effects on fish which also affect the consumer. Despite growing interest in the use of *S. cerevisiae* as a probiotic in aquaculture, limited information is available on its dietary effects in African catfish. Therefore, this study evaluated the effects of graded dietary supplementation with *S. cerevisiae* on growth performance nutrient utilization of *Clarias gariepinus* fingerlings.

2 Results

2.1 Proximate composition of the experimental diets

Table 1 shows the Proximate Composition of the four diets formulated and prepared for the feeding trial. The protein content of the diet ranged between 39.20 and Moisture content 9.79 to 12.00, ether extra on fat 10.00 to 19.50, crude fibre 1.00 to 7.50, Ash 1.00 to 1.50, Nitrogen free extract 23.66 to 32.79.

Table 1 Proximate composition of the experimental diets

	Inclusion level of *Saccharomyces cerevisiae*				
Parameters (%)	0%	0.5%	1.0%	1.5%	2.0%
MC	11.92	9.79	10.21	10.77	12.00
CP	39.20	43.66	40.00	44.07	41.10
EEF	10.00	19.00	14.00	19.50	16.50
CF	7.50	1.00	1.50	1.00	1.00
ASH	1.50	1.00	1.50	1.00	1.00
NFE	29.88	25.55	32.79	23.66	28.4

Means with the same superscript (s) across the same row are not significantly different ($p > 0.05$)

Table notes: MC = moisture content, CP = crude protein, EEF= ether extract on fat, CF= crude fibre, NFE= Nitrogen Free Extract

2.2 Growth performance and nutrient utilization of *Clarias gariepinus* feed diet containing *Saccharomyces cerevisiae*

The growth performance and nutrient utilization of *Clarias gariepinus* fed diets containing *Saccharomyces cerevisiae* are indicated in Table 2. The results show that the highest initial weight recorded was 3.13 g in the 1.5% inclusion level of *Saccharomyces cerevisiae*, followed by 3.06 g and 2.96 g in the 2.0% and 0% inclusion levels of *Saccharomyces cerevisiae*, respectively. The lowest weights were recorded in the 0.5% and 1.0% inclusion levels, with values of 2.93 g. No significant difference ($P > 0.05$) was observed among the treatments. The highest initial length recorded was 7.70 cm in the 2.0% inclusion level of *Saccharomyces cerevisiae*, followed by 7.60 cm and 7.40 cm in the 1.0 % and 0.5% inclusion levels of *Saccharomyces cerevisiae*, respectively. The lowest lengths were recorded in the 1.5% and 0% inclusion levels, with values of 7.33 cm and 7.11 cm, respectively. No significant difference ($P > 0.05$) was observed among the 0.5%, 1.0%, and 1.5% inclusion levels of *Saccharomyces cerevisiae*, whereas a significant difference was observed between the 0% and 2.0% inclusion levels of *Saccharomyces cerevisiae*. The highest final weight recorded was 71.59% in the 0.5% inclusion level of *Saccharomyces cerevisiae*, followed by 57.51% and 50.23 g in the 1.5% and 1.0% inclusion levels of *Saccharomyces cerevisiae*, respectively. The lowest weights were recorded in the 0% and 2.0% inclusion levels, with values of 48.34% and 40.73 g, respectively. No significant difference ($P > 0.05$) was observed among the 0%, 1.0%, and 1.5% inclusion levels of *Saccharomyces cerevisiae*. However, a significant difference was observed in the 0.5% inclusion level of *Saccharomyces cerevisiae*, which differed significantly from the other treatments.

Table 2 Growth performance and nutrient utilization of *Clarias gariepinus* feed diet containing *Saccharomyces cerevisiae*

