COMPENDIUM OF ABSTRACTS

12th Symposium on Diseases in Asian Aquaculture (DAA 12)

23-27 September, 2025

Transformative Innovations Shaping the Future of Aquatic Animal Health Management

Chennai, India

12th Symposium on Diseases in Asian Aquaculture:

Transformative Innovations Shaping the Future of Aquatic Animal Health Management

23-27 September, 2025

Compendium of Abstracts

Organized by:

भारत सरकार
DEPARTMENT OF FISHERIES
Government of India

NFDB

ICAR

CIBA

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This is the "Compendium of Abstracts" of the presentations at the 12th Symposium on Diseases in Asian Aquaculture (DAA 12), held from September 23-27, 2025, at the Hotel Leela Palace, Chennai, India.

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Preface

With great pleasure and deep gratitude, I extend my heartfelt thanks to all the contributors in the compendium of Abstracts for the 12th Symposium on Diseases in Asian Aquaculture (DAA'12): Transformative Innovations Shaping the Future of Aquatic Animal Health Management. We are truly grateful for your participation and sharing the wealth of knowledge, experience, and perspectives, which enrich the dialogue and strengthen our collective ability to address the challenges and opportunities before us. Your presence enriches this gathering and inspires our shared efforts toward progress and collaboration across the aquaculture community.

The Diseases in Asian Aquaculture (DAA) symposium is a premier triennial event organized by the Fish Health Section of the Asian Fisheries Society. Since its inception in 1990, it has served as a vital platform for scientists, policy makers, and stakeholders to exchange knowledge, advance research, and develop collaborative strategies for managing aquatic animal health across the Asia–Pacific region. The **12th edition DAA'12**, is being held in Chennai, India during 23 – 27th September 2025, with the theme "Transformative Innovations Shaping the Future of Aquatic Animal Health Management". The symposium brings together 389 participants representing scientists, professors, lecturers, global technical experts, consultants, fisheries officers, postdoctoral fellows and students, which includes 68 international delegates from 20 countries. The compendium of abstracts has a total of 257 presentations to share transformative innovations and strengthen regional as well as global networks for sustainable aquaculture.

We are honoured by the gracious blessings and encouragement from the Hon'ble Ministers **Shri Shivraj Singh Chouhan**, Union Minister of Agriculture & Farmers Welfare, **Sri Rajiv Ranjan Singh Alias Lalan Singh**, Minister of Panchayati Raj and Minister of Fisheries Animal Husbandry and Dairying, Government of India, and **Sri George Kurian,** Minister of state for Minority affairs and Minister of State for Fisheries, Animal Husbandry & Dairying, Government of India, whose inspiring words lend stature and motivation to this publication.

My sincere gratitude goes to **Dr. Mangi Lal Jat**, Secretary (DARE) and Director General, ICAR, for his steadfast guidance and support in making this event a reality.

I am grateful to **Dr. Abhilaksh Likhi, IAS**, Secretary, Ministry of Fisheries, Animal Husbandry & Dairying, Government of India for his support and inspiring message.

I warmly extend my heartfelt thanks to **Dr. J. K. Jena**, Deputy Director General (Fisheries), ICAR, for his constant encouragement and also the support as Chairman of Asian Fisheries Society Indian Branch (AFSIB) as co-organiser. We also thank our co-organisers **Dr. B. K. Behera,** Chief Executive, NFDB and **Sri D.V. Swamy,** Chairman MPEDA for their valuable cooperation and inspiring messages. We thank **Dr. N. Subbaiyan, IAS**, Secretary, Animal Husbandry, Dairying, Fisheries and Fishermen Welfare Department for his valuable message.

We sincerely thank, **Dr. Kua Beng Chu,** Chairperson, Fish Health Section-Asian Fisheries Society (FSH-AFS), **Dr. Eduardo M. Leaño,** Director General, NACA for their invaluable guidance and steadfast collaboration.

We extend our sincere thanks to our valued institutional partners, ICAR fisheries institutes with their director and teams, the National Fisheries Development Board (NFDB), the Marine Products Export Development Authority (MPEDA), and the Network of Aquaculture Centres in Asia-Pacific (NACA)—for their unwavering collaboration and support. Their scientific guidance, technical expertise, and cooperative spirit have been vital in shaping the DAA12 programme and enriching this abstract volume. Their commitment to fostering innovation, sustainability, and collaboration has been instrumental in the successful organization of this event.

The organisers take this opportunity to sincerely thank our sponsors for their generous support and commitment. Your contribution has been invaluable in making the DAA12 symposium possible. By supporting this platform, you have not only enabled the smooth conduct of the event but also strengthened our shared mission of advancing knowledge, innovation, and collaboration. We deeply appreciate your partnership and look forward to continuing this association in the future.

We would also like to place on record our sincere gratitude to the aquaculture farmers and stake holders, whose hard work and dedication form the very foundation of this sector. Your resilience, innovation, and commitment to sustainable practices not only feed millions but also inspire research, policy, and industry alike. This symposium is, in many ways, a tribute to your invaluable contributions. As part of this program, "DAA12 shrimp farmers conclave 2025" is being organized to show case and deliberate the need of shrimp farmers and new technology available. The conclave is co-organized by ICAR-CIBA, NFDB, MPEDA, CAA, AFSIB and SCAFi.

This symposium provides us with a unique platform to exchange ideas, strengthen collaborations, and shape a shared vision for the future. Together, let us strive to make this gathering not only a forum for discussion but also a catalyst for impactful action.

On behalf of the **ICAR–Central Institute of Brackishwater Aquaculture** and the **Organizing Committee**, we once again thank every individual and institution whose efforts and goodwill have shaped this important scientific gathering.

With sincere appreciation,

Convenor

On behalf of Editorial Committee and Organizers of DAA'12

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RAJIV RANJAN SINGH ALIAS LALAN SINGH

सत्यमेव जयते

पंचायती राज मंत्री
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Minister of Fisheries, Animal Husbandry and Dairying
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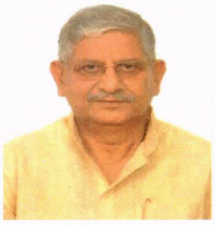

Message

It is a matter of great pride that the ICAR–Central Institute of Brackishwater Aquaculture is hosting the 12th International Symposium on Diseases in Asian Aquaculture (DAA'12) under the auspices of the Fish Health Section of the Asian Fisheries Society. This prestigious gathering brings together eminent scientists, policymakers, and stakeholders from across the globe to deliberate on issues of aquatic animal health, with a focus on diseases impacting the aquaculture sector in Asia.

India is one of the world's leading producers and exporters of farmed shrimp, and the sector is a key contributor to our blue economy. However, our shrimp industry faces complex challenges in the current global trade environment. The imposition of trade tariffs by certain importing countries, along with fluctuating international market demands, has created uncertainty for exporters. At the same time, shrimp farmers are contending with production-related hurdles such as disease outbreaks, climate variability, escalating input costs, and the need to adopt more sustainable and biosecure farming practices.

While these challenges are formidable, I firmly believe that India possesses the resilience, ingenuity, and scientific capacity to overcome them. Our research institutions, such as ICAR-CIBA, have consistently supported the sector with innovations in disease diagnostics, breeding, nutrition, and farm management. By strengthening farm-level biosecurity, diversifying export markets, investing in disease surveillance, and promoting sustainable aquaculture practices, we can ensure that our shrimp industry remains competitive and continues to thrive despite external pressures.

I am confident that the deliberations at DAA'12 will provide valuable insights and foster international collaborations that will help the aquaculture community address both present and future challenges. I extend my best wishes to the organizers, participants, and stakeholders for a highly productive symposium, and I look forward to seeing the knowledge shared here translate into tangible benefits for shrimp farmers and the aquaculture industry at large.

(Rajiv Ranjan Singh)

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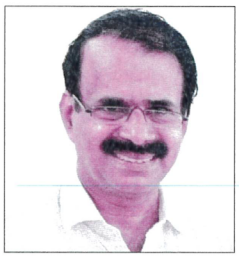

I am delighted to learn that ICAR–Central Institute of Brackishwater Aquaculture is organizing the 12th International Symposium on Diseases in Asian Aquaculture (DAA12) in collaboration with the Fish Health Section of the Asian Fisheries Society, to be held in Chennai, India during 23-27 September 2025.

Aquaculture is a lifeline for millions of households, especially in our coastal and rural communities. It plays a pivotal role in ensuring nutritional security, creating employment, and driving inclusive growth. Shrimp farming, in particular, has emerged as a flagship contributor to India's seafood exports and stands as a vital pillar of our nation's Blue Economy.

However, the challenge of emerging diseases continues to threaten productivity, profitability, and sustainability. In this context, the DAA12 symposium is both timely and significant. I am confident that the deliberations during this symposium will open new dimensions for research and technology development and will address the country need.

I extend my warm congratulations to the organisers and participants, and I wish the symposium grand success in charting the course for a healthier, more sustainable, and prosperous future for Asian aquaculture.

(George Kurian)

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Dr M. L. Jat
SECRETARY (DARE) & DIRECTOR GENERAL (ICAR)

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कृषि अनुसंधान और शिक्षा विभाग एवं
भारतीय कृषि अनुसंधान परिषद
कृषि एवं किसान कल्याण मंत्रालय, कृषि भवन, नई दिल्ली 110 001

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Message

It gives me immense pleasure to extend my warm greetings and congratulations to the organizers, participants, and collaborators of the 12th Symposium on Diseases in Asian Aquaculture (DAA'12), being hosted by the ICAR-Central Institute of Brackishwater Aquaculture (ICAR-CIBA), Chennai, under the auspices of the Fish Health Section (FHS) of the Asian Fisheries Society, Kuala Lumpur.

Fisheries sector has been playing a pivotal complementary role in ensuring food and nutritional security, employment generation, and rural development across Asia. With the growing global demand, aquaculture intensification is increasingly viewed as promising futuristic solution. While aquaculture is one of the fastest-growing food-producing sectors, its sustained growth is increasingly constrained by the emergence and spread of aquatic animal diseases.

In this context, DAA'12 intends to provide a relevant platform for bringing together scientists, academicians, industry leaders, and policymakers to deliberate on aquatic animal health threats, emerging pathogens, and innovative, sustainable solutions. It is heartening to note that the symposium will cover a wide spectrum of topics including diagnostics, epidemiology, microbial management, host-pathogen interactions, and sustainable health strategies.

I commend the efforts of Executive committee of FHS of Asian Fisheries Society, ICAR-CIBA and co-organizers for organizing this important international symposium and for spearheading India's contributions to regional and global aquatic health initiatives.

I am confident that the scientific discussions, networking, and collaborations emerging from DAA'12 will significantly contribute to the development of disease-resilient aquaculture systems, thereby strengthening food security and enhancing farmer livelihoods across Asia.

I wish the symposium great success and look forward to its valuable outcomes.

(M.L. Jat)

Dated the 04th September, 2025
New Delhi

Dr. Abhilaksh Likhi, IAS
Secretary
डॉ. अभिलक्ष लिखी, भा.प्र.से.
सचिव

सत्यमेव जयते

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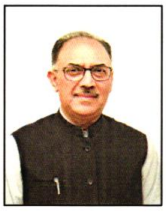

Message

I am pleased to note that the **ICAR–Central Institute of Brackishwater Aquaculture (CIBA)** and the **Fish Health Section of the Asian Fisheries Society** are organizing the **12th International Symposium on Diseases in Asian Aquaculture (DAA'12)** at Chennai from September 23-27, 2025. This symposium addresses on one of the most pressing challenges confronting the aquaculture sector: **aquatic animal health and disease management**.

The Department of Fisheries, Government of India, is actively addressing this challenge following a multi-pronged approach involving government initiatives, research, infrastructure development, and focus on biosecurity and farmer awareness. A significant initiative is the **National Surveillance Program on Aquatic Animal Diseases (NSPAAD)**, operated in collaboration with ICAR institutions, Fisheries Colleges/Universities, and the State Fisheries Departments, within the legal framework of the Prevention and Control of Infectious and Contagious Diseases in Animals Act, 2009. NSPAAD is supported by the **Pradhan Mantri Matsya Sampada Yojana (PMMSY)**, the Government of India's flagship scheme aimed at transforming the Indian fisheries and aquaculture sector through efficient and sustainable value-chain addition, promotion of science-driven innovations, and environmentally inclusive growth.

Aquaculture is a vital component of the Asian economy, contributing significantly to nutritional security, employment generation, and trade. It is, therefore, imperative to strengthen our scientific understanding and preparedness for effective disease surveillance, diagnosis, and the formulation of robust control strategies.

The **Fish Health Section (FHS) of the Asian Fisheries Society** has over the last three decades, played a pivotal role in advancing aquatic animal health research through its DAA symposium series. It is commendable that the 12th edition of this symposium is being hosted by ICAR-CIBA, in collaboration with the Department of Fisheries, Government of India, the National Fisheries Development Board (NFDB), the Marine Products Export Development Authority (MPEDA), Kochi, and important industrial partners.

This event offers a unique platform for **knowledge exchange, collaboration, and innovation**. I am confident that the scientific deliberations during DAA'12 will lead to pragmatic strategies for managing aquatic animal health, fostering sustainable aquaculture, and addressing transboundary disease threats through regional and international cooperation.

I extend my best wishes for the grand success of the symposium and fruitful outcomes for the global aquaculture community.

(Dr. Abhilaksh Likhi)

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Message

It is a matter of immense satisfaction and pride that the 12th Symposium on Diseases in Asian Aquaculture (DAA'12) is being organized in India at Chennai during 23-27 September, 2025 by the Fish Health Section (FHS) of the Asian Fisheries Society. This prestigious event which holds significant regional impact on aquaculture and disease management is being jointly organized by ICAR-Central Institute of Brackishwater Aquaculture (ICAR-CIBA) and Fish Health Section, Asian Fisheries Society.

Aquaculture has emerged as a vital pillar of food systems across Asia, making significant contributions to nutritional well-being, rural livelihoods, and economic development. However, the increasing intensification and commercialization of aquaculture have also led to the emergence and recurrence of aquatic animal diseases, posing major challenges to the sector's sustainability.

DAA'12 presents a timely opportunity to bring together scientists, academicians, industry front-runners, policymakers, and students to exchange ideas, strengthen partnerships, and explore innovative solutions for improving aquatic animal health. The symposium's focus on advancing tools for early disease detection, understanding host–pathogen interactions, and promoting sustainable health management practices reflects its commitment to shaping a resilient and forward-looking aquaculture sector.

I appreciate the dedicated efforts of ICAR-CIBA and its partners in hosting this important international event and for advancing India's leadership in aquatic animal health research and innovation. I acknowledge with appreciation to the Department of Fisheries, Govt of India, National Fisheries Development Board (NFDB), Marine Products Export Development Authority (MPEDA) and Asian Fisheries Society Indian Branch (AFSIB) for joining hands with ICAR for co-organizing this event. The deliberations and outcomes of DAA'12 are expected to greatly contribute to the development of science-driven, disease-resilient aquaculture systems that benefit farming communities across the region.

I convey my best wishes for the successful conduct of the symposium and for meaningful scientific interactions among all participants.

(J.K. Jena)

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Message

I am delighted to note that ICAR–Central Institute of Brackishwater Aquaculture (CIBA) is organizing the 12th International Symposium on Diseases in Asian Aquaculture (DAA12) in association with the Fish Health Section of the Asian Fisheries Society and National Fisheries Development Board, Hyderabad at Chennai during 23–27 September 2025. This important forum will bring together scientists, policymakers, and industry leaders to deliberate on aquatic animal health, a cornerstone of sustainable aquaculture.

Fisheries and aquaculture are among the fastest-growing sectors in India, contributing significantly to food security, livelihoods, and exports. India is today the third largest fish-producing country and second in aquaculture production, with fish and fish products emerging as the nation's leading agricultural export.

National Fisheries Development Board (NFDB) plays a key role in promoting sustainable fish production by promoting advance technology and utilizing nation resources effectively. It is committed to develop infrastructure, enhancing the socio-economic well-being of fishing communities, fostering entrepreneurship, and increasing fish consumption. Aligned with the vision of "Aatma Nirbhar Bharat" of the Government of India, NFDB works with research institutions on species diversification to ensure higher productivity, improved livelihoods, and resilience to emerging challenges.

The DAA12 holds great relevance in advancing knowledge, strengthening collaborations, and shaping the future of responsible aquaculture. I extend my best wishes to ICAR-CIBA, the Asian Fisheries Society, and all participants for the success of this symposium.

Dated: 04.09.2025

(B. K. Behera)

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D.V. Swamy, IAS
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Message from the Chairman, MPEDA

I am pleased to note that the ICAR–Central Institute of Brackishwater Aquaculture (CIBA) together with the Marine Products Export Development Authority (MPEDA) is hosting the 12th International Symposium on Diseases in Asian Aquaculture (DAA12) in Chennai from 23–27 September 2025. This symposium will provide a platform for scientists, academicians, and industry stakeholders to discuss aquatic animal health, which is essential for sustainable aquaculture.

India is a leading seafood producer and exporter, emphasizing responsible aquaculture practices. Established in 1972, MPEDA promotes quality marine product exports and has strengthened the seafood industry's production and marketing systems. In 2024-25, India's marine product exports reached USD 7.45 billion.

The seafood export sector faces challenges such as production issues and international tariffs. The market access to certain countries are also blocked due to biosecurity issues. Addressing these requires innovative efforts, particularly in fish health management to enhance productivity and align with international standards.

I am confident that DAA12 will foster collaboration and solutions for the challenges in aquatic animal health.

Best wishes to the organizers and participants for a successful symposium.

(D V SWAMY)

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MESSAGE

It is a matter of honour that the ICAR-Central Institute of Brackish water Aquaculture (ICAR CIBA) is hosting the 12th Symposium on Diseases in Asian Aquaculture (DAA' 12) at Chennai, Tamil Nadu, from 23-27 September 2025. This prestigious triennial event, organised by the Fish Health Section of the Asian Fisheries Society (AFS-FHS) since 1990, serves as a premier global platform for addressing the challenges of aquatic animal health.

Tamil Nadu is one of the leading aquaculture hubs of India, contributing significantly to seafood production, exports, and livelihood generation. Disease management remains a critical priority for ensuring the sustainability and profitability of this sector. In this context, the DAA'12, with its theme "Transformative Innovations Shaping the Future of Aquatic Animal Health Management", is highly relevant to our aquaculture industry.

The DAA'12 will bring together an exceptional gathering of experts from research, industry, academia, and global organisations. This convergence of knowledge and experience will provide a unique platform for exchanging the latest scientific insights, exploring innovative technologies, and developing practical strategies to tackle aquatic animal health challenges. The deliberations are expected to influence future policies, strengthen disease preparedness, and pave the way for a more resilient and sustainable aquaculture sector.

On behalf of the Fisheries Department, Government of Tamil Nadu, I extend my warm greetings to all delegates, organisers, and participants. I congratulate ICAR-CIBA and AFS-HS for their efforts in hosting this prestigious event and wish the symposium all success.

(N SUBBAIYAN)

Network of Aquaculture Centres in Asia-Pacific

Message from the Director General, NACA

I extend my warm greetings to the organizers and participants of the 12th Symposium on Diseases in Asian Aquaculture (DAA12), being hosted by ICAR–Central Institute of Brackishwater Aquaculture (CIBA) and organized by the Fish Health Section of the Asian Fisheries Society (FHS-AFS), during 23–27 September 2025 at Chennai, India.

The Network of Aquaculture Centres in Asia-Pacific (NACA) is an intergovernmental organization dedicated to advancing rural development through sustainable aquaculture and responsible aquatic resource management. Its core thematic areas are: Environment & Sustainability; Health & Biosecurity; Genetics & Biodiversity; Food Safety, Security & Certification; and, Emerging Regional & Global Issues. NACA is a recipient of: the FAO Margarita Lizárraga Medal for its significant contribution to sustainable aquaculture development and to the practical and tangible application of the Code of Conduct for Responsible Fisheries; AFS Gold Medal Award for its distinguished contribution to regional aquaculture education, training and development; and, CIRDAP Aziz-Ul Haq Rural Development Medal in recognition of its remarkable contributions to rural development through sustainable aquaculture and aquatic resource management programmes, policy dialogue and regional cooperation in the Asia-Pacific. NACA also established the Regional Advisory Group on Aquatic Animal Health (AG), drawing together governments and technical experts to share information on the detection, containment and management of disease. The AG was established by the Governing Council of the Network of Aquaculture Centres in Asia-Pacific (NACA) in 2001 to provide advice to NACA members in the Asia-Pacific region on aquatic animal health management and other current and related issues. NACA, in collaboration with World Organisation for Animal Health and FAO, is also implementing the Regional Aquatic Animal Disease Reporting System which monitors the presence or absence of listed and non-listed aquatic animal diseases in the region.

The DAA symposium has evolved into a flagship platform where scientists, policymakers, farmers, and industry leaders converge to share knowledge, strengthen collaborations, and drive innovations in aquatic animal health. I am confident that DAA12 will help in fostering new partnerships, sharing emerging research, and shaping collective strategies for sustainable aquaculture growth.

On behalf of NACA, I congratulate the organizers for their dedicated efforts and wish all participants a highly engaging and fruitful symposium.

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Fish Health Section
Asian Fisheries Society

It gives me immense pleasure to welcome all the delegates, researchers, professionals, and students to the **12th International Symposium on Diseases in Asian Aquaculture (DAA'12)**, organized by **Fish Health Section (FHS)** of the **Asian Fisheries Society (AFS)**, and **ICAR-Central Institute of Brackishwater Aquaculture (CIBA), Chennai**.

Since its inception in 1990, the DAA symposium series has stood as a cornerstone of scientific exchange, innovation, and collaboration in the field of aquatic animal health. Each edition has built upon the legacy of its predecessors, forging deeper understanding and stronger networks among fish health professionals across Asia and beyond.

As Chairperson of FHS, I am deeply honoured to witness how the community continues to evolve - embracing cutting-edge technologies, addressing emerging challenges, and championing sustainable practices in aquaculture health management. DAA'12 reflects this evolution with a rich scientific program that spans from diagnostics, vaccines and host-pathogen interactions to biosecurity, epidemiology, and climate-linked aquatic diseases.

I commend **ICAR-CIBA** and the organizing committee for their unwavering commitment and meticulous efforts in bringing together a diverse and distinguished group of participants. Your dedication has ensured that this symposium not only serves as a platform for knowledge exchange but also inspires a renewed vision for resilient and healthy aquaculture systems.

Let this event be a celebration of science, partnership, and progress. May the discussions held, collaborations formed, and knowledge shared here serve as guiding lights for the future of aquatic animal health in Asia.

Wishing you all a fruitful and memorable DAA'12!

Chairperson
Fish Health Section-Asian Fisheries Society
http://www.fhs-afs.net/

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MESSAGE

It gives me immense pleasure and pride to extend a warm welcome to all the delegates, researchers, academicians, students, and stakeholders to the 12th Symposium on Diseases in Asian Aquaculture (DAA12) to be held at Chennai, India during 23-27 September 2025. This prestigious international event is being organized by ICAR–Central Institute of Brackishwater Aquaculture (CIBA) under the aegis of Fish Health Section (FHS) of Asian Fisheries Society (AFS) in collaboration with Department of Fisheries, Government of India, National Fisheries Development Board (NFDB), Hyderabad, Marine Products Export Development Authority (MPEDA), Kochi and Asian Fisheries Society, Indian Branch (AFSIB) and Society of Coastal Aquaculture and Fisheries. The program also has a satellite event on 27th September 2025 on Shrimp Farmer's and Industry Conclave, at MES of CIBA, Chennai.

The theme for DAA12 is "Transformative Innovations Shaping the Future of Aquatic Animal Health Management". Aquaculture plays a crucial role in ensuring food and nutritional security, sustainable livelihoods, and economic growth, particularly in the Asia-Pacific region, which contributes over 90% of global aquaculture production. However, disease outbreaks continue to be a major constraint to sustainable aquaculture, causing significant economic losses, threatening biodiversity, and undermining stakeholder confidence. With rising intensification and scale in aquaculture systems, the need for robust health management strategies is even more pressing, than before.

This symposium is envisioning exchange of ideas among leading experts and stakeholders from across the world to deliberate on latest research developments, and innovative solutions for the management of aquatic diseases policy inclusions and industry uptakes. With the participation over 400 delegates, this event aims to foster scientific exchange, networking and dialogue to tackle the emerging challenges in aquatic animal health. I am confident that the technical deliberations and scientific contributions presented during the conference will pave the way for improving management in Asian aquaculture.

ICAR-CIBA is privileged to host this landmark event and reaffirms its commitment to scientific excellence in aquatic animal health. I thank all the contributors, sponsors, committee members and participants for their valuable support and interest in this important endeavour.

I look forward to its long-lasting impact on the aquaculture sector.

With warm regards,

(KULDEEP K. LAL)

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Contents

DAA'12

Plenary Session

Aquatic animal health research and development in Asia: From 1975 to 2025 – How far have we come?

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The ongoing triennial Symposium on Diseases in Asian Aquaculture, held since its inception in 1990, stands as a testament to the critical importance of research in aquatic animal health for transforming aquatic food systems for ensuring optimum one health outcomes. From negligible beginnings, the field has advanced significantly, spurred by major disease outbreaks such as EUS, WSD, and AHPND. Diagnostic science has undergone a significant transformation evolving from traditional parasitology and microbiology to advanced pathology, and more recently, to cutting-edge molecular biology, metagenomics, and AI-driven diagnostics. Outbreak investigations have shifted from reactive, individual-led efforts to systematic, collaborative, and epidemiologically grounded approaches. Systems thinking is increasingly applied to identify pathways of fish exposure to harmful pathogens, pinpointing hotspots for disease and antibiotic resistance emergence, and even tracing routes of human exposure to antibiotics across Asia. Quality data collection and interpretation have enabled science-based risk assessments and development of practical farm level biosecurity plans and better management practices. National aquatic animal health programs in Asia have matured significantly from rudimentary beginnings to highly sophisticated systems that now include surveillance, emergency preparedness, AMR management, and compliance with international trade standards. Collaborative networks and national laboratories of excellence have strengthened disease response capacity, and Asia now hosts several WOAH Reference Laboratories. Despite progress, health management research in carps, tilapia, and catfish affordable, low-value aquaculture species widely farmed in Asia and Africa remain limited. Translating five decades of research advancements into tangible one health outcomes demands a fundamental shift in attitudes, practices, and behaviours of aquatic food value chain actors. As future innovations increasingly rely on advanced technologies, it remains critical not to lose sight of fundamental skills clinical observation, basic diagnostics, epidemiology, and systems thinking. We must remain proactive and adaptive to meet emerging One Health challenges and safeguard our aquatic food systems.

Keywords: One Health, Diagnostics, Epidemiology, Surveillance, Systems Thinking

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Technical Session I

Finfish Health

The hidden billion-dollar burden: Quantifying Asia's aquatic disease crisis

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Walk into any fish market in Asia and you will see the success story: endless varieties of farmed fish, competitive prices, and growing exports. What you won't see are the farms that failed, communities impacted by crop failures, or billions lost to preventable diseases. Asia dominates global aquaculture production with 125.17 million tonnes (91.92%) in 2023. Yet beneath this success lies a mounting crisis: disease outbreaks silently draining billions while threatening food security for millions. Here we begin to examine the true economic impact of aquatic diseases across Asia's 50 aquaculture-active states, moving beyond mortality figures to reveal complex costs rippling through entire production chains. By analysing the most significant health conditions, we assess their impact on Asia's 20 largest fish industries by production volume. The challenge is multifaceted. While sporadic pathogens create unpredictable devastation, predictable infections generate ongoing costs through biosecurity implementation, prophylactic treatments, and regular monitoring. Often overlooked are countless smaller mortality events dismissed as "acceptable losses" whose collective impact may exceed major disease outbreaks. Our analysis reveals that Asia's fish production (56.30 million tonnes, representing 44.98% of total Asian aquaculture) faces escalating disease pressure as intensification prioritises short-term profits over biosecurity investment. The top fish species alone account for 44.42 million tonnes, making disease-related losses economically catastrophic. For pathogens like *Streptococcus* and parasitic infections, impact estimates remain conservative due to industry acceptance and underreporting. The true cost extends beyond stock mortality, encompassing treatment expenses, production delays, market disruption, and downstream industry effects that devastate livelihoods across the supply chain. As aquaculture growth slows to 3.13% annually, the correlation between intensification and disease risk becomes critical. Without comprehensive biosecurity, health management, and control programs, Asia risks undermining its aquaculture potential. This presentation provides actionable insights for industry stakeholders to minimise losses while maximising production efficiency.

Keywords: Economic Impact, Biosecurity, Pathogen Management, Mortality Assessment, Food Security

Pathogen related biosecurity in Recirculating Aquaculture Systems (RAS): Experiences from industry, controlled trials and surveillance in commercial farms

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Disease outbreaks and presence of pathogenic microorganisms in RAS (Recirculating Aquaculture Systems) can result in number of challenges leading to fish mortality and reduced welfare for the fish in production. There are many uncertainties regarding the role of biofilters in either eliminating or supporting invading pathogens during commercial fish production. Surveys and in-depth interviews to map the experiences from the farmers gave insight into the challenges faced in commercial settings relating to regulations, design and infrastructure, fish production logistics and biosecurity measures and strategies. Challenge experiments were performed in controlled RAS facilities to investigate the establishment, survival and inactivation of pathogens. The effect of biosecurity measures such as fallowing, cleaning, and disinfection of biofilters on salmon pathogens such as *Ca. Branchiomonas cysticola*, IPNV, PRV-1, and ISAV was studied. Additionally, effect of different biosecurity measures such as changing the water and ozonation were tested in mini-scale RAS bioreactors. We investigated effect of various biosecurity measures applied in commercial RAS facilities with documented disease histories, both before and after implementation of biosecurity measures. We assessed pathogen screening on samples collected from several risk assessed areas, biomedia, water and fish, both pre- and post-implementation of the biosecurity measures to evaluate intervention efficacy. In summary, replacing all water in the system and a fallowing period between two fish generations showed reduction of most of the pathogens that were assessed in this study. There were some areas in the system where we could detect DNA/RNA for specific agents post implementation of biosecurity measures. Naïve fish, introduced to the same RAS, post biosecurity intervention often tested positive at varying times after introduction indicating that eradication of pathogens is complex. Results from our studies show that RAS facilities should be carefully designed

and incorporate specific biosecurity measures to avoid fish health- and welfare- challenges and successful operation.

Keywords: RAS, Biosecurity, Surveillance, Disinfection, Pathogens

Project: Biosecurity in Recirculating Aquaculture systems

Funding: FHF 901792, Norway

Fish vaccines: Global status, recent advances, and Indian perspectives

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The rise of infectious diseases in aquaculture has intensified the demand for effective preventive measures through vaccination to reduce economic losses and ensure sustainable fish farming. There are more than 34 commercially available fish vaccines in the world, mostly being used to prevent diseases in salmonids, catfish, and tilapia. Currently, commercial aquaculture employs a variety of vaccines targeting major viral and bacterial pathogens like IHNV, IPNV, ISAV, KHV, *Vibrio* spp., *Aeromonas salmonicida, Edwardsiella ictaluri, Yersinia ruckeri,* and *Streptococcus species*. Vaccine development against multicellular parasites particularly sea lice is advancing, though efforts targeting ciliates and endoparasites are still nascent due to complex host-parasite dynamics. While inactivated vaccines delivered mostly via intraperitoneal injection dominate, immersion and oral routes are gaining traction. Emerging mucosal vaccines enhance immunity at pathogen entry sites, and plant-based platforms enable scalable antigen production for oral vaccines. Among recent innovations, next-generation vaccines such as mRNA, vector-based, synthetic peptide, nanoparticle, and plant-derived platforms offer promising avenues for more precise, efficient, and potentially cost-effective disease prevention. Oral delivery methods, including polymer encapsulation and bioencapsulation, facilitate mass immunization, particularly in juvenile fish. Vaccine research is also exploiting bioinformatics and synthetic biology for designing multiepitope synthetic vaccines, although challenges remain in efficacy, scalability, regulatory approval, and species-specific adaptation. India has made significant progress in developing vaccines against bacterial and viral pathogens, including RNAi- and DNA-based approaches and peptide-based vaccines, addressing the challenges posed by emerging fish diseases. Several vaccines have been recently commercialized against key bacterial pathogens and viral nervous necrosis (VNN). Cutting-edge technologies, including RNA interference (RNAi)-based vaccines for white spot syndrome virus (WSSV) and DNA vaccines targeting *Edwardsiella* spp., and *Aeromonas* spp., and recombinant protein/peptide-based vaccines directed against various diseases are currently under evaluation, offering hopeful prospects for combating the disease burden.

Keywords: Disease, Fish, India, Status, Vaccine, World

The pathology and blood biochemistry of juvenile Lates calcarifer on diets contaminated with mycotoxins, histamines and rancid fats

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Mycotoxins, originating from contaminated raw materials or suboptimal feed storage, are a growing concern in tropical aquaculture. Common fungi such as *Aspergillus* spp. and *Fusarium* spp. produce mycotoxins including aflatoxin, fumonisin, deoxynivalenol and zearalenone. High doses or prolonged exposure (weeks) to low doses of these mycotoxins (*Lates calcarifer* fed two different diets (FM40 and ABS3) for 5 weeks. Analysis of these diets revealed high peroxide values, multiple mycotoxins and high histamine levels. Fish fed the FM40 diet, which was contaminated with aflatoxin B1 (13.2 μg/kg), aflatoxin B2 (1.9 μg/kg), deoxynivalenol (29.5 μg/kg), alternariol (2.2 μg/kg), elevated peroxide value (45.91 mEq/kg), and histamine (129.51 mg/kg) developed mild bile duct hyperplasia, depressed total serum proteins (50.40 $\pm$ 10.06g/L), markedly elevated blood potassium (8.2 $\pm$ 0.18 mmol/L), and heavy iron deposits in splenic melanomacrophage centres (Perl's stain) indicative of increased haemolysis. The presence of multiple cytotoxic mycotoxins in FM40 diet could explain the increased haemolysis and elevated blood potassium. In contrast, fish fed the ABS3 diet, which had high histamine levels (210.05 mg/kg), exhibited protein-losing nephropathy with multifocal fibrin plugs (Martius scarlet blue stain) indicating acute renal damage, and elevated blood calcium and phosphorus levels. Histamine is metabolized and excreted through the kidney, and known to induce renal arteriolar constriction, disrupt glomerular filtration barrier and increase permeability resulting in protein loss. This study shows that blood biochemistry and histopathology are useful diagnostic tools for assessing the impact of mycotoxins and histamines on fish health.

Keywords: Bile Duct Hyperplasia, Protein-Loosing Nephropathy, Blood Potassium, Blood Calcium, Haemolysis

Project: Based on published work in Journal of Fish Diseases, https://doi.org/10.1111/jfd.14034

Evaluating the suitability of fish cell lines in toxicity assessment of metal oxide nanoparticles

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The metal oxide nanoparticles (MONP) market is witnessing substantial expansion, with its global market size expected to reach $53.74 billion by 2030. MONPs are toxic to a range of aquatic organisms, including algae, invertebrates, and fish. The extensive use of MONPs has raised significant concerns for aquatic ecosystems due to their release into water bodies. *In vitro* cell culture methods are considered effective alternatives for preliminary toxicity screening, providing a substitute for animal testing. However, most cytotoxicity assays are typically performed on human or animal cell lines. Ideally, toxicity evaluations should also include aquatic species, as they are more directly exposed to these substances in the environment. Research on fish cell lines for toxicity detection remains limited. Sensitive fish cell lines are capable of detecting lower concentrations of toxicants, making them valuable for the early identification of harmful substances like MONPs. In this study, the toxicity of zinc oxide nanoparticles (ZnO-NPs) was compared with different cell lines of human origin (MFC-7), rodent origin (L-929), and fish cell lines, i.e., Oscar Spleen (OS), Fantail Goldfish Fin (FtGF) and *Horabagrus brachysoma* Fin (HBF) to assess suitable cell lines for monitoring the nanoparticles' toxicity. Zinc Oxide Nanoparticles (ZnO-NPs) were synthesized by sol-gel method, and their nano-size was verified through UV-Visible spectroscopy, DLS, FTIR, and SEM. For quality comparison, bulk-sized Zinc Oxide particles (ZnO-BP) were also analysed alongside ZnO-NPs. Cell cytotoxicity was assessed using the MTT assay and the findings revealed that FfGF fish cells and HBF cell lines exhibited a higher IC_{50} value, i.e., >300, indicating greater resistance or lower sensitivity to ZnO-NPs, whereas OS cell lines exhibited higher susceptibility. It is concluded that the OS cell line is more suitable for assessing the toxicity of the nanoparticles. It needs to be tested with various MONPs for further confirmation.