Treatment	Inclusion level of *Saccharomyces cerevisiae* (%)				
Parameters	0%	0.5%	1%	1.5%	2.0%
IW (g)	2.96±0.07^{a}	2.93±0.07^{a}	2.93±0.07^{a}	3.13±0.07^{a}	3.06±0.07^{a}
IL (cm)	7.11±0.15^{a}	7.40±0.15^{a}	7.60±0.15^{a}	7.33±0.15^{a}	7.70±0.15^{a}
FW (g)	48.34±8.47^{ab}	71.59±8.47^{a}	50.23±8.47^{ab}	57.51±8.47^{ab}	40.73±8.47^{b}
FL (cm)	19.00±0.96^{a}	19.66±0.96^{a}	19.33±0.96^{a}	20.00±0.96^{a}	19.33±0.96^{a}
MWG(g)	45.37±8.49^{ab}	68.65±8.49^{a}	47.30±8.49^{ab}	54.37±8.49^{ab}	37.66±8.49^{b}
PWG(%)	93.81±0.84^{ab}	95.67±0.84^{a}	94.13±0.84^{ab}	93.90±0.84^{ab}	92.23±0.84^{b}
SGR(%)	2.06±0.15^{ab}	2.45±0.15^{a}	2.12±0.15^{ab}	2.06±0.15^{ab}	1.82±0.15^{b}
CF	0.71±0.08^{ab}	0.94±0.08^{a}	0.69±0.08^{ab}	0.68±0.08^{ab}	0.58±0.08^{b}
SR(%)	100±11.83^{a}	100±11.83^{a}	96±11.83^{a}	94±11.83^{a}	96±11.83^{a}
PER	1.13±0.21^{ab}	1.71±0.21^{a}	1.18±0.21^{ab}	1.35±0.21^{ab}	0.94±0.21^{b}
FCR	0.70±0.25^{b}	0.52±0.25^{bc}	0.73±0.25^{b}	1.51±0.25^{a}	1.14±0.25^{ab}

Means within the same row with different superscript letters are significantly different (P>*0.05*)

Table notes: SC=*Saccharomyces cerevisiae*, IW= initial weight, IL= initial length, FW= final weight, FL= final length, MWG=Mean weight gain, SGR=Specific growth rate, SR=Survival rate, PWG=Percentage weight gain, PER=Protein efficiency ratio, FCR =Feed conversion ratio, CF=Condition factor

2.3 Carcass composition of *Clarias gariepinus* fed experimental diet

The carcass composition of *Clarias gariepinus* fed the experimental diet is indicated in Table 3. The highest crude protein content recorded was 44.07% in the 1.5% inclusion level of *S. cerevisiae*, followed by 43.66% and 41.10% in the 0.5% and 2.0% inclusion levels of *Saccharomyces cerevisiae*, respectively. The lowest crude protein contents were recorded in the 1.0% and 0% inclusion levels, with values of 40.00% and 37.20%, respectively. There was no significant difference between the 0.5% and 1.5% inclusion levels, but the 0%, 1.0%, and 2.0% inclusion levels showed significant differences with one another. The highest ether extract (fat) content recorded was 19.50% in the 1.5% inclusion level of *S. cerevisiae*, followed by 19.00% and 16.50% in the 0.5% and 2.0% inclusion levels of *S. cerevisiae*, respectively. The lowest ether extract (fat) contents were recorded in the 1.0% and 0% inclusion levels, with values of 14.00% and 10.00%, respectively. There was no significant difference between the 0.5% and 1.5% inclusion levels, but the 0%, 1.0%, and 2.0% inclusion levels showed significant differences with one another.

Table 3 Carcass composition of *Clarias gariepinus* fed experimental diet

Treatment	Inclusion level of *Saccharomyces cerevisiae*					
Parameters	0%	0.5%	1.0%	1.5%	2.0%	SEM
MC	37.92^{b}	25.33^{c}	31.41^{d}	33.32^{c}	44.50^{a}	0.02*
CP	37.20^{d}	43.66^{a}	40.00^{c}	44.07^{a}	41.10^{b}	0.26*
EEF	10.00^{d}	19.00^{a}	14.00^{c}	19.50^{a}	16.50^{b}	0.46*
ASH	5.50^{a}	1.00^{a}	1.50^{a}	1.00^{a}	1.00^{a}	0.48*
NFE	9.38^{c}	11.01^{a}	13.09^{b}	2.11^{d}	10.5^{e}	0.02*

Means with the same superscript (s) across the same row are not significantly different (*p >0.05*)

± = Standard Error Mean

Table notes: MC = moisture content, CP = crude protein, EEF= ether extract on fat, CF= crude fibre, NFE= Nitrogen Free Extract

2.4 Water quality parameters of culture medium

The summary of the mean values of the water quality parameters of the culture medium are presented in Table 4. There was no significant difference (P>0.05) in the physico-chemical parameters observed in this study. The temperature ranged from 29.55℃~32.38℃, the pH ranged from 5.70 to 6.16, while the dissolved oxygen ranged from 4.99 to 5.85 mg/L.