Keywords: ZnO Nanoparticles, ZnO Bulk Particles, Fish Cell Line, Cyto-toxicity, MTT

Lactoferrin supplementation for barramundi: Limited growth response but potent for immunity

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Bovine lactoferrin (bLF) is an iron-binding glycoprotein with anti-inflammatory, antioxidant, and immunomodulatory properties. Its dietary supplementation is expected to enhance immune response and growth performance in aquaculture species such as barramundi (*Lates calcarifer*). In this study, 180 juvenile barramundi were randomly assigned to four groups (A-D) and maintained in 12 tanks (500 L each), with 15 fish per tank. Fish were fed diets containing bLF at doses of 15 μg (A), 5 μg (B), 2.5 μg (C), and 0 μg (D, control) per fish per day for 7 days. Parameters evaluated included specific growth rate, feed conversion ratio, feed efficiency, survival rate, haematological parameters, histopathological analysis, and gut microbiota composition, as determined by 16S rRNA metagenomic sequencing. The results showed no statistically significant differences in growth performance, feeding efficiency, or survival rate among the treatment groups ($p > 0.05$). However, haematological data revealed elevated leukocyte and lymphocyte levels in the high-dose group. Most notably, metagenomic profiling showed a reduction in overall microbial diversity and a marked increase in the relative abundance of Gram-negative pathogenic genera, such as *Aeromonas* and *Moraxella*, in bLF-treated fish. These changes suggest that while bLF may stimulate immune activation, it may also disrupt gut microbial balance and promote the colonisation of opportunistic pathogens. This highlights the species-specific nature of bLF responses and underscores the need for further optimisation of lactoferrin-based formulations for barramundi.

Keywords: Barramundi, Feed Efficiency, Growth Rate, Immune Response, Metagenomics

Project: Rumah Program Purwarupa Alat Kesehatan BRIN T.A. 2022

Funding: National Research and Innovation Agency, Indonesia (BRIN)

Use of benzalkonium chloride as a broad-spectrum therapeutic agent in different life stages of Indian major carp, *Labeo rohita*

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Infections with different microorganisms are one of the major challenges in aquaculture, especially in hatcheries and during larval rearing, where they affect early life stages. In the present study, we evaluated the efficacy of a broad-spectrum disinfectant, benzalkonium chloride (BKC) on hatching and larval survival of the Indian major carp, *Labeo rohita*, and studied its effectiveness against infections caused by *Saprolegnia parasitica* and *Aeromonas veronii*. The results revealed that a prolonged bath treatment with BKC at varying concentrations (0.1, 0.5, and 1 ppm) showed the highest hatching percentage and larval survival at 0.5 ppm. Short immersion treatment of eggs for a period of 20 min with BKC at different concentrations (1, 2, 5, 10, 20 and 40 ppm), indicated that eggs can be treated up to maximum concentration of 5 ppm of BKC. Dip treatment of eggs with BKC for a period of 2 min at different concentrations (5, 10, 20, 50, 100, 500, 1000 ppm) revealed that a 2 min dip treatment at a concentration of 10 ppm or 20 ppm increased the hatching and larval survival. Additionally, it was observed that both the prolonged bath at 0.5 ppm BKC and the dip treatment at 10 ppm for 2 minutes are promising strategies for minimizing infection with *S. parasitica* and improving hatching success. Further, to understand the efficacy of BKC against bacterial infection, the fingerlings of *L. rohita* were challenged with a virulent strain of *A. veronii* through immersion, and subsequently treated with different concentrations of BKC (0.25, 0.5 and 1 ppm). Significant reduction in mortality was observed in the BKC-treated groups and the therapeutic efficacy observed in different groups of BKC-treated rohu was further corroborated with the reduced bacterial count. The findings underscore the potential of BKC as a viable alternative to conventional antifungal and antibacterial treatments in aquaculture.

Keywords: Benzalkonium Chloride, Disinfectant, *Saprolegnia parasitica*, *Aeromonas veronii*, Larval Survival, Route of Treatment

Project: All India Network Project on Fish Health

Funding: Indian Council of Agricultural Research, India

Granulocyte tropism and lymphocyte depletion highlight the immunopathogenesis of tilapia lake virus infection in Nile tilapia

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Tilapia Lake Virus (TiLV), a highly virulent pathogen, has caused severe economic losses by increasing mortality in farmed populations. Despite a growing body of research into TiLV pathogenesis, detailed understanding of the immunopathological mechanisms of this virus in Nile tilapia remains limited. This study aimed to elucidate the hematological impact of TiLV infection, focusing specifically on alterations in leukocyte subpopulations. Seven Nile tilapias were included in the study, and hematological parameters including hematocrit and white blood cell (WBC) counts, were compared pre- and post-intraperitoneal injection with TiLV (10^6 $TCID_{50}$/mL). Clinical signs, including lethargy and reduced appetite, were observed in all fish from 1 day post-infection (dpi). TiLV RNA was detectable in blood samples from 1 dpi and significantly increased by 5 dpi. Notably, the hematocrit levels significantly decreased from 28.86% ± 0.03% to 19.71% ± 0.04% ($p < 0.01$), while WBC counts declined from 2.16 x 10^5 ± 1.66 x 10^4 cells/μL to 1.06 x 10^5 ± 1.63 x 10^4 cells/μL ($p < 0.001$). Histopathological examination of anterior kidney, spleen, and liver revealed lymphoid depletion, increased melanomacrophage centers, and eosinophilic inclusion bodies, indicating damage to hematopoietic and leukopoietic organs. Flow cytometric analysis of isolated peripheral blood leukocytes indicated significant lymphopenia and granulocytopenia from 10.27 x 10^4 ± 4.71 x 10^4 cells/μL to 5.27 x 10^4 ± 1.95 x 10^4 cells/μL ($p < 0.01$), and 2.08 x 10^4 ± 8.15 x 10^3 cells/μL to 1.06 x 10^4 ± 7.11 x 10^3 cells/μL ($p < 0.05$), respectively. Interestingly, lymphocytes was the primary subpopulation which showed virus-induced apoptosis *in vitro*. In contrast, granulocytes displayed the highest TiLV detection rate (46.4%) with minimal apoptosis, suggesting its potential role in viral dissemination. These findings advance our understanding of TiLV immunopathogenesis and support the development of targeted strategies to control the disease in tilapia aquaculture.

Keywords: Granulocytes, Lymphocytes, Hematological Profiles, Tilapia, Tilapia Lake Virus

Project: Study on the alterations in leukocyte subpopulations and the mechanisms leading to leukocyte depletion in Nile tilapia infected with tilapia lake virus

Funding: Kasetsart University, Thailand

Challenges in therapeutic management of Oomycetes disease in Indian aquaculture

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Members of Oomycetes, particularly Order *Saprolegniales*, cause frequent and severe diseases in finfish. Since 1990s, panzootics of Epizootic Ulcerative Syndrome (EUS) with heavy morbidity and mortality of many freshwater and brackishwater fish species have brought huge loss to the aquaculture and threatened resilience of inland open water fisheries in the Asia-Pacific. The disease presently continues as enzootic to epizootic in several parts of the world. Our survey detected EUS outbreaks in 80.3% of floodplain wetlands of Assam, India. Saprolegniasis is another common water mold infection affecting several fish species and fish hatcheries; however, barring scattered reports of occurrences the geographical spread, epidemiology, economic loss, etc. of the disease are yet to be estimated. In India, saprolegniasis has been recorded to cause 5-100% mortality in cold waters and ponds and 7-12% mortality in cage culture of *Pangasianodon hypophthalmus*. Unlike in human and farm animals where mycosis is often sporadic with negligible mortality, fungal diseases cause large scale outbreaks with heavy morbidity and mortality in fish demanding availability of effective therapeutics. However, there have been negligible research efforts on development of safe and effective drugs and chemicals against the fish fungi. Very recently, we have established efficacy and pharmacokinetics of clotrimazole, among several chemicals and drugs, against *Saprolegnia parasitica*. However, none of the antifungal VMP is approved for use in food animals including fish and has established MRL despite the fact that azole antimycotics are widely used in crop protection and have MRLs. Thus, besides augmented research thrusts on epidemiology, fungal pathogenesis and chemotherapy development, policy level discussions on use of antifungals in aquaculture is essential to reduce biological and other losses from fungal diseases in fish. The paper intends to highlight present state of research, including our own research, on fish fungi aiming towards drug development, and drug use policy.

Keywords: Fungal Diseases, Finfish, Drugs and Chemicals, Research, Policy

Project: All India Network Project on Fish Health

Funding: Indian Council of Agricultural Research, India

Induction of variable but non-culturable state in *Nocardia seriolae*

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Nocardiosis caused by *Nocardia seriolae* makes a severe loss of fish production in *Seriola* fish species in Japan. In our previous study, the bacteria were detected in apparently healthy juvenile fish by PCR and immunohistopathology but not isolated on 1% Ogawa medium, suggesting that *N. seriolae* infect to the host as variable but nonculturable (VBNC) state. The present study aimed to identify the condition that makes the bacteria VBNC state *in vitro*. *N. seriolae* was cultured for 60 days in BHI broth and the bacterial culture was sampled at 10, 20, 30, 40, 50 and 60 days post-incubation (dpi). The bacterial culture was subjected to measurement of turbidity at OD630, recovery culture on 1% Ogawa medium, and analysis of esterase activity. Total RNAs were extracted from the bacteria cultured for 10 and 60 days, and mRNA sequencing was performed using MiSeq sequencer. Differentially expressed genes (DEGs, $<$ 4-fold, $p < 0.001$) were detected using Trinity software and annotated using BLSAT2GO software. Turbidity at OD_{630} increased to 30 dpi and remained almost constant until 60 dpi. Bacterial colonies grew on the medium from the culture sampled at 10, 20, 30 and 40 dpi, while no colony was seen from those sampled at 50 and 60 dpi. However, the bacterial culture sampled at all time points were positive for esterase activity, indicating the bacteria were still alive even in the culture sampled at 60 dpi. There were 1,330 unique DEGs up regulated in the bacteria cultured for 60 days. These DEGs contained genes related to regulation of primary metabolism, fatty acid biosynthesis and lipid catabolism. These data suggest that *N. seriolae* enter VBNC state after long-period cultivation and the VBNC bacteria activate different biological processes from the logarithmic state bacteria in metabolism, biosynthesis and catabolism.

Keywords: *Nocardia seriolae, Seriola quinqueradiata*, Variable But Non Culturable

Fish disease scenario in coldwater fish farming in India

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Coldwater aquaculture in India, particularly the farming of rainbow trout and exotic carp, has expanded significantly in the Himalayan states over the last two decades supporting rural livelihoods and nutritional security. With the intensification of farming, the risk of disease poses a major threat to sustainable production. The unique ecological conditions of coldwater system such as low temperatures, high dissolved oxygen, and seasonal fluctuations, creates distinct host-pathogen dynamics, favouring the persistence and spread of infections. Bacterial pathogens are the predominant cause of morbidity and mortality. *Aeromonas hydrophila* is associated with hemorrhagic septicemia and ulcerative conditions in trout. *Lactococcus garvieae* has emerged as a critical pathogen, with strains harbouring multiple virulence and antimicrobial resistance genes. Occurrence of other notable bacterial pathogens, *Flavobacterium columnare, Flavobacterium tructae, Chryseobacterium balustinum, Pseudomonas koreensis, Vibrio anguillarum*, and *Aeromonas veronii* in trout and mahseer are also recorded. Fungal infections like saprolegniasis, gastrointestinal basidiobolomycosis caused by *Basidiobolus* sp., and zoonotic *Fusarium oxysporum* in rainbow trout and mahseer highlight the rising concern of opportunistic fungi. Parasitic infestations, including *Ichthyophthirius multifiliis* (white spot) and *Argulus* spp. (fish louse), are recurrent in rainbow trout, snow trout and mahseer. Lymphocystis disease virus (LCDV) has been reported from grass carp. These diseases collectively result in severe economic losses. Chemotherapeutic, antibiotic and other veterinary medicinal products (VMPs) like oxytetracycline, erythromycin, florofenicol, praziquantel, BKC, KMnO4, Chloramine-T, idophor, formalin and rock salts are commonly used for treating various microbial infections in coldwater fish farming. Experimental trials on vaccines against *Lactococcus garvieae* and other bacterial pathogens are underway. Addressing this scenario requires region-specific fish health strategies, integrating improved diagnostics, surveillance, biosecurity, and alternative therapeutics such as vaccines, probiotics, and immunostimulants. Strengthening preventive approaches and farmer awareness is also critical for safeguarding fish health and ensuring the long-term sustainability of coldwater aquaculture in India.

Keywords: Coldwater Aquaculture, Surveillance, Diagnosis, Therapeutic Measures, Veterinary Medicinal Products

Probiotic Potential of Pediococcus pentosaceus Spent Culture against *Vibrio parahaemolyticus* in Cooked Mackerel Chunks

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Probiotics are gaining increasing attention in aquaculture as sustainable alternatives to antibiotics, owing to their ability to enhance host immunity, improve gut health, and suppress pathogenic bacteria without contributing to the global challenge of antimicrobial resistance. Unlike antibiotics, which often leave residues and foster resistant strains, probiotic microorganisms such as lactic acid provide safe, eco-friendly disease control while promoting better digestion and growth in aquatic organisms. Although probiotics such as *Lactobacillus, Bacillus*, and *Pediococcus* have been shown to reduce bacterial pathogens in aquaculture, limited research has examined the direct use of their spent cultures in seafood preservation. In the present study, the spent culture of *Pediococcus pentosaceus* was evaluated for its antagonistic effect against *Vibrio parahaemolyticus*, a major seafood-borne pathogen. Fresh Indian mackerel (*Rastrelliger kanagurta*), selected as a model seafood matrix due to its commercial significance and perishability, was cooked, inoculated, and treated with the crude spent culture. Treated chunks stored at 37 °C for three days showed a marked reduction of *V. parahaemolyticus* from 6.17 $\log_{10}$ cfu/g on day 0 to 2.77 $\log_{10}$ cfu/g by day 3, with corresponding changes in pH. This significant decline highlights the bactericidal potential of probiotic metabolites. The findings suggest that the potential of *P. pentosaceus* spent culture as a biocontrol agent for seafood safety and shrimp aquaculture by improving gut health, boosting immunity, and reducing antibiotic reliance and using Indian mackerel as a model species confirmed its effectiveness in pathogen reduction, supporting the broader application of probiotics for sustainable aquaculture.

Keywords: Functional Probiotics, Seafood Safety, Sustainable Aquaculture, *Vibrio parahaemolyticus*

Impact of Tilapia Lake Virus infection on the gut microbiota of red hybrid tilapia (*Oreochromis* spp.)

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Tilapia lake virus (TiLV) is an emerging pathogen causing high mortality and economic loss in global tilapia farming. It induces systemic pathology, particularly in the liver, spleen, kidney, and notably the intestine, where damage can impair nutrient absorption and overall health. The gut microbiota plays a key role in immunity and disease resilience. However, the impact of TiLV infection on the gut microbiota and its relatedness to the fish mortality has never been studied. This study examined gut microbiota changes in red hybrid tilapia (*Oreochromis* spp., 10 ± 3 g) following TiLV challenge via two methods: intraperitoneal (IP) injection with 50 μL of 1×10^5 $TCID_{50}$/mL virus or cohabitation with infected fish at a 3:1 ratio. Mortality outcomes revealed a higher mortality rate in the IP group (81.5%) compared to the cohabitation group (49%). Correspondingly, IP-injected fish exhibited more severe intestinal pathology and viral loads at 6, 12, and 21 days post-challenge (dpc). Microbiota profiling revealed marked shifts in both alpha and beta diversity among the infected groups compared to the control. Notably, infected fish showed reduced levels of *Cetobacterium* (Phylum *Fusobacteria*) and increased abundance of *Brevinema* (Phylum *Spirochaetes*) during the early stages (6 and 12 dpc), indicating that imbalance of these bacteria play an important role in in the fish mortality. By 21 dpc, surviving fish exhibited an increased prevalence of *Bacillus* (Phylum *Firmicutes*), which is known for its probiotic properties, suggesting a potential role in recovery. Meanwhile, fish that survived the cohabitation challenge maintained a gut microbiota composition more closely resembling that of healthy controls. These finding underscore the influence of infection route on gut microbial dynamics and support the potential use of microbiota-targeted strategies such as probiotics to enhance disease resilience in tilapia farming.

Keywords: Tilapia lake virus (TiLV), Gut Microbiota, Intestinal Pathology, Tilapia, Probiotics

Project: Analysis of gut microbiome in tilapia infected with tilapia lake virus

Funding: Faculty of Veterinary Medicine, Kasetsart University

Comparative assessment of immune response to Cyprinid herpesvirus-2 (CyHV-2) infection and vaccination in goldfish (*Carassius auratus*)

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Cyprinid herpesvirus 2 (CyHV-2), the cause of goldfish haematopoietic necrosis virus (GFHNV), has led to major economic losses in goldfish (*Carassius auratus*) aquaculture. This large, double-stranded DNA virus from the *Alloherpesviridae* Family also affects other cyprinids like Prussian carp, crucian carp, and their hybrids. Owing to global goldfish trade, CyHV-2 has spread across Europe, Asia, Oceania, and South America. To counter such infections, efforts now focus on boosting fish immunity through vaccines and immunostimulants, as the host's immune response is key to controlling the disease. This study focuses on assessing the immune responses of goldfish following infection with Cyprinid herpesvirus 2 (CyHV-2) and subsequent vaccination using a heat-inactivated formulation. CyHV-2 was cultured in fantail goldfish fin (FtGF) cell lines, achieving a viral titer of $10^{7.8}$ $TCID_{50}$/mL. The vaccine was developed by inactivating the virus through heat treatment at 80°C for one hour. The efficacy of the inactivation process was validated by the absence of cytopathic effects (CPE) in FtGF cells post-treatment. The genes expression of cytokines (IL-12, IL-10), interferon gamma (IFN-γ), adaptive markers like CD8 and CD4 were observed at various time intervals (6^{th} h, 2^{nd} day, 4^{th} day, 10^{th} day, 16^{th} day and 30^{th} day) from the kidney and spleen tissues of CyHV-2 infected and vaccinated goldfish. The results showed early upregulation of IL-10 and CD4 in the kidney and significant expression of CD8 and IL-12 in the spleen of vaccinated fish, with IFN-Y elevated in the kidney by day 2 post-infection. These findings suggest that the heat-inactivated CyHV-2 vaccine effectively stimulates an early immune response. The results infer that the heat inactivated vaccine was able to boost the immune gene as that in CyHV-2 infected goldfish. Moreover, large-scale field studies are essential to evaluate the economic feasibility and practical implementation of these vaccines.

Keywords: Vaccine, Aquaculture, Virus, Prophylaxis

Project: NSPAAD Phase II

Funding: National Fisheries Development Board, India; Pradhan Mantri Matsya Sampada Yojana, Government of India

Population pharmacokinetics of antibiotic oxolinic acid following in-feed bolus administration in striped catfish *Pangasianodon hypophthalmus* (Sauvage, 1878)

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With rapid expansion of intensive aquaculture, several antimicrobials, antiparasitics and antifungal drugs are increasingly been used for finfish and shellfish disease management. However, their irrational uses can exacerbate disease resistance, alter ecological functioning and adversely affect consumer health. To curb such negative effects, a few pharmacologically active compounds are authorized/permitted by different nations for aquaculture use. However, India lacks legally permitted antibiotic(s) for use in aquaculture farms. Oxolinic acid (OA), a broad spectrum quinolone antibiotic, has been approved by some of the European countries and Japan for therapeutic purposes in freshwater fish species. The present study has investigated the pharmacokinetics of oxolinic acid following in-feed administration in widely cultured catfish *Pangasianodon hypophthalmus* with larger aim of possible use in the catfish health management in India. Following single bolus oral administration at the therapeutic dose of 12 mg kg^{-1} body weight in fingerlings of *P. hypophthalmus*, OA concentrations in serum, liver and kidneys at specified time intervals were quantified by LC-MS/MS. Analysis of kinetic profile of various pharmacokinetic parameters elucidated distribution half-life ($t_{1/2\alpha}$) and elimination half-life ($t_{1/2\beta}$) of the drug as 0.75 and 15.45 h, respectively. The drug distribution from the plasma to other tissues was found to be satisfactory with apparent volume of distribution of the drug at steady-state ($Vd_{(ss)}$) to be 3.12 L kg^{-1}. The mean residence time (MRT) of OA was short (17.78h) and the total clearance rate (Cl_T) of the drug was low (0.25 L kg^{-1} h^{-1}). For liver and kidney samples, highest drug concentrations were seen at 8 h and 12 h respectively. The fast depletion of OA suggests short withdrawal time with reference to human consumption of the treated fish. Overall, the pharmacokinetic data indicate promising applicability of the antibiotic in the catfish.

Keywords: Pangasius, Peak Concentration, Half Life, Spectrophotometry, Aquaculture

Project: All India Network Project on Fish Health

Funding: Indian Council of Agricultural Research, India

Florfenicol-induced dysbiosis in gut microflora and alterations in immunological and digestive parameters of *Pangasianodon hypophthalmus*

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The gut microbiome plays a critical role in nutrient assimilation and metabolic processes, and its disturbances have been linked with several disease conditions in human and lower vertebrates. Here the effect of oral administration of florfenicol, an antibiotic approved for aquaculture use in several countries, on digestive functions and intestinal microbiota of catfish *Pangasianodoon hypophthalmus* has been examined. Fingerlings of *P. hypophthalmus* was in-feed administered florfenicol @ 10 mg/kg biomass for 10 days, followed by a 10 days withdrawal period. Following humane sacrifice of fish, intestine was collected aseptically on day 10 of administration and post-10 days of administration, whole DNA content of gut was extracted and sequenced by next-generation sequencing (NGS) targeting V_3-V_4 regions of 16s rDNA. Blood samples were collected from caudal vein and tested for digestive enzymes in plasma. High-quality gut microbe sequence reads showed that FFL-treated fish had the highest taxonomic diversity compared to the control group. *Campylobacterota, Fusobacteriota, Firmicutes, Bacteroidota, Proteobacteria, Verrucomicrobiota, and Desulfobacterota* made up 99% of the ribotypes in healthy untreated fish share which decreased to 95.7% after antibiotic treatment. At the most fundamental taxonomic level, *Cetobacterium somerae* dominated the gut microbiota of healthy *P. hypophthalmus*. Relative abundance of *Bacteroidata* and *Proteobacteria* increased, while presence of *Crenarchaeota, Dependentiae, Gemmatimonadota, Hydrogenedentes, Spirochaetota, Deinococcota*, and few others were found only in treated fish. The blood amylase activity significantly decreased while lipase activity increased in the antibiotic treated fish; the protease activity decreased insignificantly and returned to control level by day 10 of post-dosing. The macrophage activity (respiratory burst activity), serum lysozyme, and myeloperoxidase levels showed alterations which returned to normal values upon cessation of the drug. This study is the first to disclose the unexpected consequences of an authorised antibiotic on a commercially important freshwater catfish's gut microbiome.

Keywords: Catfish, Gut Microbiome, Florfenicol, Digestive Enzyme

Project: All India Network Project on Fish Health

Funding: Indian Council of Agricultural Research, India

Effect of Ivermectin on Biosafety and HSP70 Gene Expression in *Cyprinus carpio*

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This study evaluates the biosafety of Ivermectin (IVM) in *Cyprinus carpio*, focusing on its effects on haemato-physiological parameters and expression of heat shock protein 70 (HSP70). The 96-hour median lethal dose (LD_{50}) of IVM was determined to be 8.91 ± 3.46 mg kg^{-1} body weight. Based on this, a biosafety trial was conducted using single oral doses of IVM (0.00, 0.50, 1.00, 1.25, 2.50, and 5.00 mg kg^{-1}) over 21 days. The study assessed behavioural, haematological, immunological, biochemical, histological, and HSP70 gene expression responses. Results showed that higher doses (≥ 2.5 mg kg^{-1}) significantly ($p<0.05$) reduced haematological parameters such as haemoglobin, RBC, WBC, PCV, MCV, MCH, MCHC, and lysozyme activity, while stress biomarkers serum glucose, total protein, ALP, AST, and ALT increased significantly, particularly within the first 14 days. In contrast, the 0.50 mg kg^{-1} group exhibited minimal variations compared to the control. A dose-dependent increase in HSP70 gene expression in liver and kidney was observed in all higher dose groups, excluding the 0.50 mg kg^{-1} group. The changes in biochemical markers suggested oxidative stress at higher IVM concentrations. The 0.50 mg kg^{-1} dose was comparable to control in all parameters, indicating no observable adverse effects. Thus, 0.5 mg kg^{-1} is identified as the No Observed Adverse Effect Level (NOAEL), while 1.0 mg kg^{-1} is the Lowest Observed Adverse Effect Level (LOAEL). This indicates that the lower dose had the least impact on vital physiological functions important for fish health, growth, and immunity. These results emphasize the need for cautious use of Ivermectin in aquaculture practices to mitigate adverse effects on fish health.

Keywords: Ivermectin, Biosafety, Common Carp, HSP70 Gene Expression

Project: All India Network Project on Fish Health

Funding: ICAR- Central Institute of Brackishwater Aquaculture, Chennai

An integrated approach for managing *Epistylis* sp. infestation in *Anabas testudineus* brooders using salt bath and water exchange treatments

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Epistylis sp., a pathogenic epibiont protozoan, causes significant health issues in *Anabas testudineus* brooders, often leading to co-infections. This study evaluated the efficacy of short-term salt bath (3% NaCl for 3 minutes) and water exchange strategies individually and in combination for controlling *Epistylis* sp. infestation over a 21-day experimental period. The fish used in the experiment were all infested with *Epistylis* sp., except those in the negative control group (CN). The experiment was conducted in duplicates, with 15 fishes in each replicate of the five treatment groups: CP (positive control, no treatment), T1 (3% salt bath only), T2 (daily water exchange only), T3 (3% salt bath combined with daily water exchange), and CN (negative control, comprising healthy, uninfected stock). In salt treatment groups (T1 and T3), salt baths were administered once every 3 days. At the end of the 21 days, infestation prevalence was highest in CP (50.00 ± 10.0%), followed by T2 (40.00 ± 6.6%), T1 (36.65 ± 3.35%), and lowest in T3 (23.33 ± 3.3%), while CN showed minimal infestation (6.67 ± 0.00%). Statistical analysis confirmed that T3 significantly (p <0.05) reduced infestation compared to CP. These findings have important implications for aquaculture practices, particularly in hatcheries where broodstock health is critical for larval production. An integrated management approach combining regular water exchange with periodic salt baths can be a cost-effective, non-chemical strategy to control *Epistylis* sp. infestation. However, further studies are warranted to optimise treatment frequency, duration, and salinity levels, as well as to evaluate long-term effects on broodstock health and reproductive performance.

Keywords: *Epistylis* sp., *Anabas testudineus*, Salt Bath, Water Exchange, Protozoan Control

Project: Murrel CS project

Funding: Department of Fisheries, Government of Tamil Nadu

Antagonistic activity of isolates of Lactic Acid Bacteria against Seafood borne bacteria

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Probiotics are being utilised in aquaculture as environmentally sustainable substitutes for antibiotics to enhance fish health while concurrently improving productivity characteristics. The integration of lactic acid bacteria with feed enhances its nutritional value, as these microorganisms generate various digestive enzymes that facilitate digestion and feed decomposition, positively influence growth, and stimulate reproductive activity, thereby promoting the adoption of commercial probiotics derived from LAB strains. The present study aims to study potential of various Lactic acid bacteria (LAB) against seafood-borne spoilage bacteria such as *Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Clostridium perfringens*. The results revealed that the maximum inhibition (26 mm) of *L. monocytogenes* by *Lactobacillus lactis* cells followed by *Clostridium perfringens* inhibition was observed with *L. plantarum* (21mm). *P. acidilactici* and *L. acidophilus* cell suspensions inhibited *B. cereus* equally. *Pediococcus pentosaceous* and *P. acidilactici* showed negligible inhibition against *Vibrio parahaemolyticus*. The findings of this study may essentially contribute to the understanding of the probiotic potential of LAB in effective probiotic against various fish pathogens in the aquaculture system.

Keywords: Lactic acid bacteria, spent cultures, antagonism against Seafood pathogens

Experimental infection and pathogenicity of *Aeromonas veronii* to Asian Seabass, *Lates calcarifer*

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Seabass is one of economically important cultured brackishwater finfish species in India. Many of the coastal people depend on fish farming as an important source of income and employment but farming sector is constantly under threat due to infectious diseases. Among the various bacterial pathogens of cultured fish, *Aeromonas veronii* is a gram-negative, non-motile, rod-shaped bacteria and one of the important freshwater pathogens after *Aeromonas hydrophila* in causing diseases to aquatic animals. *A. veronii* (Genbank ID PQ967979) isolated from the diseased *Lates calcarifer* cultured in low saline tanks, reported with hemorrhagic septicemia, skin ulcers and mortality. The virulence-related genes associated with the fish pathogenicity like *cytotoxic enterotoxin, cytotonic enterotoxins, aerolysin, serine protease, glycerophospholipid:cholesterol acyltransferase* were tested by PCR and only cytotonic enterotoxin alt gene was detected. Pathogenicity of bacteria in Asian seabass was studied experimentally with fingerlings (average weight 13.2g n=10/tank). Fish were treated for different bacterial concentrations in triplicates reared in 5ppt salinity and temperature of 29 ± 2°C in 100 L tanks. The lethal dose (LD_{50}) was calculated by intraperitoneal injection of 100 μL of bacterial suspension per fish with different concentrations per group and the fish in the control group injected with 100 μL sterile PBS (pH 7.4). The gross lesions like red patches in the skin, scale loss and mortalities of fish were observed after 48 hrs post-infection and monitored for 14 days. The LD_{50} of *A. veronii* to Asian seabass was calculated based on the cumulative mortality of the fish as 1.6 x 10^5 and 10^6 CFU/ml. The bacterial re-isolation with translucent pin point colonies proved Koch's postulate and confirmed as *A. veronii* by PCR, and histopathology and thus confirmed to be the cause of mortality as primary pathogen in Asian seabass.

Keywords: *Aeromonas veronii*, LD_{50}, Fish Disease, Bacterial Challenge

Project: National Surveillance Program for Aquatic Animal Diseases

Funding: Pradhan Mantri Matsya Sampada Yojana, Department of Fisheries, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India

Epidermal alterations and metabolic enzyme responses in *Cirrhinus mrigala* following *Edwardsiella tarda* infection

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This study investigated the effects of the bacterial pathogen *Edwardsiella tarda* on the epidermis of *Cirrhinus mrigala* (Indian Major Carp). Fish were assigned to three groups: a control group (untreated), a vehicle control group (injected with 50 μl of phosphate-buffered saline (PBS) on day 0), and an infected group (injected with 50 μl of PBS containing a sublethal dose of *E. tarda*, 2.2×10^6 CFU/fish - equivalent to 10% of the 96-hour LD_{50} on day 0). The study examined changes in epidermal surface structure, tissue histology, and the specific activities of two metabolic enzymes: lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH), at 2, 4, 6, and 8 days post-infection. Analysis through Scanning electron microscope (SEM) revealed hypertrophy of epidermal epithelial cells, disrupted and disorganized microridges, and exfoliation of surface cells. A significant increase in mucous goblet cell (MGC) density was observed early in the infection, while the number of club cells decreased. Club cells exhibited signs of degeneration, including vacuolization, merging with adjacent cells, and the release of their contents onto the skin surface. Biochemical assays showed a significant ($p < 0.05$) rise in LDH activity, indicating cellular stress or damage, alongside a reduction in SDH activity, reflecting impaired mitochondrial function. These findings shed light on the epidermal response of *C. mrigala* to *E. tarda* infection and enhance our understanding of host defense mechanisms. The results may aid in developing early warning strategies for managing bacterial outbreaks in aquaculture settings.

Keywords: *Edwardsiella tarda, Cirrhinus mrigala*, SEM, LDH, SDH

Funding: The Council of Scientific & Industrial Research (CSIR), India

Establishment and characterization of a cell line from brain of *Piaractus brachypomus* and its susceptibility to fish viruses

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Piaractus brachypomus, commonly known as pacu, is one of the promising candidate species for aquaculture due to its high growth rate, wide tolerance to water quality parameters, acceptability of artificial feed, and compatibility with other cultured species like Indian major carps, tilapia and pangas. Recently, the Government of India has approved the culture of pacu in the country, and formulated guidelines to regulate its farming. However, over the last few years, there have been reports of several diseases affecting this species. Considering the same, we have established a cell line from brain tissue of pacu, designated as PBB (*P. brachypomus* brain) cell line. This cell line has been subcultured for over 90 passages. The PBB cells exhibit optimum growth at 36°C in L-15 medium supplemented with 20% FBS. The morphology of the cells is epithelial, which was ascertained by their reactivity with pan-cytokeratin antibodies. The origin of the cell line was traced to *P. brachypomus* through amplification and sequencing of partial fragments of two mitochondrial genes, namely COI and 16S rRNA. Moreover, PBB cells have diploid chromosome number (2n=54), which is same as reported in this species. The cells showed high transfection efficiency with GFP vector indicating that these cells can be used for expression of foreign genes. Importantly, the PBB cells were devoid of *Mycoplasma* contamination indicating their suitability for application in *in vitro* studies. Besides, in virus susceptibility studies, the PBB cells were found to be susceptible to similar damselfish virus and snakehead rhabdovirus as revealed by cytopathic effects and specific amplicons for the respective viruses in PCR/RT-PCR of infected cell pellets. In contrast, these cells were refractory to tilapia lake virus. The developed cell line would be a vital tool for investigating disease cases suspected to be of viral etiology in this commercially important fish species.

Keywords: Pacu, Brain, Cell Line, Transfection, Virus Susceptibility

Project: National Surveillance Programme for Aquatic Animal Diseases

Funding: Department of Fisheries, Government of India

Infectivity and immunological responses induced by Tilapia ParvoVirus (TiPV) in cultivable freshwater fishes

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Tilapia Parvo Virus (TiPV) is a rising viral agent that primarily affects tilapia. It can be lethal and cause mortality ranging from 30% to 50% in farmed tilapia. The goal of the current investigation was to evaluate the infectivity potential of TiPV in freshwater fishes and to analyse the innate immune responses of tilapia following viral challenge. TiPV infection caused 25% mortality in Nile tilapia, with characteristic signs before death, while no mortality or clinical signs were observed in other freshwater fishes, such as pearl spot and common carp. Tilapia challenged with TiPV showed significant (p <0.05) changes in haematological parameters such as haematocrit (HCT), red blood cell (RBC), white blood cell (WBC), platelet (PLT) counts, haemoglobin (HGB), mean corpuscular haemoglobin (MCH) and mean platelet volume (MPV); immunological parameters such as catalase activity, respiratory burst, superoxide dismutase (SOD) and myeloperoxidase (MPO); and serum biochemical parameters such as glucose, albumin, globulin, creatinine, urea, cholesterol, total protein, alkaline phosphatase, aspartate aminotransferase (SGOT), and alanine aminotransferase (SGPT) activity at 24, 48, 72 and 96 hours post infection (hpi). Infected tilapia also exhibited differential expression patterns of immune-related genes (TLR-9, IFR-7, IFN and IL-1β) at 24, 48, 72 and 96 hpi. Further, histopathological examination revealed varying degrees of tissue damage, hemorrhages, vacuolation and necrosis consistent with viral infection in the brain, liver, spleen and kidney of experimentally infected tilapia. Changes in haematology, immunology, serum biochemistry, immune gene expression, and histopathological patterns reflect the mechanism of viral invasion and the activation of innate defense system in tilapia during TiPV infection.

Keywords: Haematology, Histopathology, Immunity, Infection, Tilapia, TiPV

Project: National Surveillance Programme for Aquatic Animal Diseases - Phase II

Funding: Pradhan Mantri Matsya Sampada Yojana, Department of Fisheries, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India; ICAR-National Bureau of Fish Genetic Resources, India

Inhibition of C-type lysozyme activity in Japanese flounder by lysozyme inhibitor Ivy derived from *Edwardsiella piscicida*

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Edwardsiella piscicida is a significant fish pathogen that causes edwardsiellosis in various species, including the Japanese flounder *Paralichthys olivaceus*, resulting in substantial economic losses in aquaculture. We investigated the direct inhibitory activity of IvyEp (Inhibitors of vertebrate lysozyme derived from *E. piscicida*) against host C-type lysozyme, and its role in host immune evasion. The results of functional assays confirmed that IvyEp significantly inhibited the activity of hen egg white lysozyme (HEWL), and kinetic analysis demonstrated that IvyEp acts as a competitive inhibitor. The ivy-deleted strain of *E. piscicida* exhibited reduced virulence in Japanese medaka *Oryzias latipes* and flounder infection models, highlighting its role in immune evasion and pathogenicity. IvyEp significantly suppressed lysozyme-like activity in the serum of Japanese flounder, yellowtail *Seriola quinqueradiata*, and Nile tilapia *Oreochromis niloticus*, suggesting broad-spectrum inhibition of C-type lysozymes across these species. Further assays confirmed that IvyEp significantly inhibited the lytic activity of purified flounder C-type lysozyme (rLyzC), supporting the hypothesis that IvyEp directly interferes with host lysozyme activity. Structural analysis predicted that IvyEp blocks the active site of rLyzC through electrostatic interactions, preventing substrate binding and thus neutralizing rLyzC function. Co-immunoprecipitation revealed that IvyEp directly binds to HEWL and rLyzC. These findings provide new insights into the potential role of IvyEp in protecting *E. piscicida* from host immune responses, suggesting its probable function as a universal C-type lysozyme inhibitor across different fish species.

Keywords: *Edwardsiella piscicida*, Inhibitor of Vertebrate Lysozyme (Ivy), Japanese Flounder *Paralichthys olivaceus*, C-type Lysozyme (LyzC)

Funding: Japan Society for the Promotion of Science (JSPS), Japan

Edwardsiella virulence protein P derived from *Edwardsiella piscicida* modulates host immune response and cell death in teleosts

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Edwardsiellosis, caused by *Edwardsiella piscicida*, causes considerable damage to aquaculture worldwide, and an understanding of its infection mechanism is needed to develop a solution. The type VI secretion system (T6SS) effector, *Edwardsiella* virulence protein P (EvpP), a type VI secretion system effector, is a major virulence factor that inhibits inflammasome formation and suppresses pyroptosis in mice. However, the relationship between EvpP and the fish inflammasome remains unclear. In the present study, the role of EvpP was examined in Japanese medaka (*Oryzias latipes*) and Japanese flounder (*Paralichthys olivaceus*) using an EvpP deletion strain (Δ*evpP*) and wild-type (WT) strain. In challenge tests, fish survival was higher in the Δ*evpP*-infected group and showed lower toxicity compared with the corresponding parameters in the WT strain. Fluorescence imaging and quantitative real-time RT-PCR (qPCR) analysis showed that although early intestinal colonization was moderate, later (after day 3), the number of bacteria in the kidney, liver, and posterior intestine was significantly lower in the Δ*evpP*-infected group; furthermore, expression analysis of immune-related genes (*il1b, il6, mcp1b*) showed an induced expression during Δ*evpP* infection. Propidium iodide (PI)-stained flow cytometry showed significant cell death in the Δ*evpP*-infected group at 1 day post-infection (dpi) and in the WT-infected group at 3 dpi. The timing of cell death induction in the Δ*evpP*-infected and WT-infected groups was different, suggesting that cell death was temporarily suppressed by EvpP. This may represent an elaborate strategy against *E. piscicida*.

Keywords: *Edwardsiella piscicida*, T6SS, EvpP, Pyroptosis, Japanese Medaka *Oryzias latipes*

Funding: Japan Society for the Promotion of Science (JSPS), Japan

Unveiling the bioactive and antibacterial properties of selected seaweed species of Andaman Islands and evaluation of its immunomodulatory potential in fish health management

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Seaweeds are recognised for their rich bioactive compounds and potent antioxidant properties. This study investigated the bioactive potential of eight seaweed species from the South Andaman coast, representing red (*Rhodophyceae: Gracilaria edulis, Acanthophora spicifera*, and *Gracilaria salicornia*), brown (*Phaeophyceae: Sargassum wightii, Padina tetrastromatica, and Turbinaria ornata*), and green (*Chlorophyceae: Halimeda opuntia* and *Dictyosphaeria cavernosa*) categories. Comprehensive analyses were conducted, including total phenol and flavonoid content, antioxidant activity, reducing power, metal chelation, carotenoid content, proximate composition, in vitro antibacterial activity, and minimum inhibitory concentration (MIC). Among these species, *G. edulis, P. tetrastromatica, and H. opuntia demonstrated* significantly higher bioactive potential in their respective categories. Carotenoid content was highest in *P. tetrastromatica* (83.81 ± 0.28 μg/g) followed by *H. opuntia* (35.28 ± 0.34 μg/g) and *G. edulis* (24.64 ± 0.18 μg/g). Antibacterial efficacy against *Aeromonas hydrophila* and *Vibrio parahaemolyticus* was notable, with the highest MIC values observed in *P. tetrastromatica, G. edulis* and *H. opuntia*. Two-way ANOVA and Principal Component Analysis identified *G. edulis, P. tetrastromatica*, and *H. opuntia* as exhibiting significantly higher *in vitro* bioactive and antibacterial properties. FTIR and GC-MS analysis confirmed the presence of bioactive constituents in these three seaweed extracts such as polysaccharides, amino acids, alkenes, fatty acids and other functional components. Mineral profiling identified 23 essential macro and micronutrients in *G. edulis* and *P. tetrastromatica*, and 19 in *H. opuntia. In vivo* trials were conducted with *Labeo rohita* using 10 treatment groups. Fish fed a diet supplemented with seaweed extract mixture at 3 g/kg feed exhibited significant improvement in growth performance, hemato-immunological responses, enzyme parameters and post-challenge survival rates against *A. hydrophila*. The bioactive properties of *G. edulis, P. tetrastromatica*, and *H. opuntia* underscore their potential as natural immunostimulants and nutrient sources, making them valuable for fish health management and biotechnological applications.

Keywords: Seaweeds, *Gracilaria edulis, Padina tetrastromatica, Halimeda opuntia*, Immunomodulation

Project: Deciphering the in vitro bioactive potential of selected seaweed species of Andaman Islands and evaluation of its immunomodulatory effect on fish
Funding: Indian Council of Agricultural Research, India

Egg disinfection-induced microbiota programming improved hatching rate, antioxidant defense and survival in Snubnose pompano (*Trachinotus blochii*) larvae

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Trachinotus blochii is a high-value mariculture finfish species. Poor larval survival is a critical bottleneck in its sustainable mariculture practices. Since early microbial colonisation plays a crucial role in the health and survival of fish larvae, the study investigated the downstream effects of pre-hatch egg disinfection using hydrogen peroxide (H_2O_2), glutaraldehyde, and iodophor on the hatching rates, antioxidant profiles, whole larval microbiome profiles, and survival rates in *T. blochii* larvae. The study initially identified the optimal disinfection conditions for improved hatchability, as 20 ppm iodophor for 10 min, 400 ppm H_2O_2 for 10 min, and 40 ppm glutaraldehyde for 5 min. The larval survival in the identified optimal disinfection protocol was the highest in glutaraldehyde (34.80 ± 1.1%), followed by H_2O_2 and iodophor treatments. Further, an enhanced catalase activity correlated positively with survival across post-hatch days, while GSH levels remained unaffected. Whole larval microbiota analysis was done on the 10th day post-hatching using 16S rRNA amplicon (V3-V4)-based sequencing. The results of principal component analysis (PCA), α-diversity, taxonomic and functional metagenomics revealed the distinct clustering based on survival rates, with higher diversity measures and enrichment of KEGG pathways related to metabolism observed in high-survival groups. Specific microbial ratios, notably lower *Proteobacteria: Bacteroidota* and higher (*Fusobacteria + Firmicutes + Bacteroidetes*): *Proteobacteria*, showed significant correlations with improved survival. Further, the results identified specific bacterial taxa at different taxonomic levels that predicted improved outcomes. Briefly, the results highlight that pre-hatching egg disinfection could program larval antioxidant capacity and microbiota composition, offering a scalable strategy to enhance the hatching and survival rates, contributing to the development of science-based sustainable hatchery management practices of *T. blochii*.

Keywords: Larval Microbiota, Marine Hatchery, Silver Pompano, Survival Enhancement, Catalase

Project: E.G. Silas Centre of Excellence and Innovation (EGS - CoEI) in Marine Fish Microbiome and Nutrigenomics

Funding: Department of Biotechnology, Government of India

Pathogenicity of *Vibrio harveyi* in Asian seabass *Lates calcarifer*

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Vibrio harveyi, a Gram-negative bacterium, is responsible for vibriosis outbreaks in several economically important aquaculture species. In the present study, *V. harveyi* SB1 was isolated from moribund Asian seabass (*Lates calcarifer*) exhibiting high mortalities. Experimental challenge assays were conducted by i/m injecting fish with bacterial suspension ranging from 5×10^4 to 5×10^5 CFU/fish, alongside a non-exposed control group. The median lethal dose (LD_{50}) was determined to be 5×10^4 CFU/fish. The affected fish exhibited severe muscular degeneration around the injection site. Histopathological evaluation showed pronounced tissue damage. For understanding the molecular mechanism of pathogenesis, *V. harveyi* SB1 was sequenced at the Illumina and PacBio platform, producing a chromosome-level assembly with 2 chromosomes and a plasmid. Genomic analysis revealed that approximately 10% of the genome was dedicated to protease synthesis, with the presence of a well-characterized empA metalloprotease gene likely contributing to muscle degeneration. These findings confirm the pathogenic potential of *V. harveyi* SB1 towards Asian seabass and highlight the critical role of proteases in disease progression. This also highlights the need for targeted management of vibriosis in Asian seabass culture.

Keywords: Asian Seabass, *Lates calcarifer*, Metalloprotease, *Vibrio harveyi*.

Project: Development of molecular diagnostics for differentiation of pathogenic and non-pathogenic *Vibrio* species in aquaculture

Funding: Consortium Research Platform on Vaccine and Diagnostics Funded by Indian Council of Agricultural Research, India

Evolutionary insights and expression of phagocyte marker genes in *Oryzias latipes* upon *Edwardsiella piscicida* Infection

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Phagocytic immune responses are the crucial component of host innate immunity against intracellular pathogens in vertebrates. In teleosts, the molecular mechanisms governing the recruitment and activation of phagocytes remain unclear, especially during infection with *Edwardsiella piscicida*, a bacterium that can survive and proliferate within phagocytes, including macrophages and neutrophils. This study investigates the evolutionary conservation and transcriptional response of three key phagocyte markers namely MCP1β (monocyte chemotactic protein 1 beta), Mpx (myeloperoxidase), and CSF1R (colony-stimulating factor 1 receptor) in Japanese medaka (*Oryzias latipes*) during *E. piscicida* infection. Medaka were infected by immersion in a suspension of *E. piscicida*, and tissue samples were collected periodically at different time points for gene expression analysis. The evolutionary relationship derived from phylogenetic analysis revealed that *mcp1b* is conserved in fish and mammals but absent in Avian lineages, indicating its evolutionary retention in lower vertebrates. The *csf1r* gene exists as two paralogs, *csf1ra* and *csf1rb*, likely arising from teleost-specific genome duplication, both implicated in macrophage development and differentiation. Similarly, the *mpx* gene has been duplicated in medaka to generate *mpx1* and *mpx2*, which may reflect functional divergence among neutrophil-related responses or inflammatory regulation. Transcriptional profiling post-infection showed that *mcp1b* is upregulated at early infection stages, supporting its role in monocyte/macrophage recruitment. Subsequently, *csf1rb* and *mpx2* were elevated in the later phase, indicating the engagement of the macrophage lineage and the progression of the innate immune response. Overall, this study provides new insights into the evolutionary and functional divergence of phagocyte marker genes in teleosts, highlighting their differential regulation in response to bacterial infection and offering potential targets for understanding innate immune response.

Keywords: Phagocyte Markers, mcp1b, mpx, csf1r, *Edwardsiella piscicida*

Funding: Japan Society for the Promotion of Science (JSPS), Japan

Activation of the TXNIP/NLRC3 inflammasome pathway contributes to inflammation in TiLV hepatitis: A novel inhibitory effect of dimethyl fumarate

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Thioredoxin-interacting protein (TXNIP) is a multifunctional regulator involved in oxidative stress, inflammation, and glucose metabolism. In this study, we investigated the role of TXNIP in TiLV infection and its potential as a therapeutic target. Our findings reveal that TiLV infection induces high ROS levels in cells, causing TXNIP upregulation. Elevated TXNIP levels promoted viral replication by disrupting interferon-beta (IFN-β) signalling and ISG expression by impacting TRAF6/NF-kB activity. The expression of NLRC3, ASC, and proinflammatory cytokines was significantly induced upon TiLV infection, and the interaction between TXNIP and NLRC3 was evaluated using immunofluorescence co-localization and immunoprecipitation. Upregulated interleukin (IL)-1β maturation, IL-18 secretion, and caspase-1 cleavage were observed, along with increased cellular senescence in cells overexpressing TXNIP. TXNIP inhibition using dimethyl fumarate blocked IL-1β and IL-18 secretion in TiLV-infected cells, indicating that the ROS-TXNIP pathway mediates NLRC3 inflammasome activation. Delineating the complexity of the TXNIP/NLRC3 inflammasome pathway in regulating the immune response to TiLV infection suggests that the protein acts as a virally manipulated host factor that negatively regulates the antiviral immune response to augment TiLV replication and highlights TXNIP inhibitors as promising candidates for antiviral therapy.

Keywords: NLRC3 Inflammasome, Tilapia Lake Virus, Thioredoxin-interacting Protein, Antiviral Response

Project: Revolving fund project on state referral laboratory for aquatic animal disease diagnosis and quality testing

Funding: Kerala University of Fisheries and Ocean Studies, India

Intraspecific variation in the characteristics of *Cryptocaryon irritans* isolated in Japan

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Cryptocaryon irritans is an obligate parasitic ciliate and the causative agent of cryptocaryoniasis, also known as marine white spot disease. This parasite is widely distributed in tropical and subtropical marine waters worldwide, and numerous outbreaks in aquaculture have been reported across different regions. Previous studies have documented differences in biological characteristics such as cell size, serotypes, and the environmental conditions under which infections occur, including salinity and temperature. These findings suggest considerable intraspecific variation in the characteristics of *C. irritans*. In Japan, although molecular and serological variation among *C. irritans* isolates has been reported, the extent of biological diversity remains largely unexplored. Clarifying this diversity is important, as intraspecific variation in biological characteristics may affect experimental outcomes and control strategies. In this study, we collected *C. irritans* from three geographically proximate coastal sites in Japan and established clonal cultures. We compared their biological characteristics including cell size, serotype, pathogenicity, and development and infectivity under different environmental conditions to assess intraspecific variation. Genetic diversity was also evaluated by phylogenetic analysis of these three strains and 20 additional isolates collected from various regions across Japan. Our results revealed differences in biological characteristics even among isolates from nearby locations, suggesting the presence of biologically distinct *C. irritans* within Japan. Phylogenetic analysis showed that isolates collected globally fall into four genetic groups, and Japanese isolates were assigned to three of them, indicating substantial genetic diversity. Moreover, distinct characteristics were observed not only between genetically distant isolates but also among those within the same group, suggesting high characteristics diversity within Japan. These findings highlight the importance of considering both biological and genetic variation in future studies and in developing effective strategies for controlling cryptocaryoniasis.

Keywords: *Cryptocaryon irritans*, Parasite, Ciliate, Characteristics, Diversity

Project: Fundamental Studies on the Serotypes of Cryptocaryon irritans

Funding: JSPS KAKENHI

Evaluating the effect of oral oxolinic acid administration on haematological and biochemical parameters of Milk fish *Chanos chanos* juveniles

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Oxolinic acid (OA) is one of the broad-spectrum quinolones that is a critically important medicine for humans and is used as a second-line treatment in aquaculture at 12 mg/kg biomass/day for 7 consecutive days. The present study was to evaluate the effect of oral oxolinic acid administration on biochemical and haematological alterations in Milk fish *Chanos chanos*. The experiments were carried out at 0-10 times the therapeutic dose (12 mg) for 21 days. Dietary OA administration caused a dose-dependent effect on fish erythrocyte morphology and haematological parameters. A significant alteration in biochemical enzymes such as catalase, alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), acid Phosphatase (ACP), superoxide dismutase (SOD), malondialdehyde (MDA) was documented during the dosing period. The majority of the alterations, however, recovered upon cessation of OA-dosing. Further, the current study hinted at the safety and tolerability of OA in *C. chanos* juveniles in tropical Indian conditions, care must be exercised for its aquacultural application because of its listing as a critically important medicine for humans.

Keywords: Aquaculture, Haematology, Enzymes, Milk Fish, Quinolones

Project: All India Network Project on Fish Health

Funding: ICAR-Central Institute of Brackishwater Aquaculture, India

Pathological effects of sequential co infection with *Streptococcus agalactiae* and *Lactococcus garvieae* in Nile tilapia (*Oreochromis niloticus*): Histopathological, serological, and immunological analyses

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This study aimed to investigate the pathological effects of co-infection with *Streptococcus agalactiae* and *Lactococcus garvieae* in Nile tilapia using different sequential exposure orders, compared to single infections, through histopathological, serological, and immunological analyses. Juvenile tilapia were assigned to a control and four treatment groups (T1-T4, triplicate). T1 received *S. agalactiae* (LD_{50}; 100 μL IP), T3 received *L. garvieae* alone, and co infection groups (T2 and T4) received both pathogens sequentially with a 2-day interval. Sampling has been done at 3, 7, and 14 days post challenge to evaluate clinical signs, mortality, serum biochemistry, tissue pathology, and immune gene expression. Clinical signs like lethargy, hemorrhages, exophthalmia, reduced feed intake were observed in both single and co infected groups. Mortality reached 100% in T1 by day 14, whereas T3 showed ∼43% mortality. Co-infection groups exhibited intermediate mortality: 53% in T2 and 47% in T4. Histopathology revealed progressive, severe tissue damage across infected groups, most pronounced in co-infection groups. Serological profiles revealed group-specific temporal dynamics: ALT and total protein surged early in T1, AST spiked in T4 by day 7, while glucose, albumin, ALP, and BUN fluctuated across treatments, reflecting distinct host responses. Immune gene analysis demonstrated that *S. agalactiae* alone triggered a rapid, robust inflammatory response, with early upregulation of pro inflammatory cytokines, TLR-7, NF-kB, and MHC-IIα. *L. garvieae* induced a slower, milder response with later cytokine elevation. Co infection (T2) generated sustained cytokine and NF-kB/TLR-7 activation, with delayed MHC-IIα upregulation; whereas in T4 cytokine peaks occurred earlier, with MHC-IIα rising by day 7 in select organs. These findings highlight the differential pathogenesis of *S. agalactiae* and *L. garvieae*, and reveal that infection sequence critically shapes tilapia immune response and disease severity. The results advance our understanding of mixed infection dynamics in aquaculture and offer insights for improved disease management strategies.

Keywords: Co-infection Dynamics, Immune Gene Expression, Histopathology, Serum Biomarkers

Project: National Surveillance Programme for Aquatic Animal Diseases

Funding: Pradhan Mantri Matsya Sampada Yojana, Department of Fisheries, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India

Effect of vitamin C and β-glucan on larval survival and immune gene expression in Asian catfish *Clarias magur* (Hamilton, 1822)

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Clarias magur is an important air breathing catfish of South-East Asian countries, particularly in Indian subcontinent. This fish has high commercial value and is considered as a potential candidate species for aquaculture due to its good taste, high protein and iron, low fat content and also having medicinal value. Seed scarcity, due to less survival of larvae, is the main constraint for expansion of culture of the species. The present study was conducted to study the effect of vitamin C and β-glucan on larval survival and pattern of immune related gene expression viz., C3, IgM, lysG and IL-1β in *C. magur*. There was six treatment groups supplemented with three levels of vitamin C (T1-10, T2-20 and T3-30 mg/ L) and three levels of β-glucan (T4-30, T5-45 and T6-60 mg/ L). One control (C) was also maintained without the vitamin C and β-glucan. Experiment was conducted for 30 days in 50 L tank with three replicates each containing 200 larvae. Larvae were exposed to vitamin C and β-glucan through immersion methods. The result showed significantly ($p < 0.05$) higher survival (%) in T2 (58.66±5.84 %) and T5 (55.66±5.04%) groups supplemented with vitamin C (20 ppm) and β-glucan (45 ppm), respectively. Immune related gene viz. IgM, lysG, IL-1β and C3 were partially amplified and sequenced and accession numbers OP354270, P374140, OP374138 and OP374139 were submitted in NCBI Genebank. In adult fish, the differential tissue expression showed significantly ($p < 0.05$) higher level of expression of IgM, lysG and IL-1β gene in kidney, while, significantly ($p < 0.05$) higher expression level of C3 gene was found in liver tissue. In case of larvae, supplemented with vitamin C (20 ppm) and β-glucan (45 ppm) showed significantly ($p < 0.05$) higher expression of all the selected immune related gene viz. IgM, lysG, IL-1β and C3, as compared to other treatments. The results collectively suggested that 20 ppm vitamin C (T2) and 45 ppm β-glucan (T5) supplementation through immersion enhanced the immunity as well as survival of *C. magur* larvae.

Keywords: *Clarias magur*, Larvae, Vitamin C, β-glucan, Survival and Immune Gene Expression

Temperature modulates the susceptibility of Nile tilapia to Tilapia Lake Virus

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The tilapia Lake Virus (TiLV) disease, also known as syncytial hepatitis of tilapia or summer mortality syndrome, is an emerging viral disease affecting tilapia populations worldwide. For understanding the effect of temperature on manifestation of the disease, in the present study, we evaluated the susceptibility of Nile tilapia to TiLV at three different temperatures (22, 27 and 32°C) in terms of mortality pattern, replication of virus, and modulation of key immune genes over an experimental period of 12-days. The results revealed that clinical signs appeared earlier and were more severe at 32°C. However, highest cumulative mortality (92%) was observed at 27°C, followed by 32°C (83%), while the lowest mortality (8%) was observed at 22°C. Similarly, viral replication, as indicated by TiLV copy numbers, was significantly higher as early as 72 hours post-infection (hpi) at 32°C, however, a more persistent infection was observed at 27°C. On the other hand, the replication of TiLV was lowest at 22°C, with significant increase of TiLV copy number observed from 6 dpi. Furthermore, immune gene (*TLR3, Mx, IL-1β, and HSP70*) expression analysis revealed that TLR3 was highly expressed during early stages of infection (up to 72 hpi) in all the groups, with highest modulation observed at 22°C. Immune genes, *Mx* and *IL-1β* were consistently upregulated from 24 hpi to 6 dpi in all the groups. *HSP70* expression was highest at 12 dpi in both the 22°C and 27°C groups. The findings of the present study indicated that elevated temperature (32°C) accelerated the onset of TiLV infection, whereas a more persistent infection with higher mortality was observed at 27°C. Further, the regulation of key immune genes might play an important role in variation in level of susceptibility to TiLV at different temperatures.

Keywords: TiLV, Susceptibility, Temperature, Virus Replication, Immune Genes

Project: Understanding molecular basis of host-pathogen-environment interaction of Tilapia Lake Virus Disease; Development of point-of-care diagnostic kit and vaccine against Tilapia Lake Virus

Funding: NASF and ICAR-Plan Scheme

Health management protocol for captive reared streaked spine foot rabbit fish *Siganus javus* during broodstock development

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Captive maturation and breeding of streaked spinefoot rabbit fish *Siganus javus*, a commercially important lower trophic level brackishwater food fish has been constrained by infection of various pathogens and parasites. While conditioning the sub adults and broodstock of *S. javus* collected from the wild, adequate quarantine protocols are needed to remove the pathogens and parasites that would help to maintain a healthy stock for broodstock development. Infestation of gill parasites such as microsporidian *Amyloodinium ocellatum* and a monogenetic trematode parasite are the commonly noticed in the wild collected rabbit fishes. A prophylactic treatment protocol was evolved to maintain *S. javus* devoid of these infestations. Initial disinfection with 0.5 ppm of $KMnO_4$ for 30 minutes, followed by 2.0 ppm $CuSO_4$ for 45 minutes and 5.0 ppm of Praziquantel for 60 minutes resulted in removal of these organisms. Continuous treatment with 0.5 ppm of $CuSO_4$ for 45 minutes on daily basis and 5.0 ppm of Praziquantel for 60 minutes once in a month have prevented the reoccurrence of these parasitic organisms. Lower dosage of the prophylactics did not create any discomfort to the fishes and their gonadal maturity was not affected and the stock was maintained healthy.

Keywords: Rabbit Fish, *Siganus javus, Amyloodinium oocellatum*, Prophylactic Treatment

Project: Broodstock development and captive maturation of Streaked spine foot *Siganus javus*

Funding: ICAR-Central Institute of Brackishwater Aquaculture, India

Characterization of *Lactococcus formosensis* isolated from infected Oscar fish (*Astronotus ocellatus*): Pathogenicity and immune modulation

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Lactococcosis is caused by *Lactococcus garvieae*, however, other species such as *Lactococcus formosensis* and *Lactococcus petauri* have also emerged as potential pathogens. *L. formosensis* is a Gram-positive bacterium closely related to *L. garvieae* in both genetic and phenotypic characteristics. Although it has been reported in various hosts including domestic animals, wildlife, humans, crustaceans, and fish, its occurrence in fish remains poorly documented. In this study, a highly virulent strain of *L. formosensis* was isolated from diseased Oscar fish (*Astronotus ocellatus*) cultured on a farm in Kerala. Affected fish exhibited clinical signs such as sluggishness, erratic swimming, ocular lesions, and hyperacute hemorrhagic septicemia, leading to high mortality. This study encompasses the characterization of the bacterial isolate, analysis of virulence-associated genes, antibiotic susceptibility profiling, disease pathology, and host immune response. Whole-genome sequencing confirmed the identity of the causative agent as *L. formosensis*. Pathogenicity testing demonstrated its high virulence in Oscar fish. Key virulence genes identified included Hemolysin 1 (521 bp), Hemolysin 2 (492 bp), Hemolysin 3 (291 bp), NADH oxidase (331 bp), Adhesin Pav (232 bp), and Adhesin Cluster 1 (264 bp). Immune gene expression analysis in kidney and spleen tissues post-infection showed significant upregulation of immunoglobulin (Ig), interleukin-8 (IL-8), major histocompatibility complex class II (MHC II), and chemokine (CC) genes. The findings from this study on virulence, antibiotic resistance, and host immune responses associated with *L. formosensis* infection in Oscar fish provide valuable insights into the pathogenic potential and evolution of this emerging bacterium. These insights could aid in the development of effective therapeutic strategies and targeted disease management practices for aquaculture systems.

Keywords: Lactococcosis, Aquaculture, Virulence, Antimicrobial Resistance, Disease Management

Project: NSPAAD Phase II

Funding: Pradhan Mantri Matsya Sampada Yojana, Department of Fisheries, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India; National Fisheries Development Board, India

Molecular and scanning electron microscopic identification of parasitic nematodes of fish from Indonesia

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The occurrence of nematodes belonging to the family *Raphidascarididae* in Indonesian waters have been garnering attention on recent years. The members of this family are generally parasitic to both marine and freshwater fishes. Several commercially valuable fishes act as a definitive host in the developmental cycle of these parasites. Some members of this family are reported to be a health hazard while some evidences implies that these parasites can affect fish health. Also, the presence of these parasites in the fish greatly reduces the market value leading to huge monetary loss to the seafood industry. In the study, nematodes were isolated from *Psettodes erumei* specimens that were collected from a traditional fish market in Kedonganan, Denpasar, Bali Island Indonesia. The morphological attributes of the worms were studied under scanning electron microscopy (SEM). The worm identified as *Hysterothulacium* spp. had a fine transversely striated cuticle without lateral alae. In the cephalic region, three round lips - a dorsal lip with two cephalic papillae along with two subventral lips with one cephalic papilla and amphidial pore were observed. Each lip had a deep post labial grooves and prominent lateral flanges which were interlocked to contain a well-developed, triangular interlabia. The esophagus was long with posterior broadening. In the posterior end of male worms, the tail was short with small caudal papillae which were arranged into 22-30 pairs of precloacal, 1 adcloacal and 7 pairs of postcloacal papillae. Based on the molecular analysis of the Internal Transcribed Spacer region (ITS) of the ribosomal RNA gene, the worms were identified as *Hysterothylacium amoyense*. The sequences were deposited to GenBank (Accession numbers, PV926762-PV926764). The occurrence of the parasitic nematodes necessitates the surveillance of this genus for monitoring infestations in the wild and aquaculture settings for maintaining fish health and to protect consumer interests.

Keywords: Nematode, Parasite, Seafood Industry, *Hysterothylacium amoyense*, SEM

Project: ASEAN-India Collaborative research project "Detection of zoonotic parasite *Anisakis* spp. through molecular tools: An emerging public health concern"

Funding: Anusandhan National Research Foundation (ANRF), Government of India

In vitro and *in vivo* propagation of Tilapia ParvoVirus (TiPV): Pathology and immune responses

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Tilapia ParvoVirus (TiPV) is an emerging fish pathogen contributing to the mortality and morbidity of Tilapia species. Studies on the host pathology and immune responses are essential for developing management measures for this critical pathogen in the Aquaculture system. This study aimed to examine the pathology *in vitro* using OnlL cell line and *in vivo* using Nile Tilapia (*Oreochromis niloticus*). An isolate of TiPV-infected Nile Tilapia tissue was used for the experimental infections. OnlL cells showed significant CytoPathic Effects (CPE) such as elongation, rounding, syncytia, and plaque formation, and the virus was confirmed through diagnostic PCR. The experimentally infected fish showed behavioural and clinical signs such as erratic swimming, septicemia in the caudal fin, hemorrhagic septicemia on the surface, and unilateral exophthalmia. On autopsy, the fish showed gill necrosis, fluid accumulation in intestine, enlargement and necrosis in liver, enlargement of gall bladder, and elongation of spleen. RNA extractions were performed from infected cells and tissue samples for relative gene expression using real-time PCR. The T cell receptor beta (TCR-β), Major Histocompatibility Complex class II beta (MHCII-β), Cluster of Differentiation 4 (CD4), elongation factor 1 alpha (EF-1α), Tumor Necrosis Factor alpha (TNF-α), Immunoglobulin M (IgM), and immunoglobulin T (IgT) genes were expressed. The results highlighted that the TiPV caused significant CPE on OnlL cells, contributing to the pathogenesis in healthy Nile Tilapia. The immune gene expressions underline that TiPV modulates the host immune response due to its virulence. The study pointed out the need for the development of control measures for TiPV in Tilapia aquaculture.

Keywords: Tilapia Diseases, Fish Virus, *Parvoviridae*, Viral Pathogenesis, Aquaculture Virology

Comprehensive protocols to control the parasitic infection in Asian seabass broodstock fishes

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Diversification of species is important for holistic and sustainable aquaculture development. Asian seabass (*Lates calcarifer*) known as Bhetki/Barramundi, is an important high-value food fish for sustainable aquaculture. Supply of hatchery-produced, healthy, disease-free seed to farmers encourages them to adopt seabass finfish farming. When developing brood fish for Asian seabass in captivity, infections from parasites are seen, which can cause the loss of these fishes and make it harder to produce healthy seeds in the hatchery. Protocols followed to control the parasitic infection have been discussed in this paper. In the tank based broodstock holding system, parasites such as *Caligus sp., Argulus sp., Amyloodinium sp.*, and gill flukes were recorded. It is observed that infections of parasites persistently noticed peaks during two seasons annually, March to April (summer period) and during the October to November (monsoon period), when sudden increase and decrease of water temperature in these periods. Periodical treatment protocols were developed to control the parasitic infection by treating fishes with antiparasitic agents such as formalin, copper sulphate and praziquantel in different weather periods. Even though the treatment protocols were found to be effective in controlling these parasites, it is necessary to undertake monthly based prophylactic treatment to avoid recurrence and manipulation of infection. It is suggested to adopt strict quarantine and biosafety protocols in the fish hatchery to maintain pathogen free healthy broodfish for seed production.

Keywords: Asian seabass, *Caligus, Argulus*, Antiparasitic Agents

Project: Reliable seed production of brackiswater finfishes

Funding: ICAR-Central Institute of Brackishwater Aquaculture, India

Exploring key biomarkers during bacterial infection in freshwater Asian seabass, *Lates calcarifer*

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Biomarkers are measurable indicators for evaluating stress factors, analyzing immune responses, disease prognosis or contaminants of environmental concerns in animals. Immune genes can function as markers to record elevated immune status, classify diseases, and analyse effects of therapeutics. To understand the expression patterns and explore their reliability as biomarkers during bacterial infection, two immune genes, Lipopolysaccharide Binding Protein (LBP) gene that binds to bacterial lipopolysaccharide during microbial infection and High Mobility Group Box Protein Gene1 (HMGB1) known to induces cytokine production and inflammatory responses when exposed to microbial infections were studied in freshwater Asian seabass. Full length characterization, ORF prediction, 3D predicted protein structure and Ramachandran Plot of the predicted structure was documented for LBP and HMGB1 genes. Molecular docking of LBP and HMGB1 with Lipid A, a component of Lipopolysaccharide of pathogenic bacteria, proved that both the proteins indeed bind to the amphipathic Lipid A moiety of LPS. This might facilitate the process of LPS monomerization thus enhancing host immune response of host to bacterial endotoxin. Tissue-specific expression patterns of LBP and HMGB1 genes were studied in eight different tissues i.e. blood, gill, liver, kidney, spleen, heart, brain and intestine. When compared, the spleen showed significantly higher expression level whereas lowest abundance was observed in blood, liver and heart tissue. HMGB1 has been identified as a significantly upregulated immune gene during the initial stages of disease compared to LBP gene which has shown a constant high level of expression during infection. Thus, solidifying it as a candidate marker gene and reliable indicator for identifying the selected bacterial disease in freshwater Asian seabass. Analyzing immune gene expression patterns of these biomarkers can definitely provide early warnings of selected microbial diseases in freshwater farmed Asian seabass, allowing for timely intervention and improved management practices in the fisheries sector.

Keywords: Biomarkers, Immune Genes, Protein Structure Predictions

Project: Identification and comparative expression analysis of novel immune-related genes against prevalent bacterial infections and development of remedial measures in Asian seabass, *Lates calcarifer*

Funding: Department of Biotechnology, Government of India

Complex co-infections in aquaculture: Case study of bacterial and parasitic pathogens in Pangas and Koi carp

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Disease outbreaks are a major obstacle for the growth of aquaculture in India and around the world. In recent days, major concern is on co-infections involving multiple types of pathogens, including bacterial-bacterial, bacterial-parasitic, and bacterial-viral pathogen combinations. These mixed co-infections complicate host-pathogen interactions and increase the risk of misdiagnosis, which can hinder effective treatment strategies. Consequently, surveillance and accurate and early diagnosis using standardized protocols are essential for disease prevention and control in aquaculture systems of India. Under the National Surveillance Programme on Aquatic Animal Diseases, fish specimens of *Pangasianodon hypophthalmus* (Pangas) and *Cyprinus carpio* var. *koi* (Koi carp) were received from the Ludhiana (30°54′16.3″ N, 75°48′05.4″ E) and Jalandhar districts (31°19′10.5″N 75°35′56.6″E) of Punjab, India, through a passive surveillance approach. Immediately, the samples were processed for disease diagnosis through internationally accepted standards outlined by the World Organisation for Animal Health (OIE). At the preliminary diagnostic level (Level I), clinical signs such as hemorrhagic lesions, excessive mucus production, scale loss, bulging eyes (exophthalmia), and skin ulcers were noted. Microscopic examination revealed the presence of *Ichthyophthirius multifiliis* (the causative agent of white spot disease) in Pangas fish (in gill and skin tissues). However, no parasitic infections were detected in the koi carp samples. In addition, bacteriological analysis revealed the presence of *Aeromonas* species infection in fish samples (culturing tissue samples on Rimler Shotts agar). Followed by identification of bacterial species through MALDI-TOF mass spectrometry and through species-specific PCR assays. The present study results showed that co-infection of the parasite *I. multifiliis* with opportunistic bacteria, including *Aeromonas hydrophila* and *A. veronii*, was detected and identified in Pangas. Whereas in Koi carp, a combination of primary and secondary bacterial pathogens such as *Citrobacter freundii* and *A. veronii* were identified. The present study's observations highlight that the emergence of co-infections with different bacterial pathogenic organisms makes the complexity in understanding the host-pathogen interactions. It also emphasizes the potential risks posed to the ornamental and food fish industry. Effective biosecurity and early diagnostic interventions are crucial in managing the complex disease challenges that have emerged in the Indian aquaculture sector.

Keywords: Aquaculture, Disease, Co-infection, Parasite, Bacteria, Disease Complexity,

Disease Diagnosis

Project: National Surveillance Programme for Aquatic Animal Diseases Phase-II
Funding: Pradhan Mantri Matsya Sampada Yojana, Department of Fisheries, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India; National Fisheries Development Board (NFDB), India facilitated through the ICAR–National Bureau of Fish Genetic Resources (ICAR-NBFGR), India

Mechanistic insights into seaweed bioactive compounds mediated immunostimulation in tilapia challenged with *Aeromonas hydrophila*

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Aquaculture is increasingly threatened by bacterial pathogens, with *Aeromonas hydrophila* being one of the most virulent agents causing mass mortalities in tilapia (*Oreochromis mossambicus*). Therefore, there is a pressing need for eco-friendly, functional feed additives to enhance fish immunity and disease resistance. This study investigates the mechanistic basis of immunomodulation mediated by dietary supplementation of seaweed bioactive compounds (SBC) in tilapia challenged with *A. hydrophila*. Fish (24 ± 2.5 g) were fed separately with three diets, commercial diet (control), diet containing different concentrations of SBC for 4 weeks. Growth performance in term of final weight (FW) specific growth rate (SGR) and feed conversion ratio (FCR), immune parameters of total protein (TP), alkaline phosphatase (ALP), myeloperoxidase (MPO), lysozyme (LYZ), reactive oxygen species (ROS), reactive nitrogen species (RNS) and antioxidant parameters of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in serum and mucus were evaluated after 2nd and 4th weeks. The FW, SGR, and FCR of fish fed with SBE significantly improved ($p < 0.05$). The activities of ALP, LYZ and MPO in the mucus were significantly higher (p <0.05) in fish that fed SBC. The TP, ROS, RNS, SOD and GPx in the serum were significantly higher (p <0.05) in fish that fed SBC. In addition, the challenge test showed that fish fed SBC enhanced significantly (p< 0.05) the resistance against *A. hydrophila* (1 x 10^7 cells ml^{-1}). In conclusion, SBC can be applied in diet to improve health status and resistance against *A. hydrophila* in tilapia farming. The outcomes pave the way for incorporating marine-derived functional ingredients into tilapia diets for improved disease management in aquaculture systems.