Table 4 Shows the water quality parameters of culture medium

	T°C	pH	DO (mg/L)
SC1	32.38[a]	6.10[a]	5.30[a]
SC2	31.28[a]	5.90[a]	5.61[a]
SC3	31.74[a]	5.78[a]	5.45[a]
SC4	29.71[a]	5.70[a]	5.85[a]
SC5	29.55[a]	6.16[a]	4.99[a]
Pr>F(Model)	0.51	0.86	0.35

Mean with the same superscript across the same row we're not significantly different (P>0.05)

Table notes: SC= *Saccharomyces cerevisiae,* T= Temperature, DO= Dissolved oxygen

3 Discussion

The growth performance of *Clarias gariepinus* fed diets containing *Saccharomyces cerevisiae* was evaluated in this study. The results showed that dietary inclusion of *S. cerevisiae* significantly improved growth performance, nutrient utilization, and carcass composition of *C. gariepinus,* particularly at the 0.5% supplementation level. The mean weight gain (MWG) recorded in this study ranged from 37.66 g to 68.65 g, with the highest value obtained in the 0.5% inclusion level of *S. cerevisiae*. This result is similar to the findings of Kela et al. (2022), who recorded a MWG of 71.60 g/fish in *C. gariepinus* fed diets containing 100% black cumin (*Nigella sativa*). However, the MWG recorded in this study is lower than the value reported by Abdullahi et al. (2024), who recorded a MWG of 120.33 g in *C. gariepinus* fed diets containing 2.5% *Nigella sativa* meal as a growth promoter. The percentage weight gain (PWG) recorded in this study ranged from 92.23% to 95.67%, with the highest value obtained in the 0.5% inclusion level of *S. cerevisiae*. This result is similar to the findings of Abdullahi et al. (2024), who recorded a PWG of 94.12% in *C. gariepinus* fed diets containing 2.5% *N. sativa* meal. The specific growth rate (SGR) recorded in this study ranged from 1.82 to 2.45, with the highest value obtained in the 0.5% inclusion level of *Saccharomyces cerevisiae*. This result is similar to the findings of Kela et al. (2022), who recorded an SGR of 1.33%/day in *C. gariepinus* fed diets containing 100% black cumin. The lowest FCR value of 0.52 in fish fed the 0.5% *S. cerevisiae* diet indicates better feed utilization, whereas the higher FCR value of 1.51 in the 1.5% group suggests lower feed efficiency.

The carcass composition of *C. gariepinus* fed diets containing *Saccharomyces cerevisiae* was evaluated in this study. The results showed that the inclusion of *Saccharomyces cerevisiae* in the diet had a significant effect on the dry matter, moisture, crude protein, ether extract, and crude fibre contents of the carcass. The highest crude protein content recorded was 44.07% in the 1.5% inclusion level of *Saccharomyces cerevisiae*, which is lower than the value reported by Abdullahi et al. (2024), who recorded crude protein contents of 61.33%, in *Clarias gariepinus* fed diets containing black seed. The ether extract content recorded in this study ranged from 10.00% to 19.50%, which is similar to the range reported by Abdullahi et al., (2024) who recorded ether extract contents ranging from 10.66% to 16.56%. The moisture content recorded in this study ranged from 25.33% to 44.50%, which is higher than the range reported by Abdullahi et al., (2024) and who recorded moisture contents ranging from 6.45% to 8.06%. The ash content recorded in this study ranged from 1.00% to 1.50%, which is similar to the range reported by Abdullahi et al., (2024), who recorded ash contents ranging from 3.38% to 7.04%.

Saccharomyces cerevisiae in the diet of *Clarias gariepinus* significantly improved growth performance and nutrient utilization. The optimal inclusion level of *Saccharomyces cerevisiae* was 0.5%, which resulted in the highest mean weight gain, percentage weight gain, specific growth rate, condition factor, protein efficiency ratio, and lowest feed conversion ratio. This study revealed that the fish fed 0.5% *Saccharomyces cerevisiae* had the best growth performance, unveiling the positive effect of *S. cerevisiae* on the growth of *Clarias gariepinus*. The improved growth and feed utilization observed with 0.5% *S. cerevisiae* supplementation could be attributed to enhanced nutrient absorption, beneficial yeast components such as β-glucans and mannan oligosaccharides, and better feed efficiency. Therefore, under the conditions of this study, 0.5% dietary *S. cerevisiae* may be considered a promising natural feed additives for improving growth performance and nutrient utilization in African catfish.

4 Materials and Methods

4.1 Experimental site

The study was conducted at fish nutrition unit of the Department of Fisheries, Faculty of Agriculture, University of Maiduguri, Nigeria. The University is located along Bama Road, Maiduguri, Borno state with the mean monthly temperature is highest (40.2 ℃) prior the onset of the rain in June and the lowest (31.3 ℃) during the peak of the rainy period of August. The area has an average mean annual rainfall of about 550 mm (Shettima et al., 2018).

4.2 Experimental fish

One hundred and fifty *Clarias gariepinus* fingerlings were procured from Aquarium Planet Agric Business Services, a private fish farm in Maiduguri, Borno State.

4.3 Source of experimental feed ingredients

Feed ingredients was purchased at Custom Market, Maiduguri Borno State which include: soybeans, fishmeal, maize, oil and *Saccharomyces cerevisiae.* Other ingredients including premix, lysine, methionine, calcium, vitamin C, salt and binder were procured at Gidan madara. The soybean was toasted and ground into powdered form separately.

4.4 Feed formulation and compounding

Pearson's square method was employed to formulate the experimental diets at 38.89cp the experimental feed ingredients were grounded separately into a powdered form and measured based on the inclusion level (Table 5), then mixed thoroughly to obtain a homogeneous product and water was added to form dough. *S. cerevisiae* was supplemented at different concentrations (0, 0.5, 1.0, 1.5 and 2.0%). The dough was then pelleted using pelleting machine. The pelleted diets were sun dried and packaged in polythene bag in well-ventilated room under ambient temperature.

Table 5 Gross composition of ingredients with *Saccharomyces cerevisiae*

	Inclusion level				
Ingredient	SC 0.0%	SC 0.5%	SC 1.0%	SC 1.5%,	SC 2.0%
Fish Meal	24.37	24.37	24.37	24.37	24.37
Soybean Meal	48.75	48.75	48.75	48.75	48.75
Maize	16.88	16.88	16.88	16.88	16.88
Lysine	2	2	2	2	2
Methionine	1.7	1.7	1.7	1.7	1.7
Vitamin premix	1	1	1	1	1
Vitamin c	0.3	0.3	0.3	0.3	0.3
Bone meal	1	1	1	1	1
Salt	0.5	0.5	0.5	0.5	0.5
Palm oil	3.5	3.5	3.5	3.5	3.5
Yeast	0	0.5	1.0	1.5	2.0

4.5 Experimental design

Complete Randomized design (CRD) was employed. One hundred and fifty *C. gariepinus* fingerings were used for the treatment. Ten fish were randomly assigned to each 1 m^2 hapa net. A total of 15 hapa net were used in polythene lined pond of 10 m × 7 m (l × b) and depth of 1.5 m, and the five formulated diets were fed at 5% body weight to the experimental fish for 8 weekss and the pond water was monitored daily.

4.6 Determination of nutrient contents

The proximate composition of the diets and carcass composition of the fish was determined using the methods of the AOAC (2019).

4.7 Determination of growth performance and feed utilization

The data was obtained on the growth performance and nutrient utilization of *Clarias gariepinus* fed on the formulated diets was determined as following the methods of Abdullahi et al. (2023).

Mean Body Weight Gain

This is the difference between the final weight and the initial weight of the fish that is been cultured. The difference between the final weight and the initial weight was determined as:

$MWG = W_2 - W_1$

Where, W_1 = Initial weight, W_2 = Final weight

Specific Growth Rate (SGR)

It is an index showing the best growth in a set of growth. This was determined to observe the best growth in a set of growth.

$SGR\ (\%/day) = [(\ln W2 - \ln W1) / (T2 - T1)] \times 100$

Where, In = Natural logarithm; W1 = Initial weight; W2 = Final weight; T1 = Initial time; T2 = Final time

Feed Conversion Ratio (FCR)

It is a numerical value used to measure the gross utilization of feed for growth in fish and other animal. It is assuming that weight gain in fish and other animals is due to increase in body weight. A lower FCR therefore implies efficient food utilization by the animal. This was measured as gross utilization of food for growth in fish as described by Olukunle (2006).