Keywords: Seaweed, *Aeromonas hydrophila*, Immunity, Feed Additives

The immune response of Asian seabass (*Lates calcarifer*) and its susceptibility to bacterial infection at different salinity levels

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The Asian seabass (*Lates calcarifer*) is a commercially valuable euryhaline species known for its fast growth and high-quality meat. Fluctuations in salinity are a significant environmental factor that affects the health and physiology of fish. Although *L. calcarifer* can tolerate a broad range of salinities, abrupt salinity changes can adversely affect growth and survival. This study investigated the immunophysiological responses of juvenile *L. calcarifer* (mean length: 5 cm; weight: 2 ± 0.1 g) under combined salinity stress and bacterial infection. Initially, fish reared at optimal salinity (30 ppt) were exposed to a wide salinity range (5-60 ppt) for 96 hours to determine tolerance thresholds. Subsequently, fish maintained at four selected salinities (5, 15, 35, and 55 ppt) were subjected to bacterial infection via intraperitoneal injection, while control groups received saline injections. Sampling was conducted at 48 and 96 hours post-challenge to assess changes in the expression of osmoregulatory and immune-related genes. The results indicated significant changes in gene expression across salinity treatments, demonstrating salinity-dependent modulation of immune and osmoregulatory pathways during bacterial infection. Fish maintained at 35 ppt exhibited optimal expression patterns of immune and stress-related genes. In contrast, higher salinity (55 ppt) led to downregulation of immune genes, including the proinflammatory cytokine interleukin-1β (*IL-1β*), and upregulation of stress markers such as heat shock protein 70 (*hsp70*). These findings provide valuable insights into the immunophysiological limits of seabass under combined salinity and bacterial stress, supporting the development of effective salinity management strategies for improved health in variable aquaculture environments.

Keywords: *Asian seabass*, Salinity, Bacterial Infection, Immunity, Gene Expression

Project: Genome editing approaches for improving growth and reproduction of brackishwater teleosts and Indian white shrimp, *Penaeus indicus*

Funding: ICAR-Central Institute of Brackishwater Aquaculture, India

Impact of acute salinity fluctuations on host immunity and pathogen susceptibility in *Etroplus suratensis*

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Etroplus suratensis (Pearlspot) is an economically important euryhaline fish, native to the Indian backwaters. Although the fish can tolerate wide salinity fluctuations, abrupt changes in abiotic and biotic environmental factors can compromise the health, survival, and physiological stability of the species, especially under hatchery conditions. This study investigated the salinity tolerance and immune response of juvenile Pearlspot (60 days post-hatch; average weight 2.2 ± 0.4 g) exposed to varying salinities. Fishes reared at <1 ppt were directly transferred to different salinities (0, 5, 15, 25 and 35 ppt) and monitored for 96 hours to assess adaptive responses. Subsequently, the fishes were challenged with a lethal dose (LD_{50}) of *Vibrio* pathogen through intra-peritoneal injection and pathological outcomes were evaluated. Higher mortality rates were observed in fish maintained at 25 and 35 ppt. Molecular analysis of osmoregulatory and immune-related genes including osmotic stress transcription factor 1 (*ostf-1*), sodium-potassium ATPase α (Na^+/K^+-*ATPase* α), heat shock protein-70 (*hsp*70), catalase (*cat*), superoxide dismutase (SOD), nuclear factor kappa B p-105 (NF-kB p105), nuclear factor kappa B p-100 (NF-kB p100) and Interleukin-1β (*IL-1*β) revealed significant differential expression in the gills, liver, kidney, and intestine at elevated salinities. Histological examination of gill tissues indicated an increased number of chloride cells and constriction of blood channels in fish exposed to higher salinities. Behavioral changes such as erratic swimming and red coloration were also noted under hypersaline conditions. The study provides insight into the adaptive strategies of Pearlspot to salinity fluctuations and suggest lower salinities (hypoosmotic environments) are more suitable for rearing *E. suratensis*, as they support better physiological stability and immune competence, especially under stress and pathogen exposure.

Keywords: Bacterial Infection, *Etroplus suratensis*, Immune Response, Gene Expression, Salinity

Project: Genome editing approaches for improving growth and reproduction in brackishwater teleosts and Indian white shrimp *Penaeus indicus*

Funding: ICAR-Central Institute of Brackishwater Aquaculture, India

Efficacy of *Curcuma longa* on growth and haematological indices of *Labeo rohita* following experimental infection with *Aeromonas hydrophila*

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Health management is a key factor for the successful production of aquatic animals. During present study, pure cultures of the bacterial species *Aeromonas hydrophila* were administered to *Labeo rohita* fingerlings at three different concentrations: 10^7, 10^6 and 10^5 CFU /ml. Each bacterial concentration challenged group was further divided into three subgroups receiving *Curcuma longa* (0.2, 0.4, and 0.6%) mixed diet. A bacterial suspension (0.1 ml) was intraperitoneally injected into healthy fishes (5 No./replicate; 3 replicates/treatment). Two controls in triplicate were used: one with 0.1 ml of the bacteria and the other with 0.1 ml of PBS. After infection in *L. rohita*, the pathogenic bacterium was re-isolated on specific media and identified. The fingerlings, averaging a weight of 12 ± 2 g fed on *C. longa* supplemented diet for one month before the challenge study and continued on this diet until the experiment concluded. At all doses of intraperitoneal method of *A. hydrophila* infection, where *C. longa* treatment was not given, several symptoms of disease appeared like discolouration of body, scale loss and tail rot, red lesions on the ventral region and mouth which spread to whole body, accumulation of fluids in the abdomen (dropsy), increased mucus on body surface, and ulcer on *L. rohita* body. Specific growth rate (0.50, 0.47, 0.47%), biomass (100.57, 94.95, 93.71g), weight increase (18.96, 16.92, 15.83%) and length increase (6.08, 5.04, 4.83%) were significantly better at 0.6 percent to *C. longa* treatments in *A. hydrophilla* challenged *L. rohita* at dose of 1x 105, 106 and 10^7 CFU/ml, respectively. The groups treated with 0.4 and 0.6 percent *C. longa* had the highest survival rates. Haematological parameters, red blood cells (1.86, 1.89, 1.87x $10^6/\mu$l), haemoglobin (7.25, 7.21, 7.12 g /dL), packed cell volume (21.90, 20.37, 19.94 %) and white blood cells (78.56, 77.39, 74.09 x $10^3/\mu$l) were significantly higher in 0.6 percent C. longa treatment after intraperitoneal infections by *A. hydrophilla* at 1x 10^5, 10^6 and 10^7 CFU/ml, respectively.

Keywords: *Aeromonas hydrophila, Curcuma longa*, Growth, Haematological Parameters, *Labeo rohita*

Anti-infective potential of sulfated galactan against *V. harveyi* in *Lates calcarifer*

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The antibacterial and antibiofilm properties of sulfated galactan (Hd-SG) derived from the red seaweed *Halymenia dilatata* were evaluated against the fish pathogenic *Vibrio harveyi* using *in vitro* and *in vivo* methods. An agar well and disk diffusion assays demonstrated that Hd-SG inhibited *V. harveyi* growth in a dose-dependent manner, with the most significant inhibition zones observed at 500 μg/ml of Hd-SG. Additionally, the antibacterial efficacy of Hd-SG was obtained through colony counting and killing kinetics tests, which indicated complete suppression of *V. harveyi* growth at 500 μg/ml. The minimum inhibitory concentration (MIC) and minimum biofilm inhibitory concentration (MBIC) of Hd-SG (500 μg/ml) against *V. harveyi* were documented in 99.72% growth inhibition and a 94.97% decrease in biofilm formation. Confocal laser scanning microscopy analysis confirmed the antibiofilm properties of Hd-SG. *In vivo* toxicity assessments of Asian seabass revealed no mortality or abnormal clinical signs at Hd-SG concentrations up to 2.0%. Asian seabass infected with *V. harveyi* and treated with Hd-SG showed improved survival rates, growth performance, and water quality parameters compared to the untreated infected group. Haematological and antioxidant enzyme analyses suggested that Hd-SG offered protection against *V. harveyi* infection, as indicated by elevated levels of haemoglobin, red and white blood cells, packed cell volume, and antioxidant enzymes in the treated groups. Histopathological examination of the liver, gills, and intestinal tissues further corroborated the protective effect of Hd-SG, which displayed fewer pathological changes in the treated groups than in the infected group. These results highlight the potential of Hd-SG as a natural antibacterial and antibiofilm agent for controlling *V. harveyi* infections in aquaculture

Keywords: Seaweed, Antibiofilm, *V. harveyi*, Asian seabass, Haematology

Transcriptome mining and characterization of antimicrobial peptides in the Indian catfish *Clarias magur*

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The emergence of antibiotic-resistant pathogens has rendered conventional treatments increasingly ineffective. Antimicrobial peptides (AMPs) have emerged as promising candidates with their broad-spectrum activity and low likelihood of resistance development. Here, transcriptomic data of *Clarias magur* was mined to identify and characterise AMPs using a comprehensive *in silico* pipeline. The previously sequenced multi-tissue transcriptome (liver, muscle, kidney, gonad, and brain; Haldar, 2022) of *Clarias magur* comprised 52,237 contigs. Using TransDecoder 27,284 short open reading frames (10-100 amino acids) were predicted. A custom AMP database was created with AMP sequences from APD3, NCBI and UniProt for BLASTp analysis. Selected sequences were screened for the presence of AMP-like domains (HMMER) and signal peptide. Fifty-five AMP-like ORFs were further screened through ten machine learning-based AMP predictors, and 15 robust candidates were profiled for physicochemical properties. Three peptides AB_P5 (24 a.a.), AB_P16 (25 a.a.), and B_P3 (22 a.a.) had ideal amphipathicity, positive net charge, stability, low aggregation potential, and high identities with known AMPs (histone H2A-derived, hepcidin and chemokine-derived AMPs). Structural models were generated using AlphaFold, iTasser and Pepfold and validated in silico. Molecular docking was carried out in ClusPro with selected bacterial OMPs of *Escherichia coli, Pseudomonas aeruginosa, Mycobacterium tuberculosis, Bacillus subtilis, Staphylococcus aureus, Aeromonas hydrophila, Edwardsiella tarda*, and *Vibrio parahaemolyticus*. Peptide B_P3 had strong binding affinities and hydrogen bond interactions comparable to those of the established human AMP LL-37 used as a reference. Wet lab validation of B_P3 is being carried out against selected bacterial pathogens and will be presented. This study could lead to novel fish-derived AMPs for combating antimicrobial resistance.

Keywords: Bioactive Peptides, Anti-Bacterial Peptides, Alternative Therapy

Funding: ICAR-Central Institute of Fisheries Education, India

Decoding Physiological and immunological responses of critically endangered *Hypselobarbus pulchellus* to stocking density in biofloc systems

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Stocking density is a key operational parameter that governs fish physiology, immune status, and water quality dynamics in biofloc aquaculture systems. In this study, critically endangered *Hypselobarbus pulchellus* early fry were cultured under five stocking densities (50 to 150 fish/m^3) for 90 days. To decipher these complex biological interactions, multivariate analyses were applied. Treatment-level growth metrics, digestive and antioxidant enzyme activities, immune responses and water quality parameters were analyzed using multivariate statistical approaches. Principal Component Analysis (PCA) revealed clear separation among treatments, driven primarily by antioxidant biomarkers (SOD, CAT), digestive enzyme activity (amylase, protease, lipase), and nitrogenous waste concentrations (TAN, NO_2, NO_3). Lower densities (SD50) were associated with strong physiological profiles and immune (lysozyme) response, while higher densities (SD150) clustered with stress markers and deteriorated water quality. Heatmap visualization further emphasized density-dependent shifts, illustrating coherent patterns of enzyme suppression and oxidative imbalance under elevated stocking pressure. The integration of multivariate and heatmap analyses enabled clear identification of physiologically optimal stocking density. Densities between 100-125 fish/m^3 emerged as the threshold range balancing biomass output with immune and digestive health. These findings underscore the value of integrative multivariate approaches in resolving the complex interplay between density stress and functional health in biofloc aquaculture systems.

Keywords: Stocking Density, Biofloc, PCA, Critically Endangered, *Hypselobarbus pulchellus*
Project: Production performance of peninsular carps in biofloc based seed rearing system
Funding: Indian Council of Agricultural Research, India

Effect of sunlight, salinity and turbidity on the depleting efficacy of sodium hypochlorite and potassium permanganate

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Disinfectants are commonly employed in foot dips and hand dips across food processing plants and aquaculture farms to reduce the risk of cross-contamination. Over the time, the efficacy of disinfectants declines due to factors such as light, salinity, turbidity etc. The study evaluated the effect of sunlight, salinity and turbidity on the depletion rates of two commonly used disinfectants, potassium permanganate ($KMnO_4$) and sodium hypochlorite (NaOCl) under simulate field conditions. The study was conducted in sunlight and dark conditions with concentrations of the disinfectants at 12.5, 25, 50, 100 and 200 ppm, the salinity range of 10, 20, 30, and 40 ppt and the turbidity levels of 0, 50, 100, and 200 NTU. The study revealed as indicated by the half-life, that the depletion rates of both disinfectants significantly accelerated under sunlight (4-6 h) compared to dark (>24 h). Additionally, both salinity and turbidity were found to influence the stability of the disinfectants and a negative correlation was observed between disinfectant stability and increasing salinity levels, suggesting that higher salt concentrations may accelerate chemical breakdown or reduce disinfectant efficacy. Similarly, increased turbidity was also associated with faster depletion of the disinfectants, likely due to increased chemical demand and physical adsorption onto particulates. Based on the observed depletion patterns of potassium permanganate and sodium hypochlorite under varying environmental conditions, the study strongly recommends re-dosing disinfectants at appropriate intervals and concentrations to maintain their effective residual levels in hand dips and foot dips used in fish processing plants and aquaculture ponds.

Keywords: Disinfectants, Depletion, Potassium Permanganate, Sodium Hypochlorite, Aquaculture

Project: All India Network Project on Fish Health

Funding: Indian Council of Agricultural Research, India

Aquatic ecosystem stress leads fish disease occurrence: The hidden web in freshwater wetland

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Deterioration in aquatic environment health make the fish and other biotic community vulnerable to various biotic stress. Bacterial pathogen mediated infection in fish cause mortality and subsequent economic loss for wetland based community. Such changes are resulted from aquaculture activities, human disturbances, and seasonal variations. Further, the limited mitigation option in natural wetland during the disease occurrence has become another great concern. In this study, a natural wetland in West Bengal was examined during outbreaks of fish mortality throughout the year to identify disease-causing pathogens. Bacterial infections were confirmed using 16S rRNA amplification and sequencing techniques. Infected fish species included *Labeo rohita, L. catla, Cirrhinus mrigala, Hypophthalmichthys molitrix*, and *Puntius sophore*, which were found to carry *Aeromonas hydrophila, A. veronii*, and *Plesiomonas shigelloides*. To analyze patterns of disease occurrence water and sediment chemistry in different season throughout the year as well as agricultural practices surrounding the wetland were studied. Representative bacterial pathogens were also tested for their degree of virulence in the respective fish host and correlated with the disease signs and symptoms. Therefore, the study concludes that understanding disease patterns and maintaining the aquatic environment can help to prevent fish disease and associated economic losses. The concern of disease in fish and their transmission amongst wetland ecosystem needs suitable mitigation approach considering both biotic and abiotic mediated stressors.

Keywords: Wetland Ecosystem, Fish, Disease, Bacteria, Pathogen

Project: Introducing 'Ecosystem based integrated wetland management' in lower Gangetic Plains of West Bengal: A tool to empower women of fishermen community

Funding: Department of Biotechnology, Government of India

Enhancing the growth and health of *Etroplus suratensis* in tank system through the supplementation of fish waste hydrolysate

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Green chromide or pearlspot (*Etroplus suratensis*) is considered a delicacy in many regions. However, its slow growth rate remains a major challenge for fish farmers. In this study, Fish Waste Hydrolysate (FWH) derived from marine fish trimmings was used as a plankton booster to enhance the growth performance and health status of *E. suratensis* in an outdoor tank system. A 90-day trial was conducted with seven treatment groups, receiving FWH at varying concentrations: 0 ppm (control), 5 ppm, 10 ppm, 20 ppm, 40 ppm, 80 ppm, and 160 ppm. The results demonstrated that fish in treatments supplemented with 20 ppm FWH and above showed significantly improved growth performance including Specific Growth Rate (SGR), Percentage Weight Gain, Average Daily Gain (ADG), and Feed Conversion Ratio (FCR) compared to the control and lower doses ($p < 0.01$). The best growth performance was observed in the 40 ppm FWH treatment, where SGR, Percentage Weight Gain, and ADG were significantly higher ($p < 0.01$) than in all other treatments, including the control. Notably, this treatment resulted in a 40% increase in final body weight compared to the control group. Additionally, floc densities were significantly higher in all FWH-supplemented treatments compared to the control, with floc levels increasing proportionally with FWH concentrations up to 40 ppm. The abundance of both phytoplankton and zooplankton also increased with higher FWH doses, suggesting that enhanced plankton availability may have contributed to better floc formation and improved fish growth. Moreover, the various haematological parameters revealed better health status of fishes in the treatments supplemented with FWH as compared to the control. In conclusion, supplementation with 40 ppm FWH significantly enhances the growth and health of *E. suratensis* and could offer farmers a practical strategy to improve productivity and profitability.

Keywords: Pearlspot, Fish Waste, Circular Economy, Plankton Growth, Health Enhancement

Project: Sustainable aqua feed formulations and feeding approaches for improved growth and health

Funding: Indian Council of Agricultural Research, India

Evaluating DNA extraction protocols for fish gut microbiome studies

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The symbiotic relationship between gut microbiota and their fish hosts has fuelled extensive research into microbial distribution followed by their vital contributions to host metabolism, supporting sustainable aquaculture and the blue revolution to ensure food security. This study aims to optimize and evaluate DNA extraction techniques for characterizing the gut microbiota of fish with diverse feeding habits: Hilsa (planktivorous), Catla (zooplankton feeder), Rohu (herbivorous), and Mrigal (illiophagus). Microbial genomic DNA was extracted using five traditional methods, PLICKS A, B, C, and CTAB (Methods D and E) and three commercial kits (MN® Microbial, MN® Soil, and MN® Faecal), each with modifications. The efficacy of these methods was assessed based on DNA yield (traditional: 74-3070 ng/μL; commercial: 8.8-224 ng/μL), purity (traditional: A260/280: 1.38-1.92, A260/230: 1.03-2.21; commercial: A260/280: 1.30-3.25, A260/230: 0.5-2.0), and successful PCR amplification, a key step for downstream 16S rRNA gene sequencing. Among traditional methods, PLICKS A (Catla), PLICKS C (Hilsa), CTAB (Mrigal and Catla), and PLICKS B (Catla, Rohu, Hilsa, Mrigal) delivered the highest DNA recovery (342-2080 ng/μL) and purity across all species. Similarly, among commercial kits, the MN® Microbial Modified Kit (Catla, Hilsa), MN® Soil Kit (Hilsa), MN® Soil Modified Kit (Catla, Rohu), MN® Faecal Kit (Catla), and MN® Modified Faecal Kit excelled, achieving optimal DNA recovery (108-224 ng/μL) and purity across various feeding habits. These findings identify effective DNA extraction methods tailored to different fish feeding habits, laying a foundation for future metagenomic research in fisheries and aquaculture.

Keywords: Gut Microbiota, 16S rRNA, DNA Extraction, Metagenomics, Microbiome

Funding: ICAR- Central Institute of Fisheries Education, India

02

Technical Session II

Shrimp Health

Application of scientific knowledge to control EHP infection in shrimp

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Hepatopancreatic microsporidiosis (HPM) is a disease threat to the global shrimp aquaculture sector. The causative agent is an intracellular fungus named *Enterocytozoon hepatopenaei* (EHP). Shrimp with severe EHP infections results in growth retardation indicated by poor pond performance in terms of reduced average daily growth (ADG) and average body weight (ABW) together with high size variation, i.e., high % coefficient of variance (% CV) and weakness leading to secondary infections by opportunistic pathogens. As with other major shrimp diseases, prevention is still the best line of defense against EHP. The research around the world on EHP's mechanism of proliferation, transmission and interaction with its hosts resulted in more knowledge leading to the development of potential strategies and commercial products to control EHP infection in shrimp. In this talk, we summarize current knowledge of EHP infection and transmission dynamics and currently recommended practical control measures that are being applied to limit its negative impact on shrimp cultivation. We also point out the major gaps in knowledge and technologies as well as the urgent needs of strong collaborations among stakeholders at national and international levels to be bridged to improve control measures.

Keywords: *Enterocytozoon hepatopenaei* (EHP), Virulence Mechanism, Control and Prevention

Funding: National Science and Technology Development Agency, Thailand

Reverse genetics approaches in developing oral vaccines and therapeutics in fish and shrimp

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Viral diseases are a major cause of economic losses in shrimp and fish farming worldwide. While vaccination is available for some high-value fish species such as salmon and trout, oral vaccines remain limited. Unlike fish, shrimp lack adaptive immunity, making vaccination ineffective. Therefore, preventative measures remain as the cornerstone for mitigating disease outbreaks in shrimp aquaculture. We have developed an oral vaccine and therapeutic delivery platform to control fish and shrimp diseases using an engineered replication-deficient Nodavirus system.

The method involves expressing nodavirus like *Macrobrachium rosenbergii* nodavirus (MrNV) infecting shrimp and red grouper nervous necrosis virus (RGNNV) infecting fish using a baculovirus expression system and insect Sf9 cells. Infection of Sf9 cells with recombinant baculovirus carrying nodaviral genomes resulted in the expression of both baculovirus and nodavirus. Insect cell-derived MrNV was infectious to shrimp. The RdRp gene in MrNV was replaced with green fluorescent protein (GFP, MrNV$^{\Delta RdRp}$-GFP). Oral administration of MrNV$^{\Delta RdRp}$-GFP via commercial diet led to GFP expression in shrimp hemocytes. Subsequently, GFP was replaced with hairpin RNA targeting white spot syndrome virus (WSSV). Oral delivery of this construct significantly reduced WSSV load in challenged shrimp, highlighting its RNAi-based therapeutic potential and the need for further optimization. The availability of oral therapeutics will be immensely beneficial to control viral diseases in shrimp. Like MrNV, recombinant RGNNV produced in Sf9 cells was infectious to hybrid sea bass, inducing histopathological lesions characteristic of wild-type RGNNV. A replication-deficient RGNNV was then engineered by deleting the RNA-dependent RNA polymerase (RdRp) gene. When Sf9-derived biomass containing RdRp-deleted RGNNV was mixed with commercial diet and fed to hybrid sea bass, it elicited immune gene expression in liver, spleen and head kidney. This finding shows that engineered replication-deficient RGNNV has potential as oral vaccine against wild-type RGNNV in fish, with efficacy trials currently in progress.

Keywords: MrNV, WSSV, RGNNV, Shrimp, Oral Vaccine, RNAi

Project: A viral vector for an oral delivery of RNAi-based therapeutics in shrimp; Marketing, Trade, and Management of Aquaculture and Fishery Resources; Developing an oral vaccine platform for fin fish.

Funding: USDA NIFA & Tech Launch Arizona,-University of Arizona

White spot syndrome virus endogenous viral elements (EVE) revealed by circular viral copy DNA (cvcDNA) in shrimp

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Circular viral copy DNA (cvcDNA) can be used to identify endogenous viral elements (EVE) within the genomic DNA of shrimp. In this study, we isolated and sequenced cvcDNA from a breeding stock of whiteleg shrimp (*Penaeus vannamei*) that had been selectively bred from individuals that survived white spot disease outbreaks and tested free of white spot syndrome virus (WSSV). This stock is known for its high tolerance to WSSV. Genomic DNA from 10 shrimp was pooled for cvcDNA extraction, followed by high-throughput sequencing. The results revealed DNA fragments covering large portions of the ~300,000 bp WSSV genome. Notably, a high frequency of reads (high-frequency read fragments, HFRF) mapped to a concentrated region of approximately 1,400 bp. We hypothesized that this region may play a role in conferring WSSV tolerance. To investigate further, four PCR primer sets were designed to target the 1,365 bp region within the HFRF. Primer Set 1 spanned the entire 1,365 bp, while Sets 2-4 targeted shorter, overlapping regions within it. These primers were used to screen individual DNA samples from 36 shrimp from the same breeding stock, including the 10 used for cvcDNA extraction. PCR results varied among individuals, with some shrimp showing amplification from only one primer set, while others showed amplification from up to four sets. Only one shrimp produced an amplicon covering the full 1,365 bp region. In other cases, the positive amplicons represented both continuous and discontinuous regions, suggesting that the corresponding genomic sequences are not contiguous and may vary in insertion length. Importantly, none of the shrimp tested positive for active WSSV infection. These findings support the use of cvcDNA profiling to identify EVEs potentially associated with WSSV tolerance, which can inform future screening and selection of resilient breeding stocks.

Keywords: Endogenous Viral Element(s) (EVE), White Spot Syndrome Virus (WSSV), *Penaeus vannamei*, Shrimp Breeding Stock

Project: The investigation of viral accommodation mechanisms in shrimp to survive virus infection

Funding: National Science and Technology Development Agency, Thailand

Impact of hyperthermia on white spot syndrome virus replication and host response in Indian white shrimp (*Penaeus indicus*)

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White spot syndrome virus (WSSV) is one of the most virulent pathogens of cultured shrimp. Amongst abiotic factors the temperature is one of the most important environmental parameter factors influencing viral pathogenesis. Flow cytometry analysis was carried out to study host response of Penaeus indicus immune parameters (apoptosis, respiratory burst, cytoplasmic free calcium concentration (cf-Ca^{2+}), cell cycle analysis, and phagocytosis) against WSSV infection at different temperatures and time points. Reduced viral load was observed when the shrimps were exposed to higher temperatures (33 °C). At the host level, shrimp responded with elevated apoptosis rate and higher percentage of cf-Ca^{2+} activity. Phagocytosis analysis revealed that the maximum percentage of semi granulocytes at 33 °C and at late stage of WSSV infection. Hyperthermia also resulted in WSSV infected shrimps to respond with an increased respiratory burst activity, deregulated cell cycle and reduced viral load. The flow cytometric based immune analysis of WSSV infected shrimps suggests hyperthermia induce host defense reaction and inhibit WSSV replication.

Keywords: *Penaeus indicus*, White Spot Syndrome Virus, Flow Cytometry, Temperature

Funding: Indian Council of Agricultural Research, India

Evidence of DIV1-ATPase PCR-positive decapods in the absence of pathognomonic lesions associated with DIV1 infection

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Decapod iridescent virus 1 (DIV1) is an emerging pathogen first reported in China. Infection with DIV1 resulted in mortality of shrimp in the pond. Recent study aims to use the PCR method for DIV1 detection recommended by the World Organization of Animal Health (WOAH) for a DIV1 surveillance program in the whiteleg shrimp, *Penaeus vannamei* and the giant river prawn, *Macrobrachium rosenbergii* in Thailand. We found 66.0% of shrimp and 98.5% of prawn to be ATPase-PCR positive but they were grossly normal, showing no signs of disease during cultivation. In addition, histological analysis revealed no pathognomonic DIV1 lesions in the hematopoietic tissue (HPT), the target tissue of DIV1 in both species and no typical accompanying lesions in the lymphoid organ (LO) of *P. vannamei*. Absence of DIV1 was also confirmed by negative *in situ* hybridization ISH results in the cytoplasm of cells of the HPT and LO. However, ISH signals were instead detected in the nuclei of epithelial cells in the gill and hepatopancreatic tubules. Altogether, these results suggest an existence of DIV1 viral inserts in shrimp genomes as endogenous viral elements (EVE) in both *P. vannamei* and *M. rosenbergii*. It is urgent to revise current DIV1 detection methods to avoid trade restrictions resulting from false-positive PCR results.

Keywords: Decapod iridescent virus 1 (DIV1), *Penaeus vannamei*, ATPase gene, PCR detection, False positive

Funding: National Research Council of Thailand (NRCT): High-Potential Research Team Grant Program

The impact of immunomodulators on gut microbiome of pacific white shrimp, *Litopenaeus vannamei*

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Pacific white shrimp, *Litopenaeus vannamei* a most common species cultured around the Asian countries. Even though it's not a native species of India, it is the most cultured species all over the country due to its adaptability to extreme conditions and comparatively good tolerance to many diseases. But despite all its advantages and qualities, disease occurrence is inevitable. Underdeveloped immunity of shrimp is the root cause of many existing and emerging diseases. Use of antibiotics and chemicals were strongly discouraged due to residual and antimicrobial resistance aspect. Characterization and subsequent manipulation of the gut microbial community are becoming popular in aquaculture research. As productivity is intimately related to health and the gut microbiome, manipulating gut microbiota to produce healthy populations in aquaculture is suggested. The use of probiotics and other immunomodulators have also been found to improve the gut health ultimately resulting in enhancing the host immune system.

In the present study, different immunomodulators such as Probiotic - *Bacillus subtilis*, Prebiotic - Inulin and Immunostimulant - β glucan were coated onto feed individually and over a combination of T1 (prebiotic & probiotic), T2 (prebiotic & immunostimulant), T3 (Immunostimulant & probiotic), T4 (prebiotic, probiotic and immunostimulant) along with control group (regular feed). After a course of 30 days feeding trial, all the gut samples from all the groups were processed for metagenome studies. The gut microbiome data revealed that all the immunomodulators enhance the microbial diversity within the gut of the animals, and out of all the combinations T1, which is probiotic and prebiotic combination given in a certain concentration to shrimp gave comparatively higher abundance of beneficial bacterial community and reduction of certain pathogenic community in the gut.

Keywords: Shrimp, Metagenome, Immunomodulators, Probiotic, Prebiotic, Immunostimulant

Project: Novel approaches for disease free health certification in finfish and development of high health shrimp for sustainable aquaculture

Funding: ICAR-NASF

Testing of modified diets for recovery of *Penaeus vannamei* hepatopancreatic tissue post starvation

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We have established a method to evaluate and compare dietary efficacy on shrimp health performance using a starvation protocol. The experiment consists of two phases: an initial starvation period followed by recovery through re-feeding with test diets. In this study, shrimp underwent a 21-day starvation period. Mortality in the starved group was first detected on day 14, reaching an average cumulative rate of approximately 25% by day 21. There was no mortality observed in the non-starved group. A statistically significant weight difference was observed between starved shrimp (2.63 ± 0.56 g) and non-starved shrimp (3.33 ± 0.79 g) (p = 0.01). Hepatopancreatic (HP) cell deterioration was first detected on day 7 of starvation, characterised by thin and collapsed epithelial tubules. By the end of the starvation period (day 21), all shrimp in the starved group exhibited severe muscle atrophy (100% prevalence). During the re-feeding phase, shrimp fed with Feed A demonstrated superior performance compared to those fed Feed B, including higher survival rates and faster hepatopancreatic recovery. Additionally, shrimp fed Feed A showed lower feed conversion ratios (FCR) post-recovery, indicating greater efficacy of Feed A during the shrimp recovery process.

Keywords: Shrimp, Starvation, Re-feeding, Hepatopancreatic Recovery, Feed Efficacy

Funding: INVE Aquaculture

Comparative studies for susceptibility and growth performance of three crustacean species infected with *Enterocytozoon hepatopenaei* (EHP)

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This study investigated the impact of *Enterocytozoon hepatopenaei* (EHP) infection on growth performance and EHP replication levels in three shrimp species: *Penaeus vannamei*, Penaeus monodon, and *Macrobrachium rosenbergii*. Shrimp (2 g body weight) were experimentally infected via oral intubation of EHP spores (1×10^5 spores/shrimp) and reared for 30 days. Growth performance indicators, including weight gain, average daily gain (ADG), and feed conversion ratio (FCR), were evaluated. Concurrently, EHP infection level was quantified by quantitative PCR (qPCR) on days 0, 10, 20, and 30 post-infection. Results demonstrated that *P. vannamei* exhibited significantly higher EHP loads and more severe growth suppression compared to the other two species ($P < 0.05$). Both *P. monodon* and *M. rosenbergii* showed significantly lower EHP replication levels and better growth performance under identical conditions. These findings suggest species-specific susceptibility to EHP, which has important implications for disease management strategies in shrimp aquaculture.

Keywords: *Enterocytozoon hepatopenaei*, *Penaeus vannamei*, *Penaeus monodon*, *Macrobrachium rosenbergii*, Growth Performance, EHP Infection

Interactive effects of ammonia and salinity stress on immune profile and white spot syndrome virus susceptibility in *Penaeus vannamei*

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The knowledge about the combined action of environmental stressors on shrimp health is limited. The current study investigated the effect of stressors, salinity (S1-3, S2-20, S3-35 ppt), ammonia as total ammonia nitrogen (TAN1, TAN3, TAN6 mg/L), and SxTAN interaction (TAN1S1, TAN1S2, TAN1S3; TAN3S1, TAN3S2, TAN3S3; TAN6S1, TAN6S2, TAN6S3), and control (S-15; TAN-0.011) on *Penaeus vannamei* (8.1±1.4g) survival, immunological and biochemical parameters for two weeks in triplicate. Shrimp haemolymph was assayed for total haemocyte count (THC), phenol oxidase (PO), total superoxide dismutase (SOD), glucose and lactate on 1st, 3rd, 7th and 14th day of experiment as a measure of stress. After completion of stressors exposure experiment, the remaining shrimps in respective treatments were pooled and challenged with white spot syndrome virus (WSSV), and survival was recorded for 10 days. Cox proportional hazard (CPH) model results expressed as Exp (β) showed a risk factor of about 1-5, 2-4 and 6-29 times in TAN, salinity and combined treatments, respectively compared to control indicating high survival rate in individual treatments. Unionized ammonia increased ($p \leq 0.05$) from TAN1 to TAN6 and decreased with increase in salinity among the combined treatments. Hemolymph osmolality increased with increasing TAN and salinity irrespective of exposure time. THC, PO and SOD decreased with increasing TAN ($p \leq 0.05$) and decreasing salinity, and combined treatments reported significantly less immunological activity. An increase in lactate and decrease in glucose concentration was observed from day-1 to day-14 in combined compared to individual treatments. Post-challenge WSSV shrimp survival results showed a median survival (days) of 4 to 5, 5 to 5.5 and 2.5 to 4 in salinity, TAN and SxTAN treatments, respectively. The CPH i.e., the risk of dying was 1-11 times in SxTAN compared to 1-4 times in individual treatments indicates that shrimp exposed to combination of stressors are more susceptible to WSSV.

Keywords: Cox proportional hazard, Lactate, Pacific white shrimp, Superoxide dismutase, WSSV

Project: National Innovations in Climate Resilient Agriculture (NICRA)

Funding: Indian Council of Agricultural Research, India

The white spot syndrome virus (WSSV) transmission from blood cockle (*Tegilarca granosa*) to Pacific white shrimp (*Litopenaeus vannamei*)

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The White Spot Syndrome Virus (WSSV) is the most devastating pathogen of farmed shrimp in the world and infects a broad range of benthic invertebrates. Blood cockle (*Tegilarca granosa*) is a common bivalve found in the shrimp ponds environment and frequently co cultured with shrimp. In previous study we detected WSSV in farmed blood cockles but no information on the transmission status. The objective of current study was to determine the transmission of WSSV from Blood cockles to pacific white shrimp through feeding route. The experiment was conducted in laboratory, consisted of three treatments: Treatment 1 (commercial feed), treatment 2 (naturally WSSV-infected shrimp) and treatment 3 (naturally WSSV-infected blood cockles) each was applied in quad triplicate with the ration of 3% of shrimp's total body weight. The experimental feeding transmission was conducted for seven days and continued with observation for the next 2 weeks during which period the shrimp were fed commercial pellet. The occurrence of WSSV was detected with nested PCR. The result showed that WSSV was transmitted from blood cockle to shrimp. Prevalence of WSSV occurrence was 58,97 % in shrimp fed with blood cockles (treatment 2), compared to 100 % in shrimp fed with infected shrimp (treatment 3) and 0% of shrimp fed with pellet. Survival rate for each treatment was 100 (Treatment 1), 75% (Treatment 2) and 5 % (Treatment 3). Results of histopathology analysis are consistent with that of PCR and survival rate. Pathological changes observed were similar between shrimp in treatment 2 and treatment 3 included necrosis in the gills and delayed clotting time of the haemolymph. This study gave conclusive result that WSSV transmitted from naturally infected blood cockle to healthy shrimp upon feeding and caused light infection. This study highlights the potential for mitigating such transmission in polyculture system.

Keywords: Polyculture, Brackishwater pond, Shrimp

Project: Investigation of WSSV transmission from blood cockle to shrimp

Funding: Indonesia Ministry of Education

Genomic characterization of *Vibrio parahaemolyticus* causing translucent post-larvae disease (TPD) in *Penaeus vannamei*

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Translucent post-larvae disease (TPD) is a new emerging disease-causing massive mortality in shrimp at the larval stage. A highly virulent strain of *Vibrio parahaemolyticus* is reported to be associated with TPD (Vp_{TPD}). Although the protein sequences and nucleotide sequences encoding the TPD-causing toxins have been deposited in the GenBank, no comprehensive genomic studies are yet to be carried out. This study characterized the whole genome sequence of *V. parahaemolyticus* strain causing TPD (Vp_{TPD}). The whole-genome sequence of VpTPD was ∼5.5 Mb, consisting of two chromosomes and three plasmids. One of the three plasmids encodes 3 (VHVP) proteins, causing TPD in shrimp. Genomic characterization revealed that VHVP is a Tc-like toxin complex. Laboratory bioassay conducted using Vp_{TPD} revealed that bacterial isolate used in this study cause mortality at the late stage of post larvae (PL10-PL20). The findings broaden our understanding of the pathogenicity of the Vp_{TPD} strain and diagnosis of TPD.

Keywords: Translucent Post Larvae Disease, Whole Genome Sequence, VpTPD

Unravelling the pathogenesis mechanism of Wenzhou Shrimp Virus 8 (WzSV8)

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Wenzhou shrimp virus 8 (WzSV-8) is a novel positive-sense single-stranded RNA virus classified under the order Nidovirales, identified through metagenomic analysis of farmed shrimp. The primary target for this virus is hepatopancreas. So far, the direct association of this pathogen with shrimp mortality has not been reported. However, it is assumed that WzSV8 may play a potential role as co-infections and contribute to subclinical impacts on the physiology of shrimp. To study this, healthy shrimps were infected using Wenzhou viral inoculum. Subsequently, these shrimps were infected with EHP by horizontal transmission. Status of co-infection were confirmed by molecular methods and pathogen loads were quantified. Subsequently, the co-infected animals reared in different salinity stress environments. At the end of the experimental periods, samples were taken for immune gene expression and histopathology for comparison among control, single pathogen infection and co-infection animals. This research provides foundational insights into pathogen interactions in shrimp and their implications for disease control and farm-level biosecurity.

Keywords: EHP, WzSV-8, Co-infection, qPCR, Histopathology.

Project: National surveillence project on aquatic animal diseases, Phase II

Funding: National Fisheries Development Board, India

Effect of silymarin compound as hepatoprotectant in shrimp against microsporidia

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Ecytonucleospora hepatopenaei (EHP) is a microsporidian pathogen primarily affecting the hepatopancreas of cultured shrimp leading to tubular damage, dysfunction and reduced growth. Silymarin a phyto-extract of *Silybum marianum* known as milk thistle was fed prophylactically as hepatoprotectant to prevent damage of hepatopancreatic (HP) cells by EHP and to enhance rejuvenation of HP tubules in shrimp. The powdered silymarin derivative mixed in four different concentrations with commercial feed and soya-based binder was used for the experimental feed trial. Shrimps (150 nos.) free of EHP and other pathogens were distributed in 200 litre FRP tanks holding 15 nos./tank were acclimatized to the experimental conditions with water salinity of 25±1 ppt and temperature ranging from 28 to 30 °C with continuous aeration and 70% water exchange. Treatment and control groups of silymarin supplementation in feed 0.01%, 0.02%, 0.05%, 0.10% and 0.00% in duplicates were fed four times daily with 6% of total biomass for each tank. The shrimps in all treatment groups were fed for twenty days as prophylactic supplemented feed and body weight gain recorded at regular intervals before the *per os* challenge with EHP. Gross clinical signs were observed, tissue samples for PCR analysis, immune gene profiling and histopathological studies were collected from challenged groups at every ten days post challenge period of thirty days. All the groups were positive for EHP from fifth day of post infection from the faecal matter collected for quantitative PCR. There was no significant difference in the weight gain in the treatment and control group. The immune gene expression for lysozyme, SOD, penaeidin, prophenol oxidase and crustin and histopathological studies of HP tubules were analyzed, the study revealed that silymarin derivative supplementation in feed was found to be a hepatoprotectant at the concentration of 0.02% and above against the damages caused by EHP infection in shrimp.

Keywords: EHP, *Ecytonucleospora hepatopenaei*, Silymarin, Shrimp

Project: Interaction of *Enterocytozoon hepatopenaei* and *Vibrio* spp. in disease outcome and their therapeutics

Funding: Indian Council of Agricultural Research, India

Phytobiotic intervention for the effective control of *Ecytonucleospora hepatopenaei* (EHP) in shrimp aquaculture

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Ecytonucleospora hepatopenaei (EHP), a shrimp microsporidian pathogen, causes the disease hepatopancreatic microsporidiosis. The disease leads to severe growth retardation and is associated with white feces syndrome (WFS) resulting in severe production losses. The annual economic loss due to EHP in Indian shrimp farms was estimated to be around Rs 4800 crores. However, the studies on prophylaxis and therapeutics remain very limited. In this study, 'CIBA EHP CURA gro ^{plus}' a phytobiotic formulation has been developed for the treatment and control of EHP. This unique blend includes a bioactive phytochemical, a hepatoprotective nutraceutical, an antioxidant, and fatty acids exhibiting anti-microsporidian, antimicrobial, immune modulating, and anti-inflammatory properties. The phytochemical component completely inhibited the EHP spore germination *in vitro* and exhibited strong binding to multiple targets of EHP such as spore wall protein (SWP), ATP/ADP carrier, protein kinase, and endochitinase *in silico*. In laboratory experiments, this formulation significantly reduced the EHP proliferation and improved the immunity, growth, and survival of shrimp. Subsequently, the product underwent extensive field validation in the shrimp farms of Tamil Nadu, Andhra Pradesh, West Bengal, Gujarat, and Punjab of India. The product has been commercialized to four private enterprises for commercialization. As a safe and environmentally friendly phytobiotic, this formulation offers a promising solution for the effective control of EHP.

Keywords: *Ecytonucleospora hepatopenaei* (EHP), CIBA EHP CURA gro plus, Phytobiotic, Therepeutictobiotic.

Funding: Indian Council of Agricultural Research, India

Vibrio spp., associated with white feces syndrome in cultured *P. vannamei* and evaluating the anti-*Vibrio* activity of probiotics

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Vibrio species are natural inhabitants of brackishwater and includes several pathogenic species that are harmful to cultured shrimps. The present study investigated the incidence of *Vibrio parahaemolyticus* associated with White Feces Syndrome (WFS) affected cultured *Penaeus vannamei* and evaluated the anti-Vibrio potential of probiotics. Real-time PCR detected the presence of *Enterocytozoon hepatopenaei* (EHP) infection in the WFS affected shrimp with a Cq value of 2.84. A total of 78 suspected *Vibrio* spp., were isolated from the WFS affected shrimp which were tested with triplex PCR for the identification of *Vibrio* spp., *V. parahaemolyticus* and further, species level identification was performed using 16S rRNA sequencing. The results revealed that *V. parahaemolyticus* accounted for 79.3% while *Shewanella* spp., comprised 20.7% of the total isolates. Antimicrobial resistance profiling of the *Vibrio* isolates showed relatively higher resistance towards ampicillin (83.7%), cefoxitin (36%) and cefotaxime (24.4%) but showed 100% sensitivity to tetracycline. Different categories of probiotics viz., commercial probiotics (n=5), on-farm formulated probiotics (n=2) and millet-based home-made fermented probiotic (n=1) were tested, both as whole probiotics (WP) form and as individual probiotic strains (n=9) against 58 *Vibrio* spp. Results revealed that individual probiotic strains showed 4.7 times superior efficacy, and their cell-free supernatants (postbiotics) showed 11.9 times greater efficacy compared to whole probiotics. Pertinently, 22% of the probiotics showed multidrug resistance and one individual probiotic strain harboured *dfrA* and *sul1* genes suggesting the need for cautious formulation to prevent unintended dissemination of resistance. The results of this study imply that vigilantly formulated probiotics can be used as an alternative to antibiotics in shrimp aquaculture against *Vibrio* spp., associated with WFS.

Keywords: Probiotics, Aquaculture, *Vibrio parahaemolyticus*, Antimicrobial Resistance, White Feces Syndrome

Project: Diagnostic development of important pathogens, emerging AMR and other pathogens in aquatic environment and seafood

Funding: Indian Council of Agricultural Research, India

Experimental investigation of *Artemia franciscana* in the transmission dynamics of *Enterocytozoon hepatopenaei*

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Ectyonucleospora hepatopenaei (EHP) is an emerging microsporidian pathogen of significant concern in shrimp aquaculture, responsible for hepatopancreatic microsporidiosis in *Penaeus vannamei*. The use of live feeds such as *Artemia franciscana* in shrimp hatcheries has raised questions regarding their role as potential vectors for pathogen transmission. This study investigated the host susceptibility and vector potential of *A. franciscana* for EHP under controlled laboratory conditions. Three developmental stages of *A. franciscana* (Instar I, pre-adult, and adult) were exposed to EHP spores through immersion challenge. Post-exposure sampling was performed at regular intervals and analysed using PCR and histopathology to assess the presence and persistence of EHP spores. Early post-challenge PCR results indicated transient EHP positivity, likely due to surface adherence or ingestion of spores. However, all life stages, including nauplii produced from exposed adults, tested negative by day 16 post-infection, suggesting *A. franciscana* is not a true biological host. To evaluate vector potential, EHP-challenged artemia were fed to EHP-free *P. vannamei* post-larvae (PL-11). Subsequent PCR testing confirmed EHP positivity in shrimp, indicating passive transmission via contaminated artemia. These findings confirm that *A. franciscana* cannot be a host for EHP but may act as a passive vector. The study emphasizes the importance of screening live feeds and supports the need for Specific Pathogen-Free (SPF) artemia production systems to mitigate pathogen introduction risks and enhance hatchery biosecurity.

Keywords: Live Feeds, Vector Potential, *P. vannamei*, Biosecurity

Differential alternative splicing events under salinity stress reveal disease vulnerability in *Penaeus indicus*

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Alternative splicing (AS) is an important post-transcriptional mechanism to produce different mRNA transcripts and has a role in cell differentiation, development, and disease. The mRNA transcripts thus produced get translated into different proteins with distinct structures and functions. Though the recent advances in transcriptomics made it possible to rapidly study the alternative splicing mechanisms, it is still less understood in crustaceans with regard to biotic and abiotic stress conditions. Here, we conducted an experiment to understand the AS mechanisms in *Penaeus indicus* under three salinity regimes. Shrimp grown in control salinities (30ppt) were acclimatised to high (45 ppt) and low (5 ppt) salinities in an indoor flow-through system. Once the desired salinities were achieved, the experiment was continued for 21 days. At the end of the experiment, RNAseq data from hepatopancreas tissues were generated, quality-trimmed, aligned to the reference genome. The aligned BAM files were subjected to detect and quantify the differences in splicing patterns between the two salinity stress conditions with rMATS (replicate Multivariate Analysis of Transcript Splicing) tool. A total of 324 significantly differential spliced genes under low salinity and 254 genes under high salinity were identified. Pathway enrichment revealed that the low salinity stress triggered complex metabolic reprogramming that demands energy expenditure, involving 81 unique pathways, including increased glycolysis, steroid biosynthesis, and neurotransmitter signaling. In contrast, high salinity stress activated only 26 unique pathways that were mainly linked to innate immune responses like NOD-like receptors and TNF signaling. GO enrichment analysis showed that low salinity stress boosted autophagy and membrane remodeling, while the high salinity stress disrupted reproductive processes and mitochondrial function. Pathway changes observed with AS events indicated a weakened animal's ability to cope up with additional challenges due to abiotic stresses and make them vulnerable to diseases.

Keywords: Alternative Splicing, *Penaeus indicus*, Abiotic Stress, Pathway Enrichment
Project: Network Project on Agricultural Bioinformatics and Computational Biology
Funding: Indian Council of Agricultural Research

Metagenomic profiling reveals severe gut microbiota disruption in WSSV infected wild Indian white shrimp compared to healthy *Penaeus indicus* counterpart

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Shrimp aquaculture faces increasing threats from viral infections, which not only impair animal health but also disturb the intestinal microbial ecosystem. The gut microbiota plays a pivotal role in digestion, immunity, and overall physiological resilience. Disruptions in this microbial balance termed dysbiosis can exacerbate disease progression and reduces growth performance. This study investigates the compositional changes in the gut microbiota of wild-caught *Penaeus indicus* compared to WSSV infected shrimps, aiming to identify microbial biomarkers and potential probiotic targets. Wild brooders were procured from Kakinada, Andhra Pradesh and screened for viral pathogen WSSV employing nested PCR amplification of VP28 and grouped the animals into WSSV-Positive (PSG), and WSSV-Negative (NSG), 16S rRNA metagenomic profiling was performed on pooled gut samples using Illumina Platform, Operational Taxonomic Units (OTU) were compared between groups. Relative abundance, diversity trends, and differential presence of microbial taxa were analyzed using QIIME2 and visualized by R Studio. The gut microbiota of NSG wild shrimp was dominated by diverse and potentially beneficial genera such as *Enhydrobacter, Acinetobacter, Dialister* and *Bifidobacterium*, which were substantially reduced or completely absent in PSG shrimp. In contrast, PSG shrimp exhibited a drastic over-representation of *Photobacterium* (88,100 OTUs vs. 556 in NSG), along with elevated levels of *Vibrionaceae, Escherichia/Shigella,* and *Allostreptomyces*, suggesting severe microbial imbalance. Several probiotic-associated genera, including *Lactobacillus, Faecalibacterium*, and *Ruminococcaceae*, were notably flourished in NSG shrimp. These shifts indicate a collapse in microbial homeostasis and proliferation of pathogenic taxa in response to viral stress. This study reveals that viral infection in *P. indicus* (PSG) leads to significant gut microbial dysbiosis, characterized by a loss of beneficial commensals and a bloom of potential pathogens like *Photobacterium*. These findings underscore the importance of maintaining gut microbial balance for shrimp health and propose microbial biomarkers and probiotic candidates for future disease mitigation strategies in aquaculture.

Keywords: Gut microbiota, *Penaeus indicus*, Photobacterium, WSSV, Metagenomics

Project: Development of indigenous shrimp (Indian white shrimp) Aquaculture: Genetic improvement program of *Penaeus indicus*

Funding: Pradhan Mantri Matsya Sampada Yojana, Government of India

Advancing shrimp aquaculture biosecurity: Efficacy of SANGUARD V against *Vibrio*, WSSV, and EHP

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Shrimp aquaculture faces major threats from pathogens like *Vibrio* spp., White Spot Syndrome Virus (WSSV), and *Enterocytozoon hepatopenaei* (EHP), resulting in high mortality rates and annual losses of approximately 0.21 million tons, valued at USD 1.02 billion. To address these challenges, a disinfectant product formulated with Triple Salt and Sodium Dichloro isocyanurate (NaDCC) was developed and evaluated for its efficacy under various conditions. Suspension and surface tests demonstrated complete microbial elimination, reducing pathogen loads from (10^6) CFU to NIL. The Minimum Inhibitory Concentration (MIC) test confirmed that a 10% concentration effectively inhibited microbial growth from (10^8) CFU to NIL. In antiviral plaque reduction assays, the product reduced viral load from 4.51 $TCID_{50}$ to 2.152 $TCID_{50}$ within five minutes, indicating rapid antiviral action. Anti-EHP activity was validated through RT-PCR analysis of hepatopancreas tissues in infected shrimp, showing EHP presence at 1 µL/L and 2 µL/L, but complete absence from 4 µL/L onward. Additionally, a plankton crash assay confirmed ecological safety, with no adverse effects observed 24 hours post-application. The product's mode of action involves disruption of microbial cell walls, penetration into DNA, denaturation of membrane proteins, and inactivation of nucleic acids, leading to rapid cell lysis and microbial death. These mechanisms contribute to significant pathogen load reduction and effective control of Vibrio, WSSV, and EHP across all shrimp life stages. SANGUARD V demonstrates broad-spectrum antimicrobial, antiviral, and anti-parasitic efficacy, making it a reliable disinfectant for shrimp aquaculture. Its rapid action, ecological safety, and comprehensive pond protection support enhanced biosecurity and sustainable, disease-free production.

Keywords: Shrimp, Disinfectant, Disease, Aquaculture

Project: Advancing shrimp aquaculture biosecurity: Efficacy of SANGUARD V against *Vibrio*, WSSV, and EHP

Funding: Sandhya Marines Ltd.

Effect of calcium hypochlorite on inactivation of white spot syndrome virus in shrimp culture ponds of varying soil texture

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Application of bleaching agents during pond preparation is the common practice of disinfection as part of better management practices in shrimp farming. The bleaching requirement of the soil varies with the soil properties such as texture, pH and organic matter content. With the objective optimizing the dose, three different dose of calcium hypochlorite (10, 20 and 30 ppm Cl) was applied to 4 kg pond soil of varying texture namely loamy sand, clay loam and silty clay in 50 L tanks. Prior to bleaching, soils were inoculated with white spot syndrome virus (WSSV) @10^7copies/ml and 10 ml of inoculum was added for each tank. The residual chlorine content in water of the treatment received 30 ppm chlorine was 1.8, 2.7 and 3.8 ppm in silty clay, clay loam and loamy sand soils, respectively. The challenge study with *Penaeus vannamei* (size of 1.9 to 2.0 g) @ 10 animals per tank showed 100% mortality in all the soils in control and 10 ppm Cl treated clay loam and silty clay soils. Chlorine requirement for WSSV inactivation was 10 ppm for loamy sand and 20 ppm for clay loam and silty clay soils. The study indicated that chlorine demand of fine textured soil was higher than that of coarse textured soil and require higher dose of calcium hypochlorite for WSSV inactivation.

Keywords: Bleaching powder, Soil property, Chlorine demand, WSSV, *Penaeus vannamei*

Project: Abiotic stress management for enhanced productivity and environmentally sustainable shrimp farming

Funding: ICAR- Central Institute of Brackishwater Aquaculture, India

Marine macroplastics as vectors of white spot syndrome virus and other pathogenic bacteria: A metagenomic perspective

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Marine macroplastics have emerged as persistent anthropogenic pollutants that not only alter coastal ecosystems but also serve as novel substrates for microbial colonization. This study utilized high-resolution shotgun metagenomic sequencing to investigate the potential role of macroplastics as reservoirs and vectors of White Spot Syndrome Virus (WSSV), a major viral pathogen impacting global shrimp aquaculture. Biofilm samples were collected from macroplastic debris and organic matter across four locations in cochin cost of Arabian sea and subjected to Illumina HiSeq-based sequencing, generating between 108-146 million high-quality paired-end reads per sample (16.3-22 Gb), with Q30 scores above 88.6% and average base quality exceeding 35. Comparative metagenomic analyses revealed substantially elevated microbial biomass and taxonomic diversity in plastic-associated biofilms compared to those on organic substrates. Notably, sequences corresponding to WSSV were detected exclusively in plastic-derived metagenomes. Out of 92,652 processed reads, 6,044 reads (6.52%) were assigned to White Spot Syndrome Virus (WSSV) highlighting its presence exclusively in plastic-derived metagenomes. Functional annotation further identified 132 unique virulence genes and 64 antimicrobial resistance (AMR) determinants predominantly efflux pumps and beta-lactamases within the plastic biofilms. These findings suggest that marine macroplastics may act as passive carriers of viral pathogens, contributing to horizontal transmission of WSSV in natural ecosystems. Future work will examine the adsorption kinetics and surface-binding characteristics of WSSV across different plastic polymers.

Keywords: Macroplastics, Shotgun Metagenomics, White Spot Syndrome Virus, Biofilm

Project: NSPAAD-PMMSY

Funding: Pradhan Mantri Matsya Sampada Yojana, Government of India; National Fisheries Development Board, India

Optimization of indoor low-salinity (28–30 ppt) culture systems for commercial-scale production of artemia biomass and cysts as live feed for aquatic hatcheries

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This study presents the optimization of an indoor low-salinity (28-30 ppt) raceway flow-through culture system for the commercial-scale production of artemia biomass and cysts as live feed for aquatic hatcheries. The system was implemented in multiple 10 m^3 cement tanks, operating under controlled conditions including salinity (28-30 ppt), DO (3.5-5 ppm), and a regulated photoperiod of 8L:16D. A standardized mixed feeding regime was adopted using wheat flour and a live algal consortium comprising *Thalassiosira weissflogii* and *Isochrysis galbana*. Over an eight-month production cycle, the system achieved a cumulative biomass yield of 6,232.81 kg. Peak productivity reached 2.9 kg/m^3, with an average yield of 2.25 ± 0.13 kg/m^3. The survival rate averaged 65 ± 3.13 %, and the feed conversion ratio was optimized at 1:2.25. Partial broodstock maturation (45-50 %) was observed by 15-21 days of culture. Cyst production was carried out over a 35-day period, yielding 250-350 g per tank. Wet cysts were collected from the tank bottom by discharging water through a 100-micron mesh. The harvested wet cysts were then dehydrated via a gradual saline water changeover, increasing salinity from 50 ppt to 300 ppt, with hatchability range of 65-79 %. Nutritional analysis of the harvested biomass revealed a high-value composition with 45.74 g protein, 34.15 g carbohydrates, per 100 g. Essential micronutrients included 792.6 mg potassium, 2862.8 mg sodium, 117.7 mg calcium, 144.2 mg vitamin C, and 5.42 mg iron. Energy content was 314.36 Kcal/100 g, moderate cholesterol levels (43.36 mg/100 g). Saturated, monounsaturated, polyunsaturated, and trans fatty acids were each measured at 1.08 g/100 g, indicating a balanced lipid profile. The results confirm the viability of biosecure indoor *Artemia* culture at low salinity, offering stable yields and a scalable alternative to saltpan systems for quality live feed in inland and coastal hatcheries.

Keywords: Low-salinity Culture, Raceway System, Indoor Aquaculture, Hatchery Feed, Commercial Aquaculture

Funding: Sapthagiri hatcheries, Kakinada

Isolation and characterization of candidate probiotic bacteria from gastro-intestinal tract and culture environment of pacific white shrimp, *Penaeus vannamei*

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Aquaculture has attained a major position in the world economy over the past decades. Shrimp and shrimp farming became one of the most cultured varieties due to many reasons, like the shorter culture days, the ease of maintenance, and also its high export values. However, the main disadvantage in this industry is disease outbreak and shrimps are more prone to it because of their non-specific immunity. Therefore, the only possible thing to be done is improving the gut health which alternatively helps in growth and defense mechanism. This could be done by using probiotics. The probiotics used in aquaculture are microorganisms that provide a variety of advantages and are crucial for enhancing water quality, gut microbiome, health status, immunity, disease resistance, growth performance, and intestinal epithelial barrier integrity. In this background, the present study attempted to isolate and characterize bacteria with probiotic potential. A series of bacterial were isolated from the gastrointestinal tract of healthy shrimp. Bacteria isolation was also attempted from water and soil samples of different shrimp farms. All isolates were subjected to different assays like microbial adhesion to hydrophobicity, aggregation, antagonistic test against pathogenic bacteria, different tolerance tests. From all the assays, the better-performing isolates were further identified by 16S rRNA sequencing. Three bacterial isolated which showed better performance were identified to be of *Bacillus* group that includes *Bacillus cereus* - PV 336007, *Bacillus subtilis* - PV022332 and *Bacillus toyonensis* - PV344719. These isolates were also challenged with shrimps to show that these are non-pathogenic. Their effect on gut microbiota and disease resistance of shrimps were tested.

Keywords: Shrimp, Probiotic, *Bacillus* sp., Characterization, Gut Microbiota

Project: Novel approaches for disease free health certification in finfish and development of high health shrimp for sustainable aquaculture

Funding: ICAR-National Agricultural Science Fund

Pathogenicity of translucent post-larvae disease-causing *Vibrio parahaemolyticus* in *Penaeus vannamei*

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Emergence of new diseases is an ongoing threat to shrimp aquaculture, leading to economic losses and highlighting the challenges in disease preparedness and response. Translucent Post-larvae Disease (TPD) is a bacterial disease caused by *Vibrio parahaemolyticus* carrying a virulent plasmid that encodes the very high virulent protein (vhvp). The clinical signs include a transparent or pale body, reduced feeding, and high mortality. The shrimp cephalothorax homogenate was used to isolate pathogenic bacteria on selective media and screened using the specific PCR primers for vhvp-1 and vhvp-2 genes associated with TPD. The *V. parahaemolyticus* strains TPD-negative (N10, N37.13) and TPD-positive (P15.31, P40.49) were used for experimental pathogen challenge. The white shrimp challenged with strain P40.49 displayed the TPD clinical signs and increased mortality compared to the P15.31 strain. The gene expression of vhvp-1 and vhvp-2 was significantly increased in the stomach and hepatopancreas of TPD-infected shrimp. Histopathology analysis of the TPD-infected shrimp showed collapsed hepatopancreatic epithelial cells, necrosis, and hemocytic infiltration in both hepatopancreas and intestine, consistent with previous reports. Taken together, our data provide valuable insights on TPD pathogenesis and emphasize the need for developing rapid detection methods, improved biosecurity measures to help prevent the spread of TPD.

Keywords: Shrimp Disease, Vhvp Gene, Pathogen Isolation

Project: The construction of an economical nursery with biosecurity management and the evaluation of the quality and cost of the shrimp seedlings produced

Funding: National Science and Technology Council, Taiwan

Manipulation of gut microbiome by feeding fermented rapeseed meal for high health shrimp, *Penaeus vannamei*

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The gut microbiome is crucial for shrimp immunity, digestion, and overall health. This study evaluated the effect of feeding fermented rapeseed meal (RSM) on the gut microbiome, growth, and nutrient utilization. RSM was fermented with *Bacillus subtilis* and *Saccharomyces cerevisiae* to ameliorate antinutritional factors and enhance its nutritional value. Fermentation increased the levels of limiting amino acids lysine, methionine, and tryptophan by 19.66%, 17.18%, and 10.91%, respectively, while the antinutrient factors phytic acid, tannins, and glucosinolates decreased by 47.69, 46.59, and 43.76 %, respectively. Five experimental diets were prepared with 0%, 5%, and 7.5% levels of raw and fermented RSM. A 45-day feeding trial was conducted on juveniles (2.86±0.06 g) of *Penaeus vannamei*. After the experiment, digestive enzyme and immune profiles were analyzed. Total genomic DNA was extracted from shrimp gut samples, and the bacterial 16S rRNA (V3–V4 region) was amplified for microbiome analysis. Libraries were prepared using the Nextera XT Index Kit and sequenced on the Illumina NovaSeq platform. Sequencing data were processed using the QIIME2 pipeline for bioinformatic analysis. The linear discriminant analysis (LDA) effect size (LEfSe) method was employed to identify differentially abundant bacterial taxa between the treatment groups. The Venn diagram shows that feeding shrimp with 7.5% fermented RSM results in the highest number of unique OTUs. Shrimp fed fermented RSM exhibited lower levels of *Vibrionaceae*, a family of significant disease-causing bacteria, compared to those fed unfermented or control diets. *Firmicutes* and *Bacteroidetes* are both involved in the fermentation process, contributing various nutrients to the host, particularly short-chain fatty acids, which increase with the feeding of fermented RSM. The inclusion of fermented RSM improved gut microbiome diversity (Simpson, Shannon, Fisher, Chao1), enhancing gut health, functional stability, and disease resistance. Improved gut microbiome from fermented RSM enhanced digestion, nutrient utilization, immunity, and growth in shrimp.

Keywords: *Penaeus vannamei*, Intestinal Microbiota, Solid State Fermentation, Rapeseed Meal, Immunity

Project: Solid state fermentation technology for development of cost effective customized plant protein products as fish meal alternate for shrimp feed & Unravelling signatures of dietary protein sparing and fibre tolerance in *Penaeus vannamei* for development of cost effective feeds through omics approaches

Funding: Department of Biotechnology, Government of India

Characterization of *Vibrio parahaemolyticus* isolated from *Penaeus vannamei* affected with white feces syndrome

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White feces syndrome (WFS) has emerged as major threat to global shrimp aquaculture. Recent reports suggest association of *Vibrio* and other bacterial species along with *Enterocytozoon hepatopenaei* (EHP) as possible causative agents for WFS. To understand the pathogenesis and etiological agents we isolated several bacterial isolates from the gut and hepatopancreas of WFS affected shrimp. Challenge study revealed that few strains of V. parahaemolyticus are highly pathogenic and cause maximum upto 32% mortality by immersion method. For gaining deeper insight, the most pathogenic strain *V. parahaemolyticus* F10G1 was sequenced at PacBio Sequel II platform in circular consensus sequencing (CCS) or HiFi mode. The genome was assembled using long read assembler Flye which produced chromosome level assembly. The strain has two chromosomes and one extra chromosomal plasmid. The total genome size is of 5.366 MBp and encode for 4843 protein coding and 169 rna genes. The study identified presence of toxins, toxin carrying prophages, CRISPR-Cas system over the chromosome and several colonization factors over the plasmid. The study is underway to analyze the role of these virulence factors in the pathogenesis of white feces syndrome.

Keywords: *Vibrio parahaemolyticus*, Virulence, White feces syndrome, Whole genome sequencing

Project: Development of molecular diagnostics for differentiation of pathogenic and non-pathogenic *Vibrio* species in aquaculture

Funding: Consortia Research Platform on Vaccines and Diagnostics, Indian Council of Agricultural Research, India

Identifying infectious pathogenic entities associated with reduced growth in farmed shrimp from Latin America

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Infectious diseases causing growth retardation and chronic mortalities are becoming more common than large-scale mortalities in shrimp aquaculture. Often such diseases remain unnoticed until active surveillance is conducted, or mortalities become gradually more pronounced. We report here on a case study from Latin America where severe growth retardation was documented in *Penaeus vannamei*. Clinical signs included high size variation (e.g. 4 gm to 20 gm), soft cuticle, weakness, dark body coloration, and red tails and pleopods. Survival rates at harvest were 30-50%. Fecal strands were seen floating on the water, similar to what is observed in white feces syndrome affected shrimp. Since *Enterocytozoon hepatopenaei* (EHP) had been reported in the region, there was concern that this facility might also have been affected.

Juvenile and post larvae samples were submitted to our laboratory for a health assessment by histopathology and PCR analyses. By histopathology, the most significant finding included the presence of lesions diagnostic of infection by necrotizing hepatopancreatitis (NHP). Additionally, lesions characteristics of Decapod Hepanhamaparvovirus (DHPV) were found in the mucosal epithelium of the anterior midgut caecum. Cowdry A type inclusion bodies, pathognomonic for IHHNV, were suspected in a few samples. No other known lesions of concern to penaeid shrimp were detected including EHP, white spot syndrome virus (WSSV) and acute hepatopancreatic necrosis disease (AHPND) that have been reported in Latin America.

PCR analysis revealed high levels of NHP, DHPV, and IHHNV. Few samples had very low levels of AHPND and WSSV, but no EHP was detected. The DHPV isolate detected in these samples corresponds to a genotype circulating in Latin America. This study exemplifies why health assessment of farmed shrimp should combine histological and molecular analysis. If PCR analysis had been performed only for EHP, based on the presumptive clinical signs, NHP and DHPV would not have been detected.

Keywords: White feces syndrome, Shrimp, NHP, DHPV, IHHNV

Project: Shrimp disease diagnosis

Funding: Aquaculture Pathology Laboratory

Dietary fucoidan from Okinawa mozuku enhances disease resistance and modulates host responses in kuruma shrimp

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Fucoidan, a sulfated polysaccharide from brown algae, has attracted considerable attention as a functional material due to its diverse physiological activities, which vary with the algal source. Among these, fucoidan derived from Okinawa mozuku (*Cladosiphon okamuranus*, O-FCD) is notable for its stable industrial-scale production and its demonstrated immunomodulatory and disease-resistance effects. While such effects have been reported in various shrimp species, evidence for its efficacy in Kuruma shrimp (*Penaeus japonicus*), the predominant species in Japanese shrimp aquaculture, remains limited. This study aimed to evaluate the protective effects of O-FCD against pathogenic infections in *P. japonicus* and to examine its influence on host immune responses and gut microbiota composition.

Experimental diets were prepared by incorporating 0.2% or 1.0% O-FCD into a commercial shrimp feed, extruding into noodle-like shapes using a catheter syringe, and then drying in a hot-air oven. Three groups were established: control (no O-FCD), 0.2% O-FCD, and 1.0% O-FCD. Shrimp were individually reared in 250-liter tanks and fed once daily at approximately 1% of body weight. After 20 days of feeding, five shrimp per group were sampled for transcriptomic and gut microbiota analyses. Subsequently, challenge tests were conducted by exposing shrimp to *Vibrio penaeicida* or white spot syndrome virus (WSSV) under controlled conditions.

In the *V. penaeicida* challenge, survival was 8% in controls, 30% in the 0.2% group, and 19% in the 1.0% group. After WSSV infection, survival reached 94% (0.2%) and 85% (1.0%), compared to only 5% in controls. Transcriptomic analysis revealed upregulation of genes involved in microtubule formation, intracellular transport, and protein phosphorylation. Although overall microbiota composition remained largely unchanged, microbial diversity increased, notably with a significant rise in *Agarivorans* spp., capable of degrading sulfated polysaccharides. These findings suggest that O-FCD supplementation enhances host immunity and disease resistance via immunomodulation and beneficial gut microbial shifts.

Keywords: Fucoidan, Kuruma Shrimp, Immune Responses, Gut Microbiota

Project: Assessment of the effects of Fucoidan on infectious diseases in farmed fish and shrimp

Funding: Yakult Honsha Co. Ltd.

Role of apoptosis in white spot disease progression in *Penaeus vannamei* shrimp

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White spot syndrome virus (WSSV), one of the important shrimp pathogens, causing white spot disease resulting in massive mortalities of farmed shrimp all over the world, with severe financial loss to the farming community and jeopardizing the sustainability of shrimp farms. Apoptosis or programmed cell death plays a crucial role in shrimp immunity, especially during WSSV. To elucidate the role of apoptosis during this disease pathogenesis in *Penaeus vannamei*, an experiment was carried out in shrimp (n=315), with seven groups of different WSSV viral copy numbers (ranging from $4.2x10^1$ to $4.8x10^6$) by intramuscular injection and control group with PBS. The animals were observed for any gross changes and clinical signs and samples were collected from 6, 24, 48 and 72hrs post injection. The group injected with 10^6 viral copies gave first step PCR amplification while group injected 10^4viral copies gave nested positive from 24 hours onwards. However, all the groups gave PCR positive amplification from 72hrs onwards. Mortality noticed from 48hrs onwards in10^4viral copies injected group. The apoptosis genes expression of p53, caspase 2, caspase 3, caspase 4 and caspase 5 in gill tissues was studied at various time intervals. It was found that all the apoptotic genes were upregulated in all the time intervals. During 6hr interval caspase 3 expressions was found to be more in all the copy numbers and p53 expression was found to be more during 24hr interval. The caspase 2 and caspase 5 gene expressions in gill tissue at 72hr interval found to more with various copy numbers. The study clearly gave an indication that apoptosis was seen during infection and has definitely a major role in the white spot disease pathogenesis. These genes are upregulated in shrimp to limit infection. Understanding these dynamics is essential for developing effective strategies for shrimp health management.

Keywords: Apoptosis, WSSV, Gene Expression

Project: NSPAAD phase II

Funding: PMMSY

Anaesthetic effects of varying eugenol concentrations on adult Indian white shrimp (*Penaeus indicus*)

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Anesthesia is a common practice in shrimp hatcheries to minimize the adverse effects of stress and enhance animal welfare. However, few studies have been conducted to investigate the application of anesthetics in Indian white shrimp (*Penaeus indicus*). In the present study, the anesthetic efficacy of eugenol on adult P. indicus was investigated at the different eugenol concentrations (100, 150, 200, 300, 400 and 500 mg/L). The anesthesia and recovery time were recorded. The results indicated that anesthesia time in shrimp decreased as eugenol concentration increased, while recovery time increased with higher eugenol concentrations. Under the same eugenol concentrations, the recovery time increased with the increase of body weight. The recovery rate was 100% at eugenol concentrations ranging from 100 to 150 mg/L. The results indicated that eugenol is a safe and efficient anesthetic for *P. indicus*, which could be used to reduce stress and increase survival/success rate during transportation, tagging and artificial insemination procedures.

Keywords: *Penaeus indicus*, Eugenol, Anaesthetic time, Recovery time

Project: Development of Indigenous Shrimp (Indian White Shrimp) Aquaculture

Funding: Pradhan Mantri Matsya Sampada Yojana, Government of India

Ectyonucleospora hepatopenaei accelerates *Vibrio parahaemolyticus* infection in *Litopenaeus vannamei* shrimp

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Ectyonucleospora hepatopenaihepatopenaei (EHP) has emerged as a major threat in the recent years in Indian shrimp aquaculture, which was evident by the amount of loss it has incurred in the country. Further it was found that this microsporidian parasite either alone or with other pathogens causes morbidity in shrimp. Hence an experiment was conducted for 14 days in *Litopenaeus vannamei* (N=360) shrimp to know the effect of *Vibrio parahaemolyticus* in association with EHP affected shrimp. The disease-free shrimp were grouped into four groups, group I (control), group II (EHP), group III (*Vibrio*) and group IV (EHP + Vibrio), each with 90 shrimp. Sampling was done on 1,3,5,7 and 14 days of infection (DOI). Mortality observed from 5 DOI onwards in group III and group IV. The clinical signs like slow growth, size variation in group II animals, loose shell, melanisation in the carapace, and antennal cut in group III animals. Group IV had lesions of both group II and group III. Total hemocyte count found to be elevated in group II animals. Semi granular hemocyte found to be increased in all groups throughout the experiment. Biochemical changes in challenged shrimp had elevated levels of protein, alkaline phosphatase and aspartate amino transferase while albumin level found to be lower than the control groups. Caspase II and P53 gene expression were found to be increased in all challenged groups indicating the role of apoptosis in the disease progression. prophenol oxidase, cathepsin B, superoxide dismutase and transglutamase gene expression were found to be more in the groups which had infection than the control group. Histological lesions were observed in the hepatopancreas, and its severity was more with combined effect of the pathogen. The study concludes that the hepatopancreas is the main target organ and EHP paves way for *Vibrio* infection in shrimp and hence a complete biosecurity is essential to have a good harvest.

Keywords: *Enterocytozoon hepatopenai, Vibrio parahaemolyticus*, Hepatopancreas, Morbid

Project: NSPAAD phase 2

Funding: PMMSY

Effect of salutary shrimp feed and fish waste-based plankton booster supplementation on health status of *Penaeus vannamei*

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ICAR-CIBA has developed a nutritionally efficient and affordable shrimp feed made of locally available feed ingredients at Indian Sundarban and the same has been designated as Chingudi ^{Plus}. Additionally, ICAR-Central Institute of Brackishwater Aquaculture (ICAR-CIBA) has developed a fish waste-based product to promote plankton growth in aquaculture ponds and it has been named as Plankton^{Plus}. The health status of *Penaeus vannamei* was monitored during a 112 days culture trial in earthen ponds following two treatment sets, such as using both Plankton^{plus} and Chingudi ^{plus} feed (T1) and using commercial feed along with conventional practices (T2). The total heterotrophic bacteria (THB) and total *Vibrio* (TV) were monitored in cultured water and haemolymph of shrimp. No significant difference in the level of THB and TV was observed in cultured water except at DOC 60, when the TV level was significantly lower in T1 compared to T2. However, compared to T1, the TV level in hemolymph was observed to be significantly greater in T2 at the end of the culture period. Upon completion of culture period, the gut bacterial diversity was analysed using metagenomics based on V3-V4 region of 16S rRNA gene. The dominant bacterial genus in shrimp gut for both the groups (T1 and T2) were found to be *Acinetobacter, Clostridium, Enterococcus, Pseudomonas, Lactococcus, Gardenerella, Lichenibacterium, Proteus, Ralstonia* and *Stenotrophomonas*. Importantly, the relative abundance of *Lactococcus* spp., which is considered as beneficial bacteria, is significantly ($P<0.05$) higher in case of T1 as compared to T2. In contrast, the relative abundance of Vibrio spp., which includes several pathogenic species, was found significantly lower in T1 as compared to T2 ($P<0.05$). Based on the observation of the current study, it can be concluded that the combined use of Plankton^{Plus} and Chingudi^{Plus} could enhance the overall health status of cultured *P. vannamei*.

Keywords: Plankton^{Plus} , Chingudi^{Plus}, Gut Health, *Penaeus vannamei*

Project: Development and demonstration of sustainable and economically viable brackishwater aquaculture models for Eastern region of India

Funding: ICAR-Central Institute of Brackishwater Aquaculture, India

Pilot-scale fermented feed with mixed strains of *Bacillus subtilis* and *Saccharomyces cerevisiae* modulates the gut microbial and transcriptomic profiles in *Penaeus vannamei*

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The gut microbiota plays crucial role in facilitating nutrient digestion, regulating intestinal immunity, and preventing pathogen colonization. This study employed a combination of *Bacillus subtilis* and *Saccharomyces cerevisiae* for fermentation in a pilot-scale fermenter. Three isonitrogenous (34% CP) and isolipidic (6% CL) diets were prepared control diet without fermentation, and two fermented diets labelled as Batch 1 (72 hrs) and Batch 2 (96 hrs). All three diets were tested in triplicate groups, with each replicate tank stocked with 20 shrimp (2.04 ± 0.04 g, N = 20). After the trial, gut samples were collected for 16S rRNA metagenomic analysis and hepatopancreas for transcriptome sequencing (Illumina). Feeding fermented diets did not significantly affect alpha diversity ($P > 0.05$). The Bray-Curtis PCoA plot shows tight clustering of FB-1 and FB-2, distinctly separated from the control, indicating fermentation altered gut microbiota composition. Venn diagram analysis showed 248 ASVs commonly shared among the control, FB-1, and FB-2 groups. At the phylum level, Bacteroidetes were more abundant in the fermented group compared to the control. Bacteroidetes in high abundance helps the host catabolize carbohydrates and play a key role in the host gut in food digesting and absorption. At the genus level the abundance of potential pathogen, Vibrio was lower in the FB-2 group. ANCOMBC analysis showed significantly higher enrichment of *Flavobacteriaceae, Gammaproteobacteria, Bacteroidia*, and *Ochrobactrum* in FB-2. Transcriptome sequencing analysis identified 61 differentially expressed genes (DEGs) that were upregulated in both fermented feed groups (Batch 1 and Batch 2) compared to the control group. Furthermore, KEGG pathway enrichment analysis revealed that the FB-2 group exhibited significant enrichment in metabolic pathways, including protein digestion and absorption, carbohydrate digestion and absorption, and pancreatic secretion. The results indicate that FB-2 can alter the intestinal microflora of shrimp and hold potential for disease prevention in shrimp aquaculture.

Keywords: *Penaeus vannamei*, Pilot scale fermentation, Intestinal microbiota, 16S rRNA, Hepatopancreas transcriptome

Project: Unravelling signatures of dietary protein sparing and fibre tolerance in *Penaeus vannamei* for development of cost effective feeds through omics approaches

Funding: Department of Biotechnology, Government of India

Fermented sunflower oilcake supports beneficial gut microbiota and health in Pacific white shrimp (*Penaeus vannamei* Boone, 1931)

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The gut microbiota is often regarded as the 'second brain' of the host, playing a pivotal role in nutrient absorption, immune regulation, and protection against pathogenic infections. *Penaeus vannamei* (1.86 ± 0.01 g) fed three iso nitrogenous (37%) and iso lipidic (6%) diets: control (CON), and two experimental diets containing 2.5% raw (SF–2.5) or microbial fermented (*Saccharomyces cerevisiae* and *Bacillus subtilis*) sunflower oilcake (FSF–2.5) for 56 days. At the end of the trial, gut samples were collected for DNA extraction, followed by 16S rRNA gene amplification, library preparation, and the resulting sequences were analysed by QIIME2 pipeline. Alpha diversity metrics did not differ significantly (P> 0.05). A total of 836 ASVs were shared among all dietary groups. Based on Venn diagram, shrimp fed the CON diet had the highest number of unique ASVs (3,116), followed by FSF–2.5 (2,684) and SF–2.5 (1,430). A total of 42 phyla were identified across treatments, with Proteobacteria being the dominant phylum (71.02%), followed by Bacteroidetes (15.86%) and Firmicutes (2.38%). Inclusion of raw SF at 2.5% increased Proteobacteria abundance to 77.16% compared to the control (66.66%). In addition, the relative abundance of Bacteroidetes, and Firmicutes was higher in the FSF–2.5 group compared to the SF–2.5 group. The relative abundances of Patescibacteria, Cyanobacteria, Verrucomicrobiota, and Bdellovibrionota were higher in FSF–2.5 compared to other groups. At the genus level, shrimp fed the FSF–2.5 diet showed a higher abundance of *Ruegeria* and a lower abundance of *Photobacterium*. *Ruegeria*, a beneficial gut symbiont, contributes to carbohydrate degradation, vitamin–B12 synthesis, and enhancement of host defense against bacterial pathogens by producing tropodithietic acid, while *Photobacterium* is associated with bacterial myonecrosis and hepatopancreatic necrosis. The inclusion of fermented SF in the diet of *P. vannamei* improves shrimp health by promoting beneficial gut microbiota while suppressing harmful pathogenic bacteria.

Keywords: Microbial Fermentation, Sunflower oilcake, *P. vannamei*, QIIME2, Gut Microbiota

Project: Unravelling signatures of dietary protein sparing and fibre tolerance in *Penaeus vannamei* for development of cost effective feeds through omics approaches

Funding: Department of Biotechnology, Government of India

Debunking the myth: No evidence for vertical transmission of EHP in shrimp breeding

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Enterocytozoon hepatopenaei (EHP) is a devastating microsporidian parasite causing significant economic losses in global shrimp aquaculture, with annual damages exceeding $500 million in India alone. While horizontal transmission through contaminated environments is well-established, the potential for vertical transmission from infected broodstock to offspring remains controversial and critically important for breeding programs targeting EHP resistance. This study investigated whether EHP can be vertically transmitted through reproductive organs of Pacific white shrimp (*Penaeus vannamei*) using a controlled cohabitation infection model. Sixty healthy broodstock were exposed to EHP-infected juveniles for 35 days, after which reproductive organs and hepatopancreas tissues were analysed using quantitative PCR (qPCR), histological staining, and *in situ* hybridization (ISH). Results confirmed successful horizontal transmission, with hepatopancreas showing high EHP loads in both males (33,115±15,672 copies/10ng DNA) and females (15,622±6,465 copies/10ng DNA). While qPCR detected EHP DNA in reproductive organs - testis/vas deferens (293±121 copies/10ng DNA), and terminal ampule (35±22 copies/10ng DNA) - ISH analysis revealed no active infection within reproductive cells themselves. Crucially, ISH signals were only detected in connective tissues surrounding reproductive organs, not in gametes. This suggests that PCR-positive results likely represent phagocytosed debris or dead pathogen material rather than viable infection capable of vertical transmission. The hepatopancreas remained the primary target organ for active EHP replication. These findings provide no conclusive evidence for vertical transmission of EHP through reproductive organs. This research contributes valuable insights for developing sustainable aquaculture practices and genetic improvement strategies against this economically important pathogen.

Keywords: *Penaeus vannamei, Enterocytozoon hepatopenaei*, Microsporidian, Reproductive organs, Aquaculture.

Viability of *Ecytonucleospora hepatopenaei* spores in shrimp tissues frozen at -20 °C

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The shrimp microsporidian *Ecytonucleospora hepatopenaei* is a major pathogen in shrimp aquaculture, causing the disease Hepatopancreatic microsporidiosis (HPM). The disease associated with severe growth retardation and white feces syndrome (WFS) and resulting in severe economic losses. The pathogen primarily targets the shrimp hepatopancreas and transmits through a horizontal pathway. However, the viability of EHP spores in the extracellular environment, particularly in freezing conditions, is poorly understood. In this study, we investigated the viability of EHP in infected tissue stored at -20°C for different durations such as 0h, 24h, 48h, 72h, and 7 days. The presence of EHP in the frozen tissue was confirmed by PCR before feeding. The frozen tissues stored at different duration (24h, 48h, 72h and 7 days, and 0 hrs) were minced and fed equally to the animal (n=20 in duplicate) at 7% of their body weight. After 30 days of experiment, EHP infection was confirmed by nested PCR. Shrimp fed with tissues stored for 0h, 24h, 48h, and 72h tested positive for EHP at first step, indicating active infection. While 7 days was found to be positive at nested positive, suggesting reduced infection, but detectable level. This preliminary finding reveals that EHP spores can be viable at -20°C up to 72 hrs, which has significant implications in biosecurity and disease transmission in shrimp culture.

Keywords: *Ecytonucleospora hepatopenaei* (EHP), Viability, Horizontal Transmission, Frozen Tissue.

Funding: Indian Council of Agricultural Research, India

Computational discovery of shrimp RNAi-mediated silencing targets in white spot syndrome virus genome

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RNA interference (RNAi) serves as the primary antiviral defense in invertebrates that lack adaptive immunity, by processing viral double-stranded RNA into virus-derived small RNAs (vsRNAs). Although RNAi plays a central role in anti-WSSV defense in shrimp, the exhaustive list of White Spot Syndrome Virus (WSSV) genes targeted by RNAi pathway remains largely unexplored. This study explores the small RNA (sRNA) sequencing datasets to document the WSSV genes targeted by *Penaeus vannamei* RNAi mechanism in through *in silico* analysis. The sRNA-Seq datasets generated from gill tissues of naturally infected and experimentally infected shrimp were analyzed using vsRNAfinder, a specialized tool for the identification, annotation, and quantification of vsRNAs. In naturally infected samples, 39 vsRNAs were identified (6 miRNAs, 33 siRNAs), while 37 vsRNAs were found in lab-challenged samples (1 miRNA, 36 siRNAs). The identified vsRNAs were mapped to the genome of WSSV-CN (AF332093.3) isolate to identify targeted coding sequences. Twenty WSSV genes were targeted in natural infections, and eighteen in experimental infections, with seven common genes identified in both. The host RNAi response was found to target key WSSV genes, including envelope proteins such as VP28, VP136, VP150, and VP664, as well as early regulatory genes like E3 ligase, immediate early protein (wsv108), and wsv231. These genes are crucial for viral entry, early infection, and replication. Overall, the analyses document that at least 31 WSSV genes are targeted by shrimp RNAi defense mechanism. By revealing key viral genes targeted by vsRNAs, the study offers insights into RNAi-based strategies for improving shrimp resilience to WSSV infection.

Keywords: siRNAs, vsRNAs, *Penaeus vannamei*, WSSV

Project: Whole genome sequencing of brackishwater aquaculture candidate species and development of genomic resources

Funding: Indian Council of Agricultural Research, India

Cracking the integrin-WSSV code: Shrimp vs Mudcrab

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Understanding species-specific resistance to viral infections is crucial for improving disease management in aquaculture. While *Penaeus vannamei*, a major shrimp species, is highly susceptible to White Spot Syndrome Virus (WSSV), leading to substantial mortality and economic losses, *Scylla serrata* (mud crab), a fellow crustacean, demonstrates notably higher resistance to this virus. To explore the molecular basis of this differential susceptibility, we investigated β-integrin, a known receptor involved in WSSV binding and cellular entry. We characterized and uploaded the full-length β-integrin sequence of *Scylla serrata* (accession number OR360538.1) to NCBI, and compared it with the full-length β-integrin sequence of *P. vannamei* (accession number GQ889365.1), retrieved from NCBI. The two sequences were analyzed for similarity and compared in terms of glycosylation patterns (via the NetNGlyc 1.0 server) and protein physicochemical properties (using the ExPASy ProtParam tool). Molecular docking studies were conducted using AutoDock with the WSSV envelope protein VP24. Results showed approximately 73% sequence similarity between the two species, with notable differences in predicted glycosylation sites and protein stability. Docking analysis revealed stronger binding affinity of VP24 to the shrimp β-integrin compared to the crab counterpart. These findings suggest that structural and functional differences in β-integrin could be one of the features contributing to the natural resistance of *S. serrata* to WSSV. This study enhances our understanding of host-virus interactions and provides a basis for developing antiviral strategies and improving disease resistance in crustacean aquaculture.

Keywords: Integrin, WSSV, Mudcrab, Receptor

Project: Molecular characterisation of integrin gene in Mud crab

Funding: ICAR- Central Institute of Fisheries Education, India

Influence of nursery culture conditions on microbial dynamics and physiological responses in juvenile *Penaeus japonicus* Form II

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The Kuruma shrimp, *Penaeus japonicus* Form II, is a premium shrimp in live shrimp export market and a promising candidate for aquaculture diversification. As ideal nursery rearing systems play a key role in enhancing the growth performance of shrimp in grow out system, the present study investigated the microbial dynamics (gut, water, and sediment) and physiological response (growth, total haemocyte count, and tissue histology) of *P. japonicus* Form II juveniles (0.02 ± 0.01 g) over a 60-day nursery period under two rearing systems outdoor and indoor sandy bed units. At the end of the trial, total haemocyte counts (THC) showed significant variation among outdoor (4.26 ± 1.08 x10^6 cells/mL) and indoors (2.83 ± 0.92 x10^6 cells/mL) reared shrimps. Differential haemocyte analysis revealed a greater proportion of semi-granular cells in both the system (outdoor: 52.58 ± 8.8%; indoor: 49 ± 6.2%) followed by granular (25.5 to 26%) and hyaline cells (19.6-25.4%). Histological examination of muscle and hepatopancreas tissues indicated better structural integrity in shrimp reared outdoors. Microbial analysis showed higher total heterotrophic bacterial counts (THC) in outdoor systems, with gut, water, and sediment recording 5.8 x 10^2 ± 274 CFU/g, 1.5 x 10^4 ± 1,040 CFU/mL, and 8.1 x 10^2 ± 181 CFU/g, respectively. In indoor systems, these values were lower at 9.5 x 10^1 ± 55 CFU/g (gut), 8.1 x 10^3 ± 1,000 CFU/mL (water), and 9.3 x 10^2 ± 105 CFU/g (sediment). Vibrio counts also varied significantly between systems. Water quality parameters such as pH and temperature showed diurnal fluctuations, with outdoor systems maintaining slightly higher pH (8.13 ± 0.09) and temperature (29.7-30.4 °C) than indoor systems (pH: 8.09 ± 0.07, temperature: 28.5-30 °C). Total ammonia nitrogen (TAN) was higher in indoor systems (0.03 ± 0.02 mg/L) than in outdoor systems (0.006 ± 0.006 mg/L). Growth performance varied significantly between the rearing systems, with juveniles in outdoor units attaining a higher average body weight (0.83 ± 0.81 g) compared to those reared indoors (0.35 ± 0.60 g). These findings highlight that rearing conditions influence the microbial composition and physiological responses in juvenile *P. japonicus* offering insights on outdoor based nursery units for enhanced health and productivity.

Keywords: Microbial dynamics, Outdoor and indoor rearing system, Haemocyte count, Histology, Bacterial load

Project: Influence of nursery culture conditions on microbial dynamics and physiological responses in juvenile *Penaeus japonicus* Form II

Funding: Indian Council of Agricultural Research, India

Immunomodulatory effects of phytogenic feed additives and its effect on gut microbiome composition in *Litopenaeus vannamei*

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The role of dietary supplementation in boosting the immunity and shaping the gut microbiota of aquatic animals is critical for enhancing disease resistance and promoting overall health. This study investigates the impact of four bioactive dietary supplements—curcumin, beta-glucan, herbal extract, and garlic extract—on the growth, immune performance, and gut microbiota of shrimp (*Litopenaeus vannamei*) post-larvae (PL13). Diets were formulated with 40% crude protein and supplemented with 0.25% of each additive. Shrimp were reared under controlled conditions for eight weeks, with regular monitoring of growth performance parameters including weight gain, specific growth rate (SGR), and feed conversion ratio (FCR). Immunological assessments involved measuring total protein, prophenoloxidase (PPO) activity, respiratory burst (RB), and superoxide dismutase (SOD) activity. Quantitative PCR was employed to evaluate the relative expression of immune-related genes such as *proPO, lysozyme, SOD, crustin*, and *peneaidin 3a*. Post-trial pathogen challenges with *Vibrio parahaemolyticus* and *Enterocytozoon hepatopenaei* (EHP) were conducted to assess disease resistance. Among the treatments, the herbal extract group showed the highest growth performance, survival (88%) against *V. parahaemolyticus*, and a strong immune profile. Garlic extract also demonstrated notable results with improved survival and enhanced beneficial gut microbiota. Microbiome analysis revealed a reduced abundance of pathogenic *Vibrio* and increased presence of beneficial genera such as *Rugeria* and *Pseudoalteromonas* in the herbal extract fed and garlic extract fed groups. Overall, the results underscore the potential of specific dietary supplements to enhance shrimp health, growth, and disease resistance. The findings support the use of functional feeds as a sustainable strategy in shrimp aquaculture to promote better immunity and microbial balance, reducing the reliance on antibiotics.

Keywords: Aquaculture, Vannamei, Gut microbiome, Phytogenic Feed Additives, Immunostimulants.

Project: Immunomodulatory effects of phytogenic feed additives and its effect on gut microbiome composition in *Litopenaeus vannamei*

Funding: Arjuna Naturals

Effect of fish sauce borne *Teteragenococcus halophilus* bacteria and its efficacy to control luminescence disease causing *Vibrio harveyi* during shrimp larviculture

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Marine extremophilic bacterium, *Tetragenococcus halophilus*, was isolated from fish sauce and characterised by comparing with the type strain (RIKEN-JCM). *T. halophilus* (1.0 ml 5×10^4) was cultured in 1.0 liter of Soy sauce medium at 30 °C/48hrs in a shaking incubator, and cells of *T. halophilus* (10^7cfu/ml), 20µl were found inhibitory against *S. aureus, Bacillus cereus,* and *V. parahaemolyticus* (15mm). The total fat of *T. halophilus* cells was ascertained as 1.6%. Various unsaturated fatty acids, such as linolenic acid, linoleic acid, and eicosatetraenoic acid, were reported, and saturated fatty acids, such as palmitic acid, myristic acid, and stearic acid, were also reported from *T. halophilus* by GC-MS. The cell wall protein of *T. halophilus* varied from 25 to 250 kDa in size. The cells alone showed the highest (30 ± 0.2mm) inhibition against *S.aureus* and the lowest on *Bacillus cereus* (21 ± 0.1mm), but their extracellular bacteriocin showed identical inhibition against them (15-16 ± 0.3mm). The bacteriocin was purified in an acetone-methane solvent and then dialysed under a 100 KD membrane, which was found inhibitory to *Vibrio harveyi* at 0.5mg/ml protein concentration. *P.indicus* (n=1000) post larvae (17 days) was stocked in the 75L FRP tank with 10 ml of *T.halophilus* (10^7cfu/ml) cells and 10^6cfu/ml of luminescence disease-causing *V. harveyi*, and the experiment was conducted for 30 days. On the 30th day of culture, a gradual reduction of 10^3 cfu/ml of *V. harveyi* load was found. This strain can be used as an effective agent for controlling bacterial pathogens in an aquaculture system.

Keywords: *Tetragenococcus halophilus*, Antagonising, *Vibrio harveyi* Shrimp larviculture

Transcriptomic analysis of WSSV infected Indian white shrimp (*Penaeus indicus*) exposed to elevated temperature

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White Spot Syndrome Virus (WSSV) is one of the most virulent pathogens affecting cultured shrimp. Water temperature is known to have a significant impact on disease expression and viral pathogenesis. This study evaluated the effect of a temperature of 33 °C on viral replication and host response. Sequencing of gill tissues from WSSV-infected and control samples was performed using the Illumina NovaSeq 6000 platform with paired-end chemistry (2x150 bp). Raw reads were processed using trimmomatic v0.39 tool with default parameters for removal of adaptor contamination and low-quality reads. Differentially expressed gene (DEG) analysis identified 846 DEGs (421 upregulated and 425 downregulated), 854 DEGs (420 upregulated and 434 downregulated), and 1,291 (662 upregulated and 629 downregulated), at 12, 24, and48 hours post-infection (hpi), respectively. At the early stage of infection (12 hpi), significant Gene Ontology (GO) terms included the Wnt signaling pathway, protein-containing complex, ribosome biogenesis, binding, assembly, structural constituents, subunits, cytosolic ribosome, and intracellular anatomical structures. Ribosome-associated terms were more prominent at the early stage (12 hpi). KEGG pathway enrichment analysis of all DEGs showed significant enrichment in 17, 25, and 33 pathways at 12, 24, and 48 hpi, respectively. Venn diagram analysis, among the KEGG pathways enriched at the three time points, revealed that pathways related to oocyte meiosis, ribosome function, and the cell cycle were commonly enriched across all time points. In contrast, 10, 14, and 22 pathways were specific to the 12, 24, and 48 hpi groups, respectively. The transcriptome analysis indicated the enrichment of several pathways activated by WSSV infection, potentially influencing viral replication, the cell cycle, and host defense mechanisms in response to infection.

Keywords: *Penaeus indicus*, WSSV, Temperature, DEG, Transcriptome

Project: Evaluation of stress-mediated immunological and physiological response in brackishwater candidate species by flow cytometry

Novel wafer-based probiotic delivery systems: Enhancing bacterial colonisation and performance in aquatic environments

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Traditional probiotic delivery methods in aquaculture face significant limitations including inconsistent dosing, rapid dispersion in aquatic environments, and suboptimal bacterial colonisation rates. This research presents a novel wafer-based delivery system that addresses these fundamental challenges through innovative engineering design and targeted biological mechanisms. INVE's Sanolife^{TM} PRO-TAB wafer system consists of thin (1-2mm) discs containing multiple *Bacillus* species (*B. subtilis, B. pumilus, B. licheniformis*) at concentrations exceeding 2 billion CFU/g, encapsulated within a water-stable matrix. Key design features include slow-sinking hydrodynamic properties that create attractive movement patterns, specialised attractant coatings for enhanced palatability, and extended water stability maintaining structural integrity for over three hours in aquatic environments. Controlled trials with *Penaeus vannamei* demonstrated significant biological improvements. In broodstock studies, daily wafer application resulted in doubled copulation rates (5% to 9%), 37% increase in nauplii production, and substantially reduced pathogenic bacterial loads. Field trials showed accelerated growth cycles (98 *versus* 102 days) and 14% higher yields compared to controls. The mechanism of action involves "probiotic shock" delivery, where high-concentration bacterial doses enable rapid gut colonisation through competitive exclusion of pathogenic species. Laboratory studies confirmed clear inhibition zones against various *Vibrio* isolates, with *B. pumilus* showing particularly strong antagonistic activity. The selected *Bacillus* strains synthesize digestive enzymes that improve nutrient utilisation and continue environmental bioremediation after gut passage. This wafer-based approach represents a paradigm shift from continuous low-level probiotic exposure to strategic pulse applications that rapidly establish beneficial bacterial communities. The system demonstrates cross-species applicability and provides a standardised, error-resistant delivery method that eliminates preparation variability while maximising biological effectiveness. The integration of controlled-release mechanisms with targeted bacterial delivery offers significant potential for advancing probiotic applications across diverse aquatic systems, addressing both individual organism health and broader ecosystem management challenges.

Keywords: Probiotic delivery, Slow-sinking wafers, High pulse delivery, *Bacillus* species, Competitive exclusion

Identification of disease free wild marine Polychaete worm, *Onuphis kovala* from the Kovalam seashore suitable for mass production to restrict wild collection under the NBA Act 2002

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The present study aims to screen disease-free wild marine Polychaete worms from the Seashore. The wild worm *Onuphis kovala* was collected from the Kovalam Sea shore area and screened for OIE-listed pathogens with nutritional composition. The PCR-based investigation was carried out for twenty-five *O.kovala*, collected from the Kovalam, seashore. The extraction of DNA from different parts of the body (anterior, middle and posterior) was done using DNeasy blood and tissue kit, Lysate and CTAB methods. Among them, the lysate method fetched good quantity, anterior (231), middle (452) and posterior (115) and quality, anterior (1.84), middle (1.70) and posterior (1.78) of DNA. To avoid mis-identification, an appropriate amount of polychaete tissue samples were chosen for detection such as White spot syndrome virus (WSSV), *Enterocytozoon hepatopenaei* (EHP), Infectious hypodermal and hematopoietic necrosis virus (IHHNV), and Acute hepatopancreatic necrosis, which tested along with the appropriate positive control of PCR product of 1447 bp (I step) and 941bp (nested) and 514bp (I step) and 148bp (nested), band of 1269bp (I step) and 230bp (nested) and 309 bp (single) were noticed for WSSV, EHP-SWP, AHPND and IHHNV, respectively. It was found that major infectious shrimp pathogens such as WSSV, EHP, IHHNV, and IHHNV were free from O. kovala. Also, the worms were diagnosed for pathogenic *V. parahaemolyticus* and resulted in negative. *O. kovala* has a suitable nutritional profile such as moisture: 75.44%, protein: 15.61%, Fat: 3.51%, Crude fibre: 1.42%, Carbohydrate: 0.47%, ash: 3.54% (WWB). As this species has more fat content this may be used in shrimp hatcheries as a broodstock feed, this can be taken for mass culture for use as a potential live feed for broodstock shrimps.

Keywords: Identification, Wild, Disease Free, Marine Polychaete Worms

Project: Developement of culture methods for muddy and wild marine Polychaete worms
Funding: Department of Biotechnology, Government of India

Transcriptome analysis of the hepatopancreas in the Pacific white shrimp *Penaeus vannamei* in response to *Ecytonucleospora hepatopenaei* (EHP) infection

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Ecytonucleospora hepatopenaei (EHP), a microsporidian parasite, is the major causative agent of hepatopancreatic microsporidiosis (HPM) in penaeid shrimp. EHP infection severely impairs hepatopancreatic function, leading to stunted growth and, in combination with other factors, triggers a cascade of pathological outcomes such as white faeces syndrome (WFS) resulting epithelial damage in hepatopancreas and microbial dysbiosis, and culminating in loose shell syndrome (LSS), due to moulting disruption and nutritional imbalance. In this study, we performed comparative transcriptomic analyses of the hepatopancreas in *Penaeus vannamei* naturally infected with EHP alone (EHP group), EHP-infected shrimps with WFS (WFS group), and EHP-infected shrimps with LSS (LSS group), using healthy shrimp as control. A total of 3,221 differentially expressed genes (DEGs) were identified including 2,501 upregulated (662 in EHP, 1,020 in WFS, 819 in LSS) and 720 downregulated (119 in EHP, 382 in WFS, 219 in LSS). Among these, 644 DEGs were common, while 67, 432, and 82 unique DEGs were found in EHP, WFS, and LSS groups, respectively. Gene Ontology and KEGG pathway analyses revealed significant enrichment in pathways associated with nutrient metabolism, immune function, and cellular stress. Key downregulated pathways included aminoacyl-tRNA biosynthesis, O-antigen nucleotide sugar biosynthesis, protein digestion, and metabolism of fructose, mannose, cholesterol, amino sugars, and nucleotide sugars. In contrast, upregulated pathways included xenobiotic metabolism via cytochrome P450, lysosomal activity, steroid hormone biosynthesis, and retinol metabolism. Some of the significantly expressed genes included C-type lectin, lysozyme, cathepsin, triosephosphate isomerase, NADH-cytochrome b5, trypsin, and lipase. This study highlights the multifactorial and pathobiome-driven nature of WFS and LSS and offers molecular insights into the disruptions in immune function, energy homeostasis, and nutrient assimilation associated with EHP infection. The findings support the development of targeted diagnostics, therapeutics, and preventive strategies in shrimp aquaculture

Keywords: Shrimp, EHP Infection, Differential Gene Expression, Host Response

Project: Investigation of existing/emerging disease in candidate brackishwater species and development of preventive/treatment strategies for effective management

Funding: ICAR-Central Institute of Brackishwater Aquaculture, India

Optimizing fibre levels in shrimp diets: Effects of cottonseed meal on growth and gut microbial balance

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Shrimp diets increasingly incorporate plant-based ingredients to reduce fishmeal dependency, but their high fibre content may impact digestion and gut health. Understanding optimal dietary fibre levels is crucial for maintaining shrimp growth, immunity, and microbial balance. Thus, a study was designed to investigate the effects of increasing dietary crude fibre levels using cottonseed meal (CS) as a primary plant protein source on growth, antioxidant enzyme activity, and gut microbiome in *Penaeus vannamei*. Four isonitrogenous and isolipidic diets were formulated to contain graded levels of crude fibre: 2.56% (C2.56, control), 5.16% (CS5.16), 7.61% (CS7.61), and 10.08% (CS10.08), with cottonseed meal serving as the main fibre source. Juvenile shrimp were fed these diets for 60 days in a controlled experimental setup. Growth performance parameters such as final weight, weight gain, specific growth rate (SGR), and feed conversion ratio (FCR) showed significant differences ($p < 0.05$) among treatments. The C2.56 and CS5.16 groups exhibited superior growth, while the CS7.61 and CS10.08 groups showed reduced weight gain and poor FCR. Concurrently, antioxidant enzyme activities, specifically superoxide dismutase (SOD) and catalase (CAT), significantly increased ($p < 0.05$) with higher fibre levels, indicating elevated oxidative stress in shrimp fed CS7.61 and CS10.08 diets. Microbial diversity analysis revealed a marked decline in alpha diversity at the highest fibre level, suggesting reduced microbial evenness. Bray-Curtis-based PCoA revealed distinct clustering of microbial communities, with CS10.08 forming a separate cluster. Phylum-level taxonomic composition indicated a fibre-induced shift in microbial populations: Proteobacteria abundance decreased, while Bacteroidota and Verrucomicrobiota increased with rising fibre content. In conclusion, moderate fibre inclusion (up to 5%) had no adverse effects, whereas excessive levels (>5%) impaired shrimp health and performance.

Keywords: Crude fibre, Cottonseed meal, *Penaeus vannamei*, Gut microbiome

Project: Unravelling signatures of dietary protein sparing and fibre tolerance in *Penaeus vannamei* for the development of cost-effective feeds through omics approaches

Funding: Department of Biotechnology, Government of India

Physiological responses and microbial profile of Indian white shrimp, *Penaeus indicus* brooders reared under different culture systems

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Captive broodstock development remains a priority research area in breeding and seed production of shrimps. The present study evaluates the health, microbial profile, and physiological responses of Indian white shrimp brooders reared for 365 days under two rearing systems: indoor and outdoor units. At the end of the study, immune responses such as total haemocyte counts did not vary ($p>0.05$) in the outdoor ($3.44 \pm 1.3 \times 10^6$ cells/mL) and indoor ($3.62 \pm 1.1 \times 10^6$ cells/mL) reared brooders. The differential haemocyte counts in the outdoor had dominant semi-granular (55.98 ± 9.7%), hyaline (22.8 ± 9.9%), and granular cells (21.1 ± 3.8%), while indoor shrimp exhibited higher granular cells (29.85 ± 19.7%), semi-granular cells (51.35 ± 20.6%), and lower hyaline cells (18.7 ± 0.7%). Microbial counts in rearing water revealed higher heterotrophs in outdoor systems ($8.09 \pm 1.98 \times 10^6$ cfu/mL) compared to indoor ($6.35 \pm 2.69 \times 10^6$ cfu/mL), whereas *Vibrio* counts were higher indoors ($5.05 \pm 3.52 \times 10^6$). In gut samples, indoor shrimp had higher *Vibrio* ($8.76 \pm 1.69 \times 10^5$ cfu/g) and heterotrophic counts ($3.37 \pm 5.01 \times 10^4$ cfu/g) compared to outdoor shrimp (*Vibrio*: $4.08 \pm 5.94 \times 10^4$ cfu/g; heterotrophs: $2.71 \pm 3.67 \times 10^4$ cfu/g), indicating higher microbial load accumulation in indoor-reared shrimps. Histology of gut and HP revealed better structural integrity of hepatopancreatic tubules, increased size and number of B-cells, and elevated digestive enzyme profile in outdoor brooders compared to indoor brooders. Biochemical analysis recorded higher triglyceride accumulation in the hepatopancreas of indoor-reared brooders (59.30 ± 2.82 µmol/mL), while serum cholesterol was higher in outdoor brooders (71.70 ± 15.94 µmol/mL). Outdoor brooders had superior growth (males: 32.57 ± 3.37 g; females: 26.5 ± 1.8 g) and spawning (30%) without eyestalk ablation, while no gonad development was recorded in indoor units. The results highlight the physiological benefits of outdoor-reared brooders over indoor rearing environments, and the potential to improve indoor units through dietary and environmental cue manipulation.

Keywords: Captive Broodstock, Haemocyte Count, Clear Water System, Heterotrophic Community, Indoor Unit, Outdoor Rearing System

Project: Development of Indigenous Shrimp (Indian white shrimp) Aquaculture: Genetic Improvement Program of *Penaeus indicus*, Phase-I

Funding: Pradhan Mantri Matsya Sampada Yojana, Government of India

Unveiling the effect of functional diet containing autochthonous lactic acid probiotic bacteria in single and mixed form on the dynamics of hepatopancreas histomorphometry and its functional cells distribution in Pacific white shrimp (*Penaeus vannamei*)

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The addition of probiotics to feed minimises demand for antibiotics, making aquaculture industry more ecologically friendly. The probiotic supplementation has impact on augmenting the hepatopancreas histologic and its functional cells distribution. The present objective was to study the effect of functional diet containing probiotic bacteria on dynamics of hepatopancreas histomorphometry and its functional cells distribution in *P. vannamei*. Shrimps (630 numbers) were randomly distributed into 21 tanks @ 30 juvenile shrimps (2.5 to 2.8 g ±0.045 g) constituting three replicates per treatment and feeding trial for 60 days. Experimental diets were formulated basal diet with *Pediococcus pentosaceus* 10^9 and 10^{11} CFU/ g of feed; *Lactiplantibacillus plantarum* 10^9 and 10^{11} CFU/ g of feed; Multiple strain probiotics (*P. pentosaceus, L. plantarum, Lactococcus lactis, Enterococcus faecium, E. durans, E. hirae*) 10^9 and 10^{11} CFU/ g of feed; and control (CON) diet without supplementation of probiotics. Histomorphometry of hepatopancreas and distribution of functional cell types of *P. vannamei* were quantified from triplicates in *P. vannamei* collected on 15, 30, 60 and 90 days post probiotic supplementation. Hepatopancreas histology of shrimp fed with probiotic functional feed showed significantly ($P<0.05$) higher whole hepatopancreas on post probiotic supplementation. Correspondingly, hepatopancreas whole distal portion was significantly ($P<0.05$) lower on dietary supplementation of probiotic aquafeed additive. In addition, hepatopancreas whole middle and proximal portion as well as proximal, middle and distal tubule were significantly ($P<0.05$) higher in probiotic supplemented groups. It was found that 15, 30, 45 and 60 days post probiotic treatment, functional cells B and R cells in hepatopancreas starts differentiating from moderate to high in the distal end of tubule and this progressed well in middle end and further high differentiation was observed in 60 days. It was found that probiotic supplementation has impact on augmenting hepatopancreas histology and functional cells distribution in *P. vannamei*.

Keywords: Single Strain Probiotic, Multiple Strain Probiotic, Hepatopancreas Functional Cells, Hepatopancreas Histomorphometry, *Penaeus vannamei*

Project: Development, testing, and demonstration of newer feeds and feed management strategies

Funding: Indian Council of Agricultural Research, India

Effect of functional feed for thermal stress on immunity and gut microbiome in *Penaeus vannamei*

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Development of climate smart feeds for amelioration of global warming side effects is essential for shrimp aquaculture. The nutritional quality of plant protein mix is increased by solid state fermentation with a combination of *Bacillus subtilis* and *Saccharomyces cerevisiae* in a pilot-scale fermenter. This ingredient fermented plant protein mix (FPPM) was used at two different levels 7.5 (FPPM 7.5) and 10.5% (FPPM 10.5). Other two feeds are control feed and raw plant protein mix feed at 7.5% (PPM). A 45 days' feed trial was conducted to evaluate the efficacy of functional feeds in mitigation of temperature stress in *Penaeus vannamei* reared at two temperature regimes: 28°C (control) and 34°C (elevated). By the end of the trial period, no significant differences in immunological parameters viz., THC, SOD and PO were observed among the dietary treatments in control temperature. However, in high temperature, shrimp fed FPPM 7.5 feed exhibited significantly higher THC (142 ± 5.65 x 10^5 cells/ml), SOD (0.362 ± 0.002 U/ml) and PO (0.562 ± 0.019 U/ml), compared to other treatments, whereas FPPM 10.5 feed is associated with immunosuppression. Further, genomic DNA was extracted from shrimp gut samples of all treatment groups followed by amplification of the 16S rRNA gene and high-throughput sequencing using the Illumina platform. Sequence data were processed and analysed using the QIIME2 bioinformatics pipeline. An increase in the relative abundance of the phylum *Bacteroidota* and decrease in *Proteobacteria* was observed across all dietary treatments under elevated temperature conditions compared to the control. Among the phylum *Bacteroidota*, the genus *Meridianimaribacter*, a potential lignocellulose degrading bacteria was predominant and is considered as a beneficial microbe contributing to host nutrition and gut health. These findings suggest that FPPM 7.5 feed enhances thermal tolerance in shrimp, as evidenced by improved immunity and gut microbiome under elevated temperature.

Keywords: Climate smart feed, Temperature stress, Immune response, Gut microbiome, *Meridianimaribacter*

Project: National Innovations in Climate Resilient Agriculture (NICRA)

Funding: Indian Council of Agricultural Research, India

Effect of climate change induced temperature on white spot syndrome virus (WSSV) virulence and host immune response in shrimp (*Penaeus vannamei*)

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It is well known that rearing water temperature play critical role in viral pathogenicity as well as host immune response in the case of poikilothermic aquatic animals including shrimps. White spot syndrome virus (WSSV) has been known to cause significant loss in the shrimp aquaculture industry and temperature does play important role. In this line, we studied impact of rearing water temperature on WSSV infection in *Penaeus vannamei* by studying the mortality pattern, viral copy and key immune factors in response to the infection. A recirculatory shrimp housing system was designed and fabricated for this purpose where desired temperature was maintained with automated system. Three controlled temperature regimens were maintained @ 30, 33 and 35 °C while RT group, a treatment without temperature regulation (26-32 °C). Acclimatised shrimp (2.3±0.2g) were challenged with WSSV by immersion (10^7 copies/L) for two-hour and maintained in the respective 45 L glass tanks (25/tank) in triplicate. Mortality of the shrimp was noticed from two to seven day post challenge (dpi) with cumulative mortality of 100% in the 30 °C and RT group while 6.67% in the 35 °C group. No further mortality was observed until end of observation at 14 dpi in 35 °C group. WSSV load found to be higher in the RT and 30 °C group compared to 33 and 35 °C groups at 48 and 72h post challenge. Majority of the immune genes (crustin, penaeidin, pro-phenoloxidase, and superoxide dismutase) except lysozyme responded at an elevated level at 48 hpi in the RT and 30 °C group. Interestingly, heat-shock protein-70 responded higher in the 35 °C group in comparison to the lower temperature group indicating its potential role in anti-WSSV response. The study confirms maintenance of elevated temperature (>33 °C) can protect shrimp from WSSV and prevent mortality.

Keywords: Aquaculture, Shrimp, Viral Disease, WSSV, Water Temperature, Viral Load

Project: National Innovations on Climate Resilient Agriculture

Funding: Indian Council of Agricultural Research, India

Assessment and control of parasite infestations in brackishwater ornamental fish under captive conditions

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The major species in the brackishwater ornamental fish sector include Scat (*Scatophagus argus*), Silver Moony (*Monodactylus argenteus*), Orange Chromide (*Pseudetroplus maculatus*), Tiger Dwarf Goby (*Mugilogobius tigrinus*), Chiseltooth Goby (*Mangarinus waterousi*), and various other goby species. Brackishwater ornamental fishes are commonly infested with ectoparasites such as *Amyloodinium* sp., *Caligus* spp., *Argulus* spp., and *Lernaea* spp. Among these, Severe infections with the dinoflagellate protozoan *Amyloodinium* sp. have been observed on the gills and skin, leading to complete stock mortality within a week in silver moony. Histopathological examination of infected gill tissues revealed significant lamellar epithelial hyperplasia, lamellar fusion, and the presence of *Amyloodinium trophonts. Caligus* spp., *Argulus* spp., and *Lernaea* spp., predominantly affect silver moony, spotted scat, and orange chromide during winter months and control measures for infestations involve prophylactic treatment with 100 ppm formalin. *Amyloodinium* infections can be managed using 0.5 ppm copper sulphate administered continuously for 15 days or through 3-5 minute freshwater dips, which cause the trophonts to detach. Repeated treatments over 2-3 weeks are typically necessary to manage an *Amyloodinium* outbreak. Formalin and hydrogen peroxide have also proven effective as therapeutic agents against *Amyloodinium*. Effective maintenance of brackishwater ornamental fishes require regular health monitoring and the implementation of prophylactic treatments, including periodic formalin applications and freshwater dips, to maintain fish health and prevent parasitic outbreaks.

Keywords: Parasite infestations, Ectoparasites, Brackishwater ornamental fish, *Amyloodinium*

Project: AINP on Ornamental Fish Breeding and Culture

Funding: Indian Council of Agricultural Research, India

Isolation and identification of *Citrobacter freundii* associated with diseased *Labeo rohita* and *Clarias magur*

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Citrobacter freundii is recognized as a major stress induced opportunistic pathogen in freshwater fish. In the present report, *C. freundii* was isolated from *Clarias magur* and *Labeo rohita* with clinical signs such as skin lesions, body discoloration, haemorrhages, pale gill and discolored internal organs. Cream-colored colonies were obtained on Tryptone Soy agar (TSA) from gill rot of both *C. magur* and *L. rohita* and subsequently identified as *C. freundii* (strains BMG2 and RGS, respectively). Both of the strains were Gram-negative, short rod, catalase positive, and weakly hydrolysed gelatine. Although in case of antibiogram RGS strain from *L. rohita* showed a higher Multiple Antibiotic Resistance (MAR) index compared to the BMG2 strain from *C. magur*, indicating host-specific virulence. Isolates were tested against 11 antibiotics following the Kirby-Bauer disk diffusion method. The MAR index of RGS was 0.63, highlighting a serious concern in terms of Multidrug Drug Resistant (MDR) strain. Molecular characterization further confirmed both isolates as *C. freundii*, forming a monophyletic cluster with other *C. freundii* strains based on evolutionary phylogenetic analyses. This study represents a new record of *C. freundii* isolation and association with disease outbreaks in *C. magur* and *L. rohita* from Bihar, India. Further investigations on pathogenicity and virulence profile of *C. freundii* as an emerging opportunistic pathogen in cultured freshwater fish are necessary, which would contribute to a better understating and management strategy of the disease condition.

Keywords: *Citrobacter freundii*, *Labeo rohita*, *Clarias magur*, Opportunistic pathogen, Multidrug Drug Resistant

Project: Screening of bacterial pathogens associated with motile *aeromonas septicemia* (MAS) and flavobacteriosis in freshwater fish

Funding: College of Fisheries, Kishanganj, Bihar Animal Sciences University

Integrative flow cytometry analysis of immune dynamics and gut metagenomic modulation in *Penaeus vannamei* cultured with biofloc systems

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This study presents a comprehensive analysis of immune dynamics and gut microbial modulation in *Penaeus vannamei* reared under diverse biofloc systems, leveraging flow cytometry and metagenomic profiling. Experimental treatments included a control without biofloc (C0), CIBAfloc (BFCF), CIBAfloc with mineral mix (BFMM), and periphyton-enriched CIBAfloc (BPCF). Juvenile shrimps with ABW of 1.35 ± 0.1 were cultured over 100 days at a density of 200/m^3, maintaining a carbon-to-nitrogen ratio of 15:1 in triplicate across treatments. Post-culture, shrimp were challenged with *Vibrio parahaemolyticus* (1.1x10^4 CFU/mL), and hemolymph samples were collected at 6-, 12-, and 18-hours post-infection. Flow cytometry enabled high-resolution profiling of immune parameters including phagocytic activity, respiratory burst, intracellular free calcium levels, apoptotic cell ratios, and cell cycle progression. Concurrently, gut samples were subjected to metagenomic sequencing to assess microbial diversity and functional shifts. Results revealed that BPCF-treated shrimp demonstrated superior growth, with final average body weight of 23.81 ± 0.53 g, daily weight gain of 0.24 ± 0.01 g, and survival rate of 93.33 ± 1.53%, compared to 12.63 ± 0.25 g and 68.33 ± 1.53% in controls. Total ammonia levels were also significantly reduced in BPCF (0.13 ± 0.05 ppm) vs control (1.82 ± 0.61 ppm), indicating improved water quality and a distinct enhancement in gut microbial composition in biofloc systems, particularly BPCF, with elevated levels of beneficial bacteria and suppressed *vibrio* populations. Immunological assessments showed transient suppression of immune responses up to 12 hours, followed by marked recovery at 18 hours in all biofloc treatments. BPCF exhibited the most robust immune activation and the lowest cumulative mortality (40-46%) compared to 90% in controls ($P<0.05$). Gene expression analysis further confirmed upregulation of immune-related transcripts in biofloc-reared shrimp. Overall, the integrative approach revealed that biofloc systems, especially those augmented with periphyton-significantly modulate gut microbiota and enhance systemic immune competence in *P. vannamei*. These results highlight the potential of biofloc systems in enhancing shrimp health and supporting sustainable aquaculture.

Keywords: *P. vannamei*, Biofloc, Gut metagenomics, Challenge study, Flow cytometry Immunomodulation

Project: Evaluation and Refinement of Biofloc based new age farming technology through effective microbial management, recirculation, and input optimization for sustainable intensification across different aquaculture system
Funding: ICAR-National Agriculture Science Fund, India

Zinc biofortification for improving growth performance and immune response in *Penaeus vannamei* reared in biofloc system

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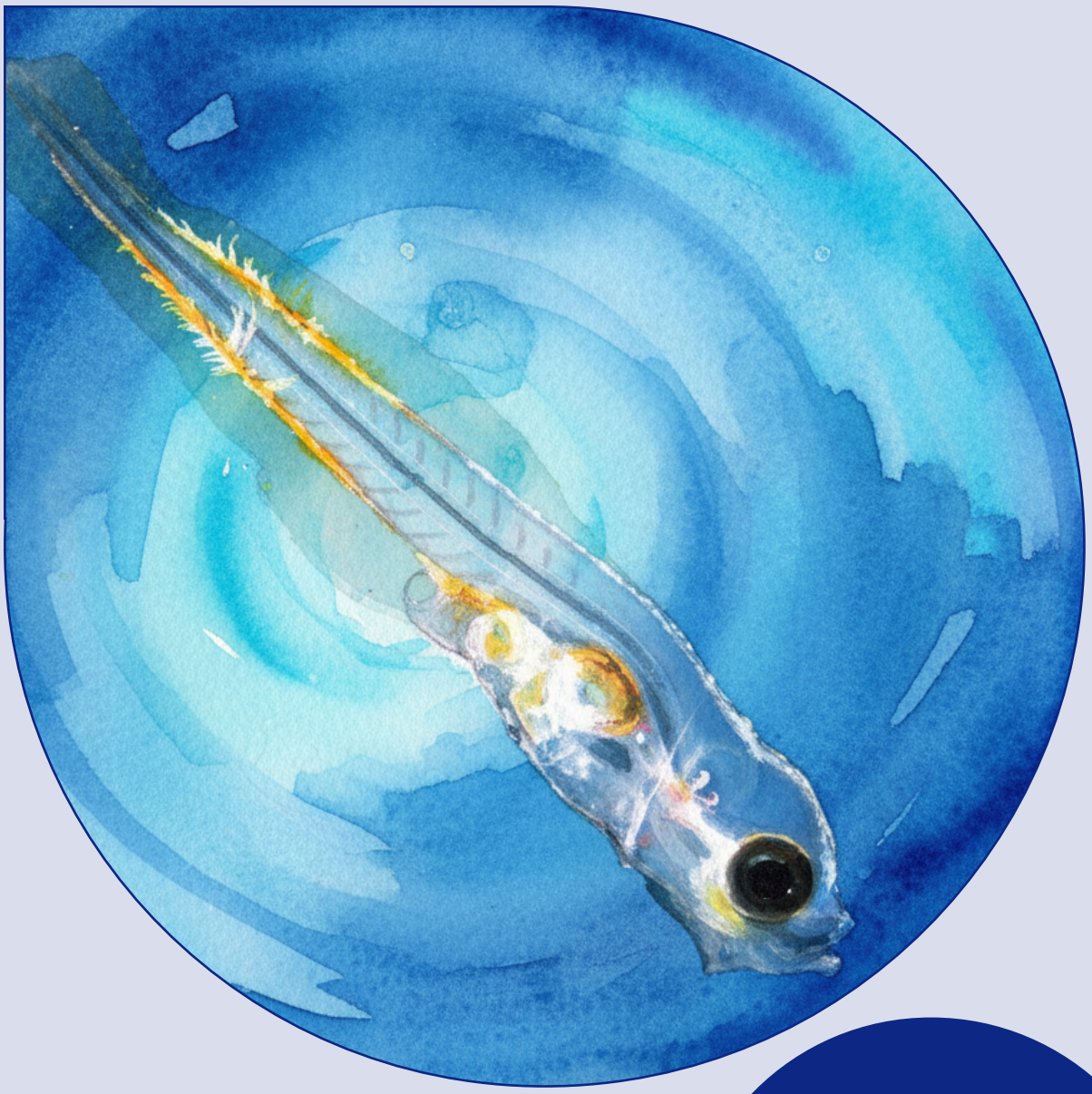
Sustainable intensification of Indian aquaculture necessitates innovative approaches that enhance productivity while safeguarding the environment. This study evaluated the combined effects of biofloc technology (BFT) and zinc (Zn) supplementation on the growth performance, muscle quality, and immune response of *Penaeus vannamei* post-larvae (PL). A total of 60 PL (0.35±0.05g) were stocked in 100-L tanks and cultured for 30 days under two treatment conditions: Control (clear water) and Biofloc supplemented with 20 ppm $ZnSO_4$ (BFT+Zn), each in triplicate. The BFT+Zn group exhibited significantly improved performance, including higher body weight (1.46±0.13g), specific growth rate (SGR 4.31±0.2), and feed conversion ratio (FCR 1.64±0.08), as well as increased survival (88.33±1.6). Immune gene expression analysis revealed a significant upregulation of *penaedin, stylicin*, and *crustin* in the BFT+Zn group, suggesting a robust immune response. Inductively coupled plasma optical emission spectrometry (ICP-OES) revealed a higher Zn accumulation (115.51 mg/100 g) in shrimp muscle. Histological examination shows increased cellular and metabolic changes in the zinc-fortified animals, thereby enhancing muscle quality and structural integrity. BFT+Zn systems exhibited more effective performance compared to the control, substantiating their advantages in water quality management and nutrient recycling. These results suggest that Zn biofortification within BFT systems improves shrimp growth, immunity, and nutritional value, offering a promising strategy to enhance both aquaculture productivity and the dietary zinc intake of consumers.

Keywords: *Penaeus vannamei*, Biofloc technology, Zinc supplement, Immune response, Biofortification

Project: Biofortification of trace elements in biofloc based Aquaculture: Microbial mediated approach for value added healthy shrimp and fish production

Funding: Department of Biotechnology, Government of India



03

Technical Session III

Aquaculture: New Directions

Aquaculture future: An African focus

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By 2050, the global population is projected to reach 9.7 billion, which will have significant implications for the supply of aquatic animal foods. To maintain the 2022 per capita consumption level of 20.7 kg, the total supply of aquatic animal foods would need to increase by 36 million tonnes, representing a 22% rise. Meeting this demand will require higher production, sourced from increased domestic output or imports, depending on the region. For instance, achieving the 74% increase in supply required to maintain Africa's current per capita consumption through domestic production alone presents a major challenge. This would require substantial investment and sector transformation. A more probable scenario is that Africa will need to import from other regions, assuming that additional supply is available and affordable. Without access to this supplementary supply, the region risks declining per capita consumption, which is already significantly lower than the world average. To raise Africa's per capita annual consumption from its current 9.4 kg to the 2022 world average of 20.7 kg, the supply would need to increase by about 38 million tonnes. This daunting task of aligning food supply with future demand requires strategic planning, including expansions in aquaculture and investments in sustainable resource management.

Keywords: Aquaculture, Future, Global, Africa, Consumption

04

Technical Session IV

Aquatic Animal Disease Diagnostics, Prophylactics and Therapeutics

Promoting non-lethal methodologies in aquatic animal health research

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Globally, a substantial number of aquatic animals are used in research aimed at reducing disease-related mortality in aquaculture. While these studies play a critical role in improving animal health, they often come at the cost of the lives and well-being of the animals used in testing. Drawing from ethical principles, particularly those rooted in spiritual traditions that recognize all sentient beings as valuing life and seeking to avoid suffering, we are reminded to act with compassion and mindfulness toward all forms of life. Safeguarding farmed aquatic animals should not necessitate the sacrifice of others. Although shifting from lethal to non-lethal approaches presents technical and practical challenges, it is achievable and requires an open mind. By embracing non-lethal methods and establishing new research standards grounded in compassion and responsibility, we can advance scientific knowledge while respecting and preserving the lives of all beings involved. This presentation emphasizes the importance of non-lethal approaches in aquatic animal health research and discusses future directions for promoting such methodologies. This presentation discusses the relevance of these approaches to strategic priorities in finfish health research, as outlined by the World Organisation for Animal Health (WOAH) and the STAR-IDAZ International Research Consortium on Animal Health (STAR-IDAZ IRC). These finfish health research priorities were identified through a global survey and expert consultation. Ultimately, the shift toward non-lethal research is more than a technical choice, it is a reflection of our shared values, where compassion informs innovation and science serves life, not sacrifice.

Keywords: Non-lethal Methodologies, Aquatic Animal Health, Disease Mitigation, Priorities in Finfish Health Research

Ecytonucleospora hepatopenaei (EHP) in Indian shrimp aquaculture – A decadal review

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Ecytonucleospora hepatopenaei, previously described as *Enterocytozoon hepatopenaei* (EHP), has emerged as a serious pathogen in Indian shrimp aquaculture since its first report in 2016. During the past decade, though not on the scale of WSSV, significant research efforts have been made on the microsporidian. The production and economic loss due to EHP and the probability of disease occurrence were estimated to be much higher than WSSV. High prevalence and wide occurrence of EHP were reported; however, many of these studies employed non-specific PCR targeting ssu rRNA and with limited microscopic and histological evidence. Many aquatic macrofauna have been identified as potential carrier hosts. Experimental transmission of the parasite and induced germination of spores under laboratory conditions, along with the viability of EHP spores in frozen tissues, have been demonstrated. Moreover, the effect of disinfectants such as formalin, sodium hydroxide, and hydrogen peroxide on the polar tubule extrusion was investigated. Experiments to understand the possibility of vertical transmission of EHP showed that the ovaries of EHP-challenged broodstock were negative by histology and PCR. An experimental study indicated that EHP can be one of the contributing factors of white faeces syndrome (WFS). Several diagnostic methods, including sensitive molecular methods and field-deployable assays, were developed. Comparative susceptibility study indicated that *P. monodon* is less susceptible than *P. vannamei*. Pathogenesis of EHP indicating severe damage to hepatopancreas and adverse effect on several physiological functions and immunity and growth retardation in shrimp was elucidated. Further, host-pathogen interaction through metagenomic and transcriptomic approaches was investigated. The whole genome of EHP was sequenced. An epidemiological survey identified major risk factors associated with EHP infection. In silico analysis and molecular docking studies identified some of the potential anti-EHP therapeutic compounds. Significantly, a phytobiotic formulation, 'CIBA EHP Cura I', has been developed for the treatment and control of EHP.

Keywords: *Ecytonucleospora hepatopenaei*, *Enterocytozoon hepatopenaei*, EHP, Microsporidian, Shrimp

Funding: ICAR-Central Institute of Brackishwater Aquaculture, Chennai, ICAR-Central Institute of Fisheries Education, Mumbai

Antibacterial activity of gallic acid-loaded graphene oxide nanocomposite against *Vibrio* spp. and its mechanism of action

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Vibrio spp. are Gram-negative bacteria that are linked to a disease known as vibriosis. *Vibrio* spp. are marine bacteria that cause infections in aquatic species and pose serious threats to the aquaculture industry. The different currents treatments have been shown to have major drawbacks. Therefore, efforts to discover the new alternatives and investigate their potential antibacterial activity are warranted. The present study investigates the antibacterial activity and further elucidates the mechanism of action of gallic acid-loaded graphene oxide (GAGO) on several *Vibrio* spp. The antibacterial properties were investigated through disk diffusion, CFU counting method and time-kill studies. The mechanisms of action for the antibacterial effect were elucidated through TEM observations, DNA and protein leakage, ROS production, membrane integrity study, zeta potential measurement, as well as FTIR analysis. GAGO showed complete inhibition against all 3 strains at 1000 μg/mL. Over 70% of cells were dead following 4 h of incubation with GAGO, increasing to 99% after 8 h. Treatment with GAGO exhibited a significant DNA and protein leakage, compromising the membrane integrity. The increase in zeta potential value and loss of absorption spectra of FTIR indicate extensive damage in the cell membrane. TEM observations revealed morphological changes and reduction in cellular content following treatment with GAGO. In conclusion, GAGO exhibited anti-bacterial effects toward *Vibrio* spp. through membrane disruption, leading to DNA and protein leakage, and alteration of key functional groups. These results offer a new perspective on GAGO's potential as an alternative that might reduce *Vibrio* infections and reduce the use of conventional antibiotics.

Keywords: *Vibrio* spp., Vibriosis, Graphene Oxide, Gallic Acid, Antibacterial, Aquaculture

Project: Elucidating the anti-bacterial potential of gallic acid loaded graphene oxide (GAGO) nano formulation and the mechanism of action involved for management of vibriosis in aquaculture

Funding: Ministry of Higher Education Malaysia under Fundamental Grant Research Scheme (FRGS), grant number FRGS/1/2023/WAB04/UPM/02/21 and Universiti Putra Malaysia under Putra Grant (GP-IPS), grant number GP-IPS/2023/9742000

Probiotic-mediated bivalent vaccine against ciliate parasite and viral nervous necrosis in Bluefin bream (*Sparidentax hasta*)

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The disease due to marine ich resulted in losses of over 2000 kg of sobaity (*Sparidentax hasta*) and shaeim (*Acanthopagrus latus*) at the Kuwait Institute for Scientific Research (KISR). Nervous necrosis virus (NNV) is known to cause severe losses, especially in the larval phases in hamoor (brown spotted grouper, *Epinephelus coioides*). This work aimed to develop a host-friendly vaccination method using a genetically manipulated probiotic bacterium, *Bacillus subtilis*, to deliver antigenic material to the hindgut of vaccinated fish. On an out-sourcing basis, the bacterium procured was found to release the antigenic components into the culture supernatant. The bacteria were incorporated into feed and administered twice daily for optimizing the dose of vaccination, at 10^5 (LD), 10^6 (MD), and 10^7 (HD) cells/g of feed for 15 d. The fish were evaluated during the 90-d post-vaccination period for growth, survival, antibody response, and gene expression capabilities. Natural infection that occurred in the fish holding facilities in the adjacent building was used to evaluate the batches of vaccinated and control fish (cohabitation challenge). The dose of 10^6 cells/g of feed is found to be the optimum dose of vaccination. Kidney, spleen, and intestine showed localization of antigens, indicating a positive correlation with the antibody response as measured through ELISPOT assays. Relative expression of immunoglobulin (Ig) gene was higher in lymphoid tissue, such as the head kidney, spleen, and liver, at 30 days post-vaccination. However, peak expression in the intestine was seen at 60 dpv. Relative expression of recombinase-activating gene (Rag) followed a similar trend. All the genes were better expressed in MD group compared to the LD group and showed no significant difference ($p > 0.05$) with that of the HD group.

Keywords: Seabream, *Bacillus subtilis*, Immunity, Oral Vaccine, Antibody, Challenge

Field evaluation of a nanobubble-based non-invasive vaccination approach for preventing streptococcosis in tilapia

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Oxygen nanobubbles (O_2NB), combined with the positively charged polymer chitosan (CS), have recently emerged as a novel cationic and non-invasive delivery system for immersion vaccination in fish. In parallel, pre-treatment with ozone nanobubbles (O_3NB) has been shown to stimulate immune responses and enhance mucosal antigen uptake. This study evaluated a synergistic vaccination strategy integrating O_3NB pre-treatment with an O_2NB/CS-formulated heat-killed vaccine, followed by an oral booster, for the prevention of *Streptococcus agalactiae* serotypes Ia and III in tilapia under field conditions. Field trials were conducted at four commercial farms using 56,220 juvenile red tilapia (∼30 g) reared in net-pen cages. Fish were allocated into three groups with two to three replicates each: (G1) unvaccinated control, (G2) immersion vaccination with O_2NB/CS-vaccine, and (G3) O_3NB pre-treatment followed by O_2NB/CS-vaccine. All vaccinated groups received an oral booster on day 28. Antigen uptake in gill tissues, immune-related gene expression, and systemic antibody responses were assessed throughout the trial. On day 60, a laboratory-based bacterial challenge using fish sampled from each farm revealed that G3 fish exhibited significantly higher relative percent survival (RPS: 42.9%-70.0%) compared to G2 (4.5%-36.2%). Final survival rates and biomass measurements further supported these findings. Overall, the combined nanobubble-based prime-boost vaccination approach represents a promising, scalable, and non-invasive strategy for disease prevention in tilapia aquaculture.

Keywords: Immersion Vaccine, Nanobubbles, Streptococcosis, Immune Response, Field Trial, Tilapia

Funding: International Development Research Center (IDRC), Ottawa, Canada

Assessment of biological responses, accretion, depletion of residues and determination of withdrawal period in Nile tilapia, *Oreochromis niloticus* administered dietary antiparasitic drug lufenuron

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The increasing prevalence of diseases in tilapia aquaculture systems has led to a rise in the use of veterinary medicinal products, but their pathophysiological effects on fish remain poorly understood. A study was conducted to assess the effects of dietary administration of the antiparasitic drug lufenuron (LN) on the safety and biological responses of *Oreochromis niloticus* juveniles at the recommended dose (5 mg) and overdose (12.50 mg/kg biomass/day) for a consecutive 7 days. The LN caused a dose-dependent decrease in feed intake, survival, and biomass. The plasma biochemical analyses revealed an elevation in the levels of glucose, creatinine, liver enzymes, and a decrease in calcium, chloride, and acetylcholinesterase in LN-fed *O. niloticus*. The administration also significantly influenced haematology and altered the erythro-morphology. Erythrocyte alterations like vacuolation, irregularly shaped cells, tear drop-like cells, crenation, micronucleus, blebbed nucleus and notched were noted. The oxidative stress biomarkers, such as malondialdehyde, ferric-reducing antioxidant power, and total nitric oxide, increased, while levels of glutathione-S-transferase and catalase decreased during the administration. Nevertheless, the changes were reversible with the cessation. The LN residues peaked on day 7 of dose administration in the plasma, muscle, liver, and kidney. With dose suspension, residues declined in both groups, but were detectable until day 35 post-dosing. The withdrawal period for LN in *O. niloticus* was estimated to be 2 days, considering a maximum residue limit of 1350 μg/kg. Despite the short-term adverse effects, dietary administration of LN appears safe for *O. niloticus* juveniles at the recommended dose.

Keywords: Tilapia Aquaculture, Parasitic Diseases, Anti-parasitic Drug, Haematology, Drug Residues, Dose-dependent Toxicity

Project: All-India Network Project on Fish Health
Funding: Indian Council of Agricultural Research

Intratumoral tigilanol tiglate (Stelfonta TM) for the treatment of cyprinid skin tumours: A preliminary case series

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Skin tumours in ornamental fish are commonly managed through surgical excision or cryosurgery, yet recurrence remains a frequent challenge. Tigilanol tiglate (TT), marketed as Stelfonta^{TM}, is a naturally derived compound from *Fontainea picrosperma* with established efficacy in treating non-resectable mast cell tumours in dogs and other neoplasms in horses. Despite its success in terrestrial species, TT's application in aquatic animals remains largely unexplored. This study presents a preliminary case series investigating TT's efficacy in treating skin tumours in cyprinid fish. Fish presenting with observable skin tumours underwent anaesthesia and surgical biopsy for histopathological and immunohistochemical classification using markers such as S100, CD34, CD54, vimentin, and periaxin. Each tumour was treated with a single intratumoral injection of TT at 0.05 mg/cm^3 using QUATRON® multi-needle injectors or a standard 31-gauge needle for smaller masses. Post-treatment monitoring included assessment of tumour necrosis, sloughing, healing progression, and side effects. Topical treatment and debridement were applied as needed. Preliminary results indicate variable but promising responses, ranging from complete tumour necrosis and sloughing to limited cellular death. Healing outcomes appeared dose-dependent, with minimal adverse effects observed. Histopathological and IHC analyses are pending, but early findings suggest TT's potential as a targeted therapy for cyprinid skin neoplasms. This study aims to establish species-specific dosing protocols and a therapeutic window for TT in cyprinids, contributing to the development of standardised treatment approaches. These findings may pave the way for broader applications of TT in aquatic veterinary oncology and improve outcomes for ornamental fish with skin tumours.

Keywords: Tigilanol Tiglate, Cyprinid, Fish, Tumour, Treatment

Funding: The University of Adelaide, QBiotics Pty Ltd

Brown seaweed extracts improve gut microbial health, stimulate innate immunity, enhance growth performance, and disease resistance against *Vibrio* pathogen in the white shrimp *Penaeus vannamei*

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Penaeus vannamei is among the most economically significant aquaculture species; however, high-intensity farming practices has led environment and physiological stress leading to increased susceptibility to pathogens. The dietary incorporation of seaweeds offers a sustainable strategy for enhancing shrimp health and disease resistance, thereby reducing dependence on chemical agents in aquaculture. This study investigates the effects of dietary supplementation with brown seaweed extract on growth performance, gut microbiota, immune modulation, and disease resistance in *P. vannamei*. Shrimp post-larvae were fed diets containing the extract @ 370 mg/kg (T I), 37 mg/kg (T II), 3.7 mg/kg (T III), 0.37 mg/kg (T IV) and a negative control (TV)–over a 100-day period. All treatment groups exhibited improvements in average body weight and feed conversion ratio (FCR), with the T II group (37 mg/kg) showing the most significant enhancement in growth and gut microbial diversity, marked by increased abundance of *Actinobacterota* (43.58%) and *Firmicutes* (26.09%). Following a *Vibrio parahaemolyticus* challenge, shrimp in the T II group demonstrated a 70% survival rate and a reduced *Vibrio* load in the haemolymph. Additionally, seaweed-fed shrimp showed elevated expression of immune-related genes, antioxidant enzymes, and beneficial bacterial populations. These findings highlight the potential of brown seaweed extract as a functional feed additive to enhance growth, health, and disease resistance in shrimp aquaculture.

Keywords: Gut Microbiota, Immune Gene, *Vibrio parahaemolyticus*, Seaweed

Project: Development of probiotics and immunostimulants for shrimp

Funding: Consortium Research Platform on Vaccine and Diagnostics, Indian Council of Agricultural Research, India

Comparative transcriptomic and bioinformatic analysis of biocidal activity of stylicin from *Penaeus vannamei* against *Vibrio harveyi*

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The present study was conducted to evaluate the antibiofilm potential of stylicin from *Penaeus vannamei* against *Vibrio harveyi*. The minimal inhibitory concentration was determined to be 100 µg, and SEM and confocal microscopy imaging revealed that, the stylicin significantly affected cell morphology and biofilm density. Comparative transcriptomic analysis on stylicin-treated *V. harveyi* generated 74107048 (control-36354900; stylicin treated - 37752148) clean reads and resulted in 215 DEGs with 131 upregulated genes and 84 downregulated genes. The genes such as NO-inducible flavohemoprotein, NnrS family protein, cryptochrome/photolyase family protein, which mainly help to maintain the physiological activity were upregulated with stylicin. The downregulated genes were mainly associated with Type 6 secretion system, adhesion and biofilm formation, which are considered to be associated with the main virulence factors of *V. harveyi*. Further protein-protein interaction analysis identified five potential target proteins for stylicin, (CysD, MetK, GroEL, Gap, and TssJ). Docking studies showed strong binding affinity, with optimal docking scores, indicating highly favourable interactions. Molecular dynamics simulation also confirmed enhanced stability and compactness of the protein-stylicin complexes with stable hydrogen bonds and non-bonded contacts. This finding highlighted the potential of stylicin by targeting the key virulence factors of *V. harveyi*, paving the way for further validation and therapeutic potential.

Keywords: Stylicin, *Penaeus vannamei*, *Vibrio harveyi*, Virulence protein, Microscopic images, Transcriptomic analysis

Project: Healthy shrimp and GIFT Tilapia production through Biofloc based farming system: Development of technology and Standard operating procedure (DBT/PR11721/AAQ/683/2014) & Evaluation and refinement of biofloc based new age farming technology through effective microbial management, recirculation and input optimization for sustainable intensification across different aquaculture systems (NASF)

Funding: Department of Biotechnology, Government of India; ICAR-National Agricultural Science Fund, India

Molecular and histopathological confirmation of *Lactococcus garvieae* infection in *Litopenaeus vannamei* cultured in low salinity water

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Litopenaeus vannamei shrimp samples were collected from disease outbreak ponds across 10 farms in Tamil Nadu and Andhra Pradesh, India, between January 2022 and October 2024. The shrimps were cultured in low salinity water (<6 ppt), where farmers observed disease symptoms such as loss of appetite, stunted growth, discoloration, and persistent low-level mortality. The collected moribund shrimps were subjected to various assays to identify the causative agent. Wet mount examinations revealed granulomatous lesions, coagulative necrosis, and clusters of coccoid bacteria in the gills and muscle tissue. The isolated pathogenic bacteria were identified as Gram-positive cocci and tested positive for methyl red, arginine, glucose, arabinose, sucrose, lactose, sorbitol, mannitol, and rhamnose. Antibiogram results showed resistance to multiple antibiotics including ampicillin, trimethoprim/sulfamethoxazole, streptomycin, erythromycin, nalidixic acid, tetracycline, ceftazidime, amoxyclav/clavulanic acid, and azithromycin. Molecular identification using 16S rRNA gene amplification revealed 99.9% similarity to *Lactococcus garvieae*, and the sequences were submitted to NCBI GenBank (Accession Nos. OQ096627 and OQ096658). Pathogenicity tests demonstrated 100% mortality within 144 hrs at a concentration of 10^6 CFU/mL, with higher mortality observed in salinity levels between 0-5 ppt. *L. garvieae* was re-isolated from moribund shrimp to accomplish Koch's postulates. Histopathological analysis revealed multiple granulomatous lesions with bacterial masses in infected organs. Transmission electron microscopy (TEM) indicated that the *L. garvieae* strain lacked an outer capsular layer. Tests for antagonistic activity using commercial probiotic products showed no zone of inhibition. Based on these findings, *L. garvieae* is recognized as an emerging and significant pathogen in low-salinity shrimp aquaculture, warranting further investigation into preventive and control measures.

Keywords: Shrimp, Lactococcosis, Koch's Postulates, Antibiogram, Granuloma

Project: National Surveillance Programme on Aquatic Animal Diseases (NSPAAD)

Funding: Pradhan Mantri Matsya Sampada Yojana (PMMSY) through National Fisheries Development Board

Commercialization and revenue forecasting of aquaculture health technologies in India

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Aquaculture health technologies are pivotal in strengthening the industry by safeguarding fish health, boosting productivity, and fostering sustainable practices. It enables real-time monitoring, early disease detection, optimized feeding, and improved water quality management resulting in higher efficiency, reduced losses, and a lower environmental footprint. Commercializing and fostering these innovations turn research breakthroughs into economic value through licensing, strategic partnerships, and specialized professional services. The complex challenges like disease control, antibiotic resistance, environmental management, climate adaptation, biosecurity, traceability etc. has been addressed through effective partnership among researchers, industry and academia. These technologies and innovations developed and disseminated from ICAR-CIBA was found to be highly commercialized in Tamil Nadu (39%), Telangana (36%), Andhra Pradesh (18%), Karnataka (7%), Maharashtra (6%), Gujarat (5%), West Bengal (3%) and Madhya Pradesh (1%) using Social Network Analysis. The revenue generated through these technologies was evaluated and forecasted using a Grey Model; GM (1, 1). In essence, disseminating technology by ITMU across all states is fundamental for sustainable, resilient, and inclusive aquaculture development; helping India secures its leadership in global seafood production while protecting the environment, economy, and public health.

Keywords: Sustainable Aquaculture, Health Technology, Grey Model

Funding: ICAR-Central Institute of Brackishwater Aquaculture

Recombinant stylicin peptide from *Penaeus vannamei*: Anticancer and apoptotic effects on human cancer cells

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Cancer is a leading global cause of death, with treatment often limited by drug resistance, poor selectivity, and toxicity to normal cells. These challenges have highlighted the need for novel therapeutic agents. Antimicrobial peptides (AMPs), key components of innate immunity in various organisms, exhibit selective cytotoxicity, low resistance potential, and immunomodulatory effects. Recent evidence suggests that AMPs can inhibit cancer cell proliferation and metastasis. This study investigates the anticancer potential of the recombinant AMP stylicin, derived from *Penaeus vannamei*, against human prostate cancer (PC-3) cells. Cell viability and inhibitory concentration (IC_{50}) values were determined using MTT assay. Apoptotic morphological changes were visualized using propidium iodide (PI) staining. Apoptosis was further confirmed using Annexin V-FITC/PI and dual acridine orange/ethidium bromide (AO/EB) staining. Mitochondrial membrane potential was analyzed using Rhodamine 123 staining. Cell cycle distribution and apoptosis-related changes were evaluated using flow cytometry. Stylicin peptide treatment resulted in a dose-dependent reduction in prostate cancer (PC-3) cell viability, with an IC_{50} value of 250 μg/mL. Morphological and nuclear changes were consistent with apoptosis characteristics. Annexin V-FITC/PI and AO/EB staining revealed the presence of early, late apoptotic, and necrotic cells. Rhodamine 123 staining indicated mitochondrial membrane depolarization following treatment concentrations. Flow cytometry analysis demonstrated that stylicin induced cell cycle arrest at G2/M phase after 24 hours treatment. Collectively, these findings suggest that stylicin effectively induces apoptosis and inhibits proliferation in prostate cancer cells. This study highlights the potent anticancer activity of recombinant stylicin peptide against human prostate cancer cells, acting via mitochondrial disruption, apoptosis induction, and cell cycle arrest. With high selectivity and low toxicity, stylicin and similar antimicrobial peptides offer promising prospects for future cancer therapies.

Keywords: Recombinant Stylicin, *Penaeus vannamei*, MTT, Annexin V-FITC, Flow Cytometry.

Project: Evaluation and refinement of biofloc based new age farming technology through effective microbial management, recirculation and input optimization for sustainable intensification across different aquaculture systems

Funding: National Agricultural Science Fund (NASF), Indian Council of Agricultural Research

Development of a polyclonal antibody against recombinant spore wall protein (rSWP) and whole spore of *Ecytonucleospora hepatopenaei* for immunodiagnostic application

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Diseases continue to pose a major threat to shrimp farming, significantly impacting global seafood production. *Ecytonucleospora hepatopenaei* (EHP), a microsporidian parasite, the causative agent of the disease hepatopancreatic microsporidiosis causes retarded growth and size variation resulting in severe economic losses. Rapid, early and accurate diagnosis is essential for the effective management of the diseases. Among various diagnostics, immunoassay methods are rapid, cost effective and can be used at the point of care and do not need expensive equipment. This study aims at developing polyclonal antibodies against the whole spore and specific spore wall protein (SWP) of EHP. Whole spores were purified from infected shrimp by percoll gradient method and the recombinant spore wall protein (rSWP) was over expressed in *E. coli* and purified by column chromatography. Both purified whole spore and rSWP of EHP were used for immunizing rabbits. The purified polyclonal antibodies were evaluated for titre and specificity by ELISA and western blot. In ELISA, the antisera of rabbit immunized with whole spore of EHP, reached a titre of up to 1:8,19,200 with 1,00,000 spores/ well and the antisera of rabbit immunized with rSWP of EHP, reached a similar titre with 1μg/ml of antigen. The dot blot assay performed with the polyclonal antibodies developed against whole spore of EHP successfully detected antigen down to 104 spores/ml. These antibodies can be used in developing immunodiagnostic assays, such as immunohistochemistry, immunofluorescence assay, lateral flow immunoassays and Dot ELISA in combination with monoclonal antibodies to enhance sensitivity.

Keywords: Polyclonal Antibody, Spore Wall Protein, Recombinant Protein Expression, Column Chromatography.

Project: Network project on vaccines & diagnostics

Funding: Indian Council of Agricultural Research

Recombinant viral protein production against shrimp white spot syndrome virus (WSSV): Engineering microalgae *Chlamydomonas reinhardtii* against VP28 of WSSV

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White Spot Syndrome Virus (WSSV) represents one of the highest economic burden in shrimp aquaculture worldwide. Ineffective control measures underscore the pressing need for innovative approaches to combat this persistent threat. VP28, a key envelope protein plays a crucial role in viral entry, making it a promising candidate for vaccine development against WSSV. To explore a cost-effective and scalable production system, the VP28 gene was engineered for expression in green microalgae *Chlamydomonas reinhardtii* as a platform for producing recombinant proteins and high-value compounds. However, its widespread industrial use remains limited due to a lack of robust genetic engineering tools and the challenge of identifying economically viable products. The VP28 gene was cloned into the pASapI vector under the control of a high-expression algal promoter, and a codon-optimized variant was designed to enhance translational efficiency in *C. reinhardtii*. Both constructs were verified by restriction digestion and sequencing before being transformed into *C. reinhardtii* via electroporation. Positive transformants were selected using antibiotic resistance and molecular analysis confirmed successful transgene integration and transcription. This work establishes *C. reinhardtii* as a viable platform for recombinant WSSV protein production with codon optimization further improving expression yields a step toward developing sustainable algal-based vaccines for aquaculture.

Keywords: WSSV, VP28, *Chlamydomonas reinhardtii*, Electroporation, Recombinant Protein

Project: Development of microalgae based improved delivery method for control of shrimp white spot syndrome virus (WSSV) in cultured ponds

Funding: ICAR-Central Institute of Brackishwater Aquaculture

Biosafety, pharmacokinetics and tissue distribution of oxolinic acid in Milk fish (*Chanos chanos*)

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This study investigated the biosafety, pharmacokinetics, and tissue distribution of oxolinic acid in milkfish (*Chanos chanos*) following oral administration of medicated feed. A bio-safety experiment was conducted to assess the effects of oxolinic acid on milkfish. The experiment involved administering oxolinic acid at doses of 12 mg/kg, 36 mg/kg, 60 mg/kg, and 120 mg/kg over a 21-day period, including dosing and 21-day post-dosing periods. Key parameters, including animal behavior, feeding behavior, water quality, survival rate, average body weight, and histopathological changes, were systematically recorded and analyzed. No abnormal behavior was recorded. Feed consumption was 100%, and there was no mortality. The second experiment was designed to study the drug kinetics following oral administration. Acclimatized fish were divided into three replicate tanks, and after a 24-h starvation period, the medicated feed was administered once at 2% of the total body weight. Blood and tissue samples (liver, kidney, bile, and muscle) were collected at specific time intervals (0, 2, 3, 4, 6, 8, 12, 16, 24, 32, 48, 64, 96, and 128 h) after in-feed drug administration. Plasma samples were separated from blood samples by centrifugation. All samples were stored at -40°C until analysis. Tissue and plasma samples were prepared for quantification of oxolinic acid using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Pharmacokinetic parameters were estimated using compartmental modelling techniques. The results demonstrated that after oral administration, oxolinic acid concentration peaked in bile, followed by liver, muscle, kidney, and plasma. The drug was rapidly absorbed into the bloodstream, with plasma concentration peaking at approximately 6 h. The liver exhibited rapid uptake, with the concentration peaking at around 2 h, while the kidney concentration peaked at approximately 3 h. Bile exhibited a slower accumulation, with peak concentrations at 8 and 24 h. The pharmacokinetic parameters exhibited significant inter-tissue variability. The liver exhibited the highest absorption rate, while bile exhibited the lowest. In conclusion, this study provides a comprehensive pharmacokinetic characterization of oxolinic acid distribution in milkfish tissues, elucidating substantial inter-tissue variations in absorption, distribution, and elimination. The findings underscore the paramount importance of considering tissue-specific drug concentrations when determining optimal dosage regimens and withdrawal intervals in aquaculture practices.

Keywords: Antimicrobial, Oxolinic Acid, Pharmacokinetics, Biosafety, Dose Regime

Project: All India Network Project on Fish Health
Funding: ICAR-Central Institute of Brackishwater Aquaculture

Biological effects of praziquantel (PZQ) on environmental toxicity indicator organisms: Microalga, *Chlorella marina* and copepod, *Apocyclops* sp.

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Praziquantel (PZQ), a broad-spectrum anthelmintic extensively used in aquaculture, and need to be tested for its effect on non-target marine species. This study has evaluated the biological and oxidative stress responses of the marine microalga, *Chlorella marina* and the copepod, *Apocyclops* sp. following exposure to various concentrations of PZQ (0, 10, 20, 40, 80 mg/L). The estimated LC_{50} (96 h) of *C. marina* was 65.74 mg/L, and EC_{50} (48 h) of *Apocyclops* sp. was 56.09 mg/L. In *C. marina*, PZQ exposure increased the cell density and photosynthetic pigments such as chlorophyll a, chlorophyll b and total chlorophyl while it decreased the cell density and photosynthetic pigments indicating inhibited algal growth @ 40 & 80 mg/L. Biochemical assays revealed increased oxidative stress, evidenced by elevated activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), lipid peroxidation (LPO) and glutathione oxidation (GSSG), particularly at higher concentrations of 80 mg/L. *Apocyclops* sp. exposed to similar concentrations (40 & 80 mg/L) showed acute toxicity responses, including increased mortality, behavioural alterations (e.g., impaired swimming), and possible metabolic and reproductive inhibition. But PZQ was found to be neither effective nor lethal @ the concentration up to 40 mg/L during the first 24 h of exposure. The present findings highlighted that PZQ is safe to use at 20-40 mg/L but not at 80 mg/L.

Keywords: Praziquantel (PZQ), Environmental indicators, Toxicity, Microalga, Copepod

Project: All India Network Project on Fish Health

Funding: ICAR-Central Institute of Brackishwater Aquaculture

Approaches to managing parasitic infections in fish culture

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Brackishwater aquaculture relies heavily on precise and intensive management practices, which necessitate a comprehensive understanding of the complex interactions among environmental factors, host health, and pathogen behaviour. Maintaining the well-being of farmed aquatic species is vital for the long-term viability of these systems and requires a holistic approach that integrates prevention, early detection, and effective treatments. Parasitic infections pose a significant challenge in aquaculture, leading to substantial economic losses due to both morbidity and mortality. The primary parasites affecting fish in brackishwater environments include protozoans (such as ciliates and dinoflagellates), monogeneans, and parasitic crustaceans are particularly problematic in fish hatcheries and grow-out systems. Timely identification is critical in managing ectoparasitic outbreaks. Metazoan parasites are easier to detect than protozoan infections. Common treatment approaches include the application of chemicals and therapeutics. Treatment approaches commonly involve the use of chemical agents and therapeutics. Under the Network Project on Fish Health, studies have been conducted to evaluate the efficacy, withdrawal periods, and biosafety of drugs such as emamectin benzoate, praziquantel, and copper sulfate ($CuSO_4$). These treatments have been effective in controlling infestations of the crustacean parasite *Caligus minimus*, the monogenean *Diplectanum penangi*, and the dinoflagellate *Amyloodinium ocellatum* in brackishwater hatcheries and culture systems. Although not yet widely adopted, vaccination and selective breeding for parasite-resistant fish represent promising strategies for enhancing disease resistance in aquaculture.

Keywords: Fish Parasites, Drug, Immune Responses

Project: AINP-FISH HEALTH

Funding: ICAR-Central Institute of Brackishwater Aquaculture

Rapid on-site detection of WSSV and EHP using multiplex LAMP coupled with lateral flow strip

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Diseases remain a major constraint to the sustainability of shrimp aquaculture with pathogens such as white spot syndrome virus (WSSV) and *Ecytonucleospora hepatopenaei* (EHP) are causing severe economic losses globally. Early, rapid and point of care diagnostics and timely interventions are necessary for the effective management of diseases. In this study a multiplex LAMP coupled lateral flow was developed for the simultaneous diagnosis of WSSV and EHP. The LAMP primers were designed to target the specific regions such as envelope protein VP28 gene of WSSV and spore wall protein gene (SWP) of EHP. To enable multiplex detection using lateral flow strips forward inner primer (FIP) of EHP was labelled with biotin at the 5' end and backward inner primer was labelled with FAM at the 5'end and WSSV FIP was labelled with digoxigenin at the 5'end and BIP with FAM at the 5'end. This multiplex LAMP was performed in a simple dry bath and optimised at 65°C. This multiplex LAMP was found to be highly sensitive can detect as few as 10 copies of WSSV and 10 copies of EHP. This assay was rapid and can detect the disease within 55 minutes. This assay demonstrated 100% diagnostic sensitivity and specificity. This LAMP was highly specific and did not show any cross amplification with other shrimp pathogens such as IMNV, IHHNV, *Vibrio* spp. or with host genomic DNA of *P. vannamei* and *P. indicus*. No non-specific binding was observed in lateral flow. This multiplex LAMP assay coupled with lateral flow offers a rapid, sensitive, specific point of care diagnostic assay that can be effectively used for routine on-farm level surveillance and early disease intervention in shrimp culture.

Keywords: White Spot Syndrome Virus, *Ecytonucleospora hepatopenaei*, *Penaeus vannamei*, Multiplex LAMP

Project: Consorita research Platform on Vaccines and Diagnostics

Funding: ICAR-Central Institute of Brackishwater Aquaculture

Dietary strategies for immune modulation and enhanced disease resistance in red hybrid tilapia coinfected with Tilapia Lake Virus and *Aeromonas hydrophila*

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Tilapia Lake virus (TiLV) and *Aeromonas hydrophila* are two important pathogens which pose major threat to the global tilapia aquaculture. Previously, studies have demonstrated that co-infection with both pathogens can synergistically worsen the fish mortality. Improving disease management strategies is therefore important to improve the survival and health of co-infected fish. This study investigated the effectiveness of two dietary supplementation strategies in enhancing the disease resistance of red hybrid tilapia (*Oreochromis* spp.) challenged with both TiLV and *A. hydrophila*. The experimental design included a control co-infected fish fed with a standard diet, and two treatment strategies fed with a standard diet supplemented with either additive A (strategy A), a blend of organic acids and a lyso-phospholipid-based digestive enhancer or standard diet supplemented with additive B (strategy B), an organic acid blend enriched with natural immunostimulants and essential nutrients. Results showed that while the control group had a mortality of 76.3%, fish supplemented with strategy A and B exhibited a significant lower mortality at 50% and 41.7%, respectively ($p < 0.05$). Strategy B also enhanced immune responses, as indicated by the lower expression of key immune genes (*il-8*, *mx*, and *rsad2*), and reduced histopathological damage in the intestines, liver, and spleen. Although Strategy A did not significantly affect gene expression or pathogen load, it resulted in milder intestinal lesions compared to the control co-infected fish, suggesting protective effects on gut health. These findings highlight the potential of functional feed additives, particularly organic acids and immunostimulants, to mitigate the impacts of coinfections and promote healthier tilapia production.

Keywords: Coinfection, Feed Additives, Tilapia Lake Virus (TiLV), *Aeromonas hydrophila*, Aquaculture Health

Funding: Kasetsart University of Research and Development

Production and evaluation of IgY antibodies for passive immunization against Tilapia Lake Virus

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Tilapia lake virus (TiLV) remains a critical threat to tilapia aquaculture worldwide, causing considerable economic losses due to the lack of effective vaccines or therapeutic interventions. As an alternative approach to disease control, this study explored the use of passive immunization through immunoglobulin Y (IgY) antibodies targeting TiLV. Hens were immunized intramuscularly with recombinant segment 4 protein of TiLV, and the resulting IgY antibodies were subsequently isolated and purified from egg yolks using polyethylene glycol precipitation technique. Specificity testing by Western blot confirmed that the IgY antibodies selectively recognized the TiLV antigen without cross-reacting with unrelated proteins. Neutralization assay demonstrated that the purified IgY can dose-dependently decrease in TiLV infectivity from the 5.01×10^6 $TCID_{50}$/mL to 5.01×10^4 – 1.26×10^5 $TCID_{50}$/mL. Furthermore, the neutralization effects of the IgY were further confirmed by the observation of cytopathic effects in vitro, and the detection of viral antigen in fish cells using immunofluorescence analysis. Although variations in neutralizing activity were observed among individual hens, the overall findings underscore the potential of IgY as a promising antiviral agent. This study supports the feasibility of IgY-based therapies as a sustainable and ethical strategy for the prevention of TiLV in aquaculture, while emphasizing the need for further optimization regarding delivery methods and stability in practical applications.

Keywords: Tilapia Lake Virus (TiLV), IgY Antibodies, Chicken Eggs, Neutralization Assay, Passive Immunization

Funding: Kasetsart University of Research and Development

Development and application of a recombinant protein-based indirect ELISA for the detection of TiLV-specific antibodies

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The rapid growth of tilapia aquaculture worldwide is increasingly challenged by emerging viral pathogens, particularly Tilapia Lake virus (TiLV). While the infection of TiLV has caused severe mortality to the fish farms, the effective surveillance and disease management of this pathogenic virus are still limited. In this study, we developed and validated an indirect enzyme-linked immunosorbent assay (ELISA) targeting TiLV-specific antibodies using a recombinant nucleoprotein derived from segment 4 (S4) of the TiLV genome. The open reading frame of TiLV-S4 was cloned into pET28a (+) vector and expressed in *Escherichia coli* BL21(DE3). The recombinant TiLV S4 protein (∼38 kDa) was purified via affinity chromatography and confirmed for immunoreactivity using rabbit antiserum specific to TiLV. ELISA optimization determined the ideal coating concentration at 0.5 μg/mL and fish serum at a dilution of 1:100. The assay showed high repeatability and reproducibility, with intra- and inter-assay coefficients of variation values below 10%. Using 120 seronegative samples, the cut-off percent reactivity was established at ≥37.11. Diagnostic evaluation, compared against whole-virus ELISA, yielded sensitivity and specificity values of 82.5% and 90.0%, respectively. We further applied this TiLV-S4 ELISA to clinical serum samples from outbreak-affected farms. Results revealed antibody detection after 28, 39, 60, and 90 days post infection, at the rate of 80%, 39.47%, 45.24%, and 15%, respectively. The declination of antibody detectability in the fish serum over time post-infection reflected the dynamic nature of TiLV-specific humoral responses. In conclusion, the recombinant TiLV-S4 protein-based ELISA is a specific serological tool which is suitable for monitoring TiLV exposure in tilapia. Its practical application in field surveillance highlights its potential as a cost-effective component in integrated health management and disease prevention strategies in commercial aquaculture.

Keywords: Tilapia Lake Virus (TiLV), Indirect ELISA, Recombinant Protein, Antibody Detection, Aquaculture Diagnostics

Establishment and characterization of a red hybrid tilapia brain cell line for TiLV propagation

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Tilapia lake virus (TiLV) continues to pose a major challenge to the sustainability of global tilapia aquaculture. Although various fish cell lines have been employed for TiLV isolation, none had been derived specifically from red hybrid tilapia (*Oreochromis* spp.) until now. In this study, we established a novel cell line, designated RHTiB, originating from brain tissue of red hybrid tilapia. The cells exhibited a fibroblast-like morphology and were continuously subcultured for more than 50 passages over 18 months. Optimal growth occurred at 25°C in Leibovitz's L-15 medium supplemented with 10% fetal bovine serum at pH 7.4. Species identification based on cytochrome oxidase I gene sequencing confirmed the tilapia origin, and mycoplasma testing indicated no contamination. Following cryopreservation, the RHTiB cell line showed a revival rate of 75-80% after 30 days. Chromosomal examination at passage 25 revealed a diploid number (2n) of 44. While TiLV did not induce obvious cytopathic effects, both immunofluorescence microscopy and RT-qPCR confirmed effective viral replication within the RHTiB cells, reaching a peak viral load of $10^{7.82} \pm 0.22$ copies/400 ng cDNA on day 9 post-inoculation. The successful development of this red hybrid tilapia-derived cell line enhances available resources for virus propagation, offering a species-specific platform to support improved diagnostics, research, and virological surveillance in red tilapia aquaculture.

Keywords: Red Hybrid Tilapia, Cell Line, Tilapia Lake Virus (TiLV), Virus Propagation, Culture Conditions

Funding: Faculty of Veterinary Medicine, Kasetsart University

Pharmacokinetics and withdrawal period of oxolinic acid in Silver pompano (*Trachinotus blochii*) following in-feed administration

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Oxolinic acid (OA) is a widely recommended antimicrobial agent against gram-negative bacterial infections in aquaculture. The present study assessed the pharmacokinetics and withdrawal period of OA in *Trachinotus blochii*, a commercially important marine species, under tropical mariculture conditions. Drug quantification was performed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). For pharmacokinetic evaluation, a single oral dose of 12 mg/kg, the recommended dose for therapeutic applications was administered, and plasma and tissue samples from the intestine, muscle + skin, liver, kidney, and gills were collected at 12 time points ranging from 0.5 to 60 hours post-dosing. Peak OA concentrations (Cmax) were attained within 6 hours (Tmax). The concentration order was plasma (99.77) < muscle + skin (616.67) ≈ liver (666.67) < intestine (1764.67) ≈ gill (1776.67) ≈ kidney (1783.33). The elimination half-life (T½) was longest in the kidney, followed by the liver and intestine, while faster clearance was observed in plasma, muscle + skin, and gills. There was an extensive tissue distribution. OA was efficiently absorbed via the intestine and eliminated rapidly through renal, intestinal, and gill pathways, highlighting the primary role of the kidney in OA clearance. In the withdrawal study, following a 7-day oral administration at therapeutic dosage, OA levels in edible tissues exceeded the permissible residue limits at 6 hours but declined below detection within 24 hours. Accounting for a 30% safety buffer, a withdrawal period of 31.2 hours (equivalent to 37.7 °C-degree days) is advised to ensure food safety. These findings offer critical insights into the judicious use of OA in *T. blochii* culture, supporting sustainable aquaculture practices, residue management, and regulatory compliance.

Keywords: Snub Nose Pompano, Antimicrobial Residues, Therapeutic Dose, Tissue Residue

Project: All-India Network Project on Fish Health

Funding: Indian Council of Agricultural Research

Targeted biocontrol of *Vibrio parahaemolyticus* using bacteriophage in aquaculture

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The emergence of multidrug-resistant strains of *Vibrio parahaemolyticus*, a major pathogen in aquaculture, underscores the urgent need for alternative biocontrol strategies. In this study, we report the isolation and characterization of a lytic bacteriophage with high specificity against *V. parahaemolyticus*. Host range determination via spectrum analysis confirmed its narrow specificity towards *V. parahaemolyticus*, indicating its potential for targeted application with minimal impact on beneficial microbiota. Morphological examination using electron microscopy (TEM) revealed that the phage possesses an icosahedral head and a long, contractile tail, consistent with members of the *Myoviridae* family. Plaque and spot assays demonstrated clear lytic activity, producing well-defined plaques indicative of effective bacterial lysis. Phage genomic DNA was successfully extracted and is currently undergoing whole-genome sequencing to further elucidate its genetic composition and therapeutic potential. The high specificity, strong lytic capability, and distinct morphological features of this phage highlight its promise as a viable candidate for phage therapy and biocontrol of *V. parahaemolyticus* in aquaculture.

Keywords: Aquaculture, Lytic Bacteriophage, Phage Therapy, *Vibrio parahaemolyticus*

Project: Bioengineering of Polyvalent Endolysin as Therapeutics against Vibriosis in Aquaculture

Funding: Department of Science and Technology, Government of India

Antiparasitic effects of green synthesized silver nanoparticles against *Argulus siamensis* in *Labeo rohita* (Hamilton, 1822)

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Argulosis, caused by *Argulus siamensis*, is a major parasitic threat to freshwater aquaculture, particularly affecting *Labeo rohita*, one of the most susceptible species among Indian major carps (IMCs). In the present study, the argulocidal efficacy of silver nanoparticles synthesized using *Azadirachta indica* (neem) leaf extract (NL-AgNPs) was evaluated against *A. siamensis* infesting *L. rohita*. The biosynthesized NL-AgNPs had a characteristic λmax of 420 nm, Zeta potential of -28 mV, spherical shape with an average size of 20±5 nm, while Fourier Transform Infrared Spectroscopy (FTIR) identified the presence of various functional groups acting as capping and stabilizing agents. Further, an acute toxicity analysis in *L. rohita* revealed a 96 h median lethal concentration (LC_{50}) of NL-AgNPs was 28.25 ppm. No mortality was observed in control groups treated with either silver nitrate (1 mM) or in negative control group containing tap water, confirming the relative safety of NL-AgNPs at lower concentrations. Furthermore, the antiparasitic efficacy (AE) tests under *in vivo* condition were conducted at 25%, 50%, and 75% of the LC_{50} value (7.05, 14.12, and 21.19 ppm, respectively) against *A. siamensis* infested in *L. rohita* along with control groups. After 96 h of exposure, the AE was found to be 36%, 75%, and 100%, respectively. The median effective concentration (EC_{50}) was calculated to be 9.12 ppm, with a therapeutic index of 3.10, indicating a favourable therapeutic margin. The genotoxicity assays demonstrated a dose-dependent increase in DNA damage, with the highest concentration showing 15.49% DNA in the tail and a tail length of 6.35 px, indicating potential sub-lethal effects at elevated doses. Thus, neem-derived AgNPs emerge as a safe, eco-friendly, and effective alternative for controlling argulosis, supporting sustainable fish health management in freshwater aquaculture.

Keywords: Green Synthesis, AgNPs, *Argulus siamensis*, *Labeo rohita*, Safety

Project: Development of Phytotherapy against Argulus Parasite of Fish

Funding: ICAR-Central Institute of Fisheries Education, Mumbai

Essential oils based nanoemulsions against aquatic pathogenic oomycetes, *Saprolegnia parasitica* and *Saprolegina australis*: *in vitro* and *in vivo* study

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Saprolegniasis is one of the major obstacles in fish production worldwide, especially for the salmonids industry. A safe and effective treatment for saprolegniosis has yet to be established. Previously used and relatively effective treatments have been banned due to their carcinogenic or toxic properties. Natural compounds such as essential oils (EOs) are alternative to teratogenic antifungal agents, but EOs are not miscible in water, which limit their use as water soluble drug for disease management in aquaculture. Nanoemulsions are effective and promising delivery vehicles for lipophilic compounds such as EOs. Synergistic oil in water nanoemulsion compositions containing EOs i.e. thyme oil, carvacrol oil, ajwain oil, peppermint oil and cumin oil were prepared and explored for their potential against *Saprolegnia parasitica* and *S. australis*. Nanoemulsion compositions of EOs were prepared by low-energy spontaneous emulsification method. Fine oil droplets are spontaneously formed when an organic phase containing oil and a surfactant was mixed with an aqueous phase. Oil phase was prepared mixing three essential oils in different combinations to a total of 4% w/w and MCT oil 6% w/w and added to a surfactant in ratio of 1:1. The emulsions were sterile filtered and stored at room temperature. All five nanocompositions were showing *Saprolegnia* hyphal inhibitory activity. Three of the five formulations were selected further for spore inhibition and germination assay and one was found to have better efficacy for the inhibition. All three nanocompositions were nontoxic to erythrocytes and cell. The composition was safe for administering to rainbow trout eggs lesser than LC_{50} value of 59.71 mg/L and physically stable for at-least four months with particle size diameter 185 nm at room temperature. The selected composition applied at 5 and 10 ppm in-water treatment for 96 hr was safe and effective against *Saprolegnia* infection in rainbow trout eggs.

Keywords: Saprolegniasis, Nanoemulsion, Essential Oil, Natural Compounds, Toxicity

Project: Immunostimulatory potential of nanocomposition and effect of immunisation of rainbow trout against saprolegnia infections

Funding: ICAR-Central Institute of Brackishwater Aquaculture

Isolation of lytic phages against *Flavobacterium oreochromis* causing Columnaris in Thai freshwater aquaculture

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Bacterial diseases like columnaris pose a major threat to global aquaculture, particularly freshwater systems, which account for 33% of total aquatic animal production. In Thailand, *Flavobacterium oreochromis* is a primary cause of columnaris in tilapia (*Oreochromis* spp.), leading to severe economic losses. The widespread use of antibiotics in aquaculture has accelerated antimicrobial resistance, underscoring the urgent need for alternative, eco-friendly solutions. Bacteriophages, viruses that specifically infect bacteria, offer a promising alternative due to their host specificity, self-replicating nature, and minimal environmental impact. This study aimed to isolate lytic bacteriophages targeting *F. oreochromis* from Thai freshwater aquaculture. Water samples from culture ponds and rivers were centrifuged at 4500 × g for 20 minutes at 4°C and filtered through 0.22 μm filters. The clarified water was mixed with *F. oreochromis* (0.1 mL) in 10 mL Anacker and Ordal's broth (AOB) containing 1 mM $CaCl_2$ and incubated for 48 hours to amplify phages. Phage presence was confirmed using spot tests. Briefly, 0.1 mL of bacterial was mixed with 3 mL of semi-solid AOB containing 1 mM $CaCl_2$ and poured onto an Anacker and Ordal's agar (AOA) plate. Then, 5 μL of the phage solution was spotted onto the surface and incubated at 28°C for 48 hours. Double agar overlay assays were later used for phage isolation and purification. Individual plaques were picked and processed using SM buffer. In early trials, plaque formation was observed against *F. oreochromis* strain KCRT 2301(high virulent strain), indicating successful infection and lysis. These findings support further efforts to isolate and develop specific lytic phages as sustainable biological control agents against columnaris.

Keywords: Bacteriophage Therapy, Antibiotic Alternatives, *Flavobacterium* spp., Plaque Assay, Eco-friendly Disease Management

Project: Development of bacteriophages specific to pathogenic bacteria *Flavobacterium* sp. isolated from freshwater fish infected with the disease

Funding: "Graduate Scholarship Program for ASEAN or Non-ASEAN Countries" & "Center of Excellence in Fish Infectious Diseases (CE FID)", Chulalongkorn University, Thailand

Bioactive Metabolites from Purple Non-Sulfur Bacteria: A Novel Strategy to Combat *Vibrio parahaemolyticus*

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Purple non-sulfur bacteria (PNSB) exhibit metabolic versatility and have shown probiotic potential, positioning them as promising candidates for sustainable applications in aquaculture. Their capacity to generate bioactive secondary metabolites, demonstrate antagonistic effects against pathogens, and enhance host immunity establishes them as viable natural alternatives to antibiotics in the context of disease management. This study seeks to explore the antibacterial properties of extracts obtained from isolated PNSB strains in relation to *Vibrio parahaemolyticus*, a significant bacterial pathogen in aquaculture systems. The focus of our research is on assessing the inhibitory effects of PNSB extracts by conducting minimum inhibitory concentration (MIC) assays, as well as investigating their mechanism of action through the generation of reactive oxygen species (ROS) in bacterial cells. Additionally, the extracts underwent gas chromatography-mass spectrometry (GC-MS) analysis to identify bioactive compounds, which will then be computationally evaluated through molecular docking against essential *V. parahaemolyticus* virulence proteins. *In vivo* experiments utilizing shrimp models were carried out to assess the therapeutic efficacy and potential toxicity of the extracts in a controlled environment. The results shed light on the antimicrobial mechanisms of PNSB-derived compounds and facilitate their advancement as environmentally sustainable biocontrol agents in aquaculture. This study advances sustainable disease management strategies, aiming to decrease dependence on traditional antibiotics while improving the health of aquatic animals.

Keywords: Purple Non-sulfur Bacteria, *Vibrio parahaemolyticus*, Sustainable Aquaculture

Calcium phosphate nanoparticle-OMP conjugate as a potent oral vaccine inducing broad immune gene response in *Oreochromis niloticus* against *Aeromonas hydrophila*

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Aeromonas hydrophila is a major bacterial pathogen in *Oreochromis niloticus* (Tilapia), leading to significant economic losses in aquaculture. Developing a vaccine that effectively activates the host immune system is essential for disease prevention. This study evaluates the potential of calcium phosphate (CaP) nanoparticles conjugated with the outer membrane protein (OMP) of *A. hydrophila* (CaP-OMP) as a novel vaccine candidate, with particular emphasis on immune gene response. CaP nanoparticles were synthesized and conjugated with purified OMP to form the CaP-OMP vaccine. Tilapia were immunized with CaP-OMP, OMP, CaP, and a control, and monitored over a defined period. Post-vaccination, immune responses were assessed using quantitative real-time PCR (qPCR) targeting key immune-related genes such as *Cd4, Cd8, IgM, IgT, IL-6, MHC I, MHC II, TNF-α, IFN-γ*, and *β -actin* as the housekeeping gene. Opsonophagocytic killing assays (OPKA) and a challenge study using virulent *A. hydrophila* were also performed. The CaP-OMP group exhibited a significant upregulation of all tested immune genes compared to both the OMP and control groups, indicating activation of both innate and adaptive immunity. OPKA confirmed enhanced pathogen clearance in vaccinated fish. Upon challenge, the CaP-OMP group demonstrated a relative percentage survival (RPS) of 83.3%, highlighting the vaccine's protective efficacy. In conclusion, the CaP-OMP nanoparticle vaccine effectively stimulated immune gene expression and enhanced bacterial clearance in Tilapia. The upregulation of T-cell markers (*Cd4, Cd8*), immunoglobulins (*IgM, IgT*), cytokines (*IL-6, TNF-α, IFN-γ*), and antigen presentation molecules (*MHC I, MHC II*) indicates a broad immune activation. These findings support the use of CaP nanoparticles as a promising antigen delivery system for aquaculture vaccines, offering a targeted approach to disease management in tilapia farming.

Keywords: Nano Particle, Drug Delivery System, Outer Membrane Protein, Oral Vaccines

Computational insights into WSSV virulent protein interactions with perfluorotributylamine from *Halmonas salifodinae*

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The Asian subcontinents, especially India, are habitat to a large population of shrimp and other crustacean-infecting White Spot Syndrome Virus (WSSV). Shrimp aquaculture has suffered a major financial loss due to the virus. It is a highly transmissible disease that can result in total mortality 3 - 10 days after an outbreak. About 300 kb of the WSSV genome codes for several infection