FCR = Feed intake / Weight gain

Condition factor (CF)

$CF = (W / L^3) \times 100$

Where, W = Body weight (g) and L = Total length (cm)

4.8 Data analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) using XLSTAT version 2022. Duncan's multiple range tests was used to separate treatment means, and differences were considered significant at $P < 0.05$. The difference between mean was compared at 95% confidence level.

Acknowledgments

All the laboratory technicians who assisted in the analyses are hereby acknowledged.

References

Abdullahi A.I., Ali F.M., Mohammed T., Mohammed A.M., Isiyaku M.S., Wuroma M.Z., Ibrahim Y., Yusuf H.B., and Kabir S., 2024, Dietary black seed (*Nigella sativa*) promotes growth performance of African catfish (*Clarias gariepinus*) in Aquaculture, Journal of Nutrition and Food Processing, 7(14): 1-6.

Abdullahi A.I., Hassan M.Z., Usman A., Auta J., Bolorunduro P.I., and Onimisi H.U., 2023, Growth performance and nutrient utilization of Nile tilapia (*Oreochromis niloticus*) fed processed duckweed (*Lemna paucicostata*) meal as protein source, International Journal of Oceanography and Aquaculture, 7(3): 1-13.
https://doi.org/10.23880/ijoac-16000256

Andlid T., Vazquez-Juarez R., and Gustafsson L., 1995, Yeast colonizing the intestine of rainbow trout, Salmo gairdneri and turbot, Scophthalmus maximus, Microbial Ecology, 30: 321-334.
https://doi.org/10.1007/BF00171938

Anwar M., 2018, Business model innovation and SMEs performance-does competitive advantage mediate?, International Journal of Innovation Management, 22(7): 1850057.
https://doi.org/10.1142/S1363919618500573

Bolorunduro P.I., 2016, Fisheries extension service in Nigeria: The good, the bad, the ugly and the way forward, inaugural lecture, Ahmadu Bello University, Zaria, 74 pp.

El-Nobi G., 2021, Synbiotic effects of *Saccharomyces cerevisiae*, mannan oligosaccharides, and β-glucan on innate immunity, antioxidant status, and disease resistance of *Nile tilapia*, Oreochromis niloticus, Journal (details incomplete in original source), 10(5): 567.
https://doi.org/10.3390/antibiotics10050567

Gonçalves A.T., and Gallardo-Escárate C., 2017, Microbiome dynamic modulation through functional diets based on pre- and probiotics (mannan-oligosaccharides and *Saccharomyces cerevisiae*) in juvenile rainbow trout (*Oncorhynchus mykiss*), Journal of Applied Microbiology, 122: 1333-1347.
https://doi.org/10.1111/jam.13437

Kela E., Onyia L.U., and Sogbesan O.A., 2022, Effects of black cumin-additive diets on growth and nutrient utilization of *Clarias gariepinus*, Proceedings of the 37th Annual Conference of FISON, 16: 117-120.

Olukunle O., 2006, Nutritive potential of sweet potato peel meal and root replacement value for maize in diets of African catfish (*Clarias gariepinus*) advanced fry, Journal of Food Technology, 4(4): 289-293.

Pinheiro R., Rodrigues A., Lima C.E., Santos J., Pereyra C.M., Torres A.M., Cavaglieri L.R., Lopes J., and Muratori M., 2020, Saccharomyces cerevisiae as a probiotic agent and a possible aflatoxin B1 adsorbent in simulated fish intestinal tract conditions, Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 72: 862-870.
https://doi.org/10.1590/1678-4162-11280

Ringo E., and Gatesoupe F., 2010, Lactic acid bacteria in fish: a review, Aquaculture, 160: 177-203.
https://doi.org/10.1016/S0044-8486(97)00299-8

Shettima M.K., Ikusemoran M., and Daura M.M., 2018, Geospatial assessment of the impact of topography on flood vulnerability in Maiduguri, Nigeria, Jalingo Journal of Social and Management Sciences, 1(4): 129-145.

Zaineldin A.I., Hegazi S., Koshio S., Ishikawa M., Dawood M.A., Dossou S., Yukun Z., and Mzengereza K., 2021, Singular effects of Bacillus subtilis C-3102 or Saccharomyces cerevisiae type 1 on the growth, gut morphology, immunity, and stress resistance of red sea bream (*Pagrus major*), Annals of Animal Science, 21: 589-608.
https://doi.org/10.2478/aoas-2020-0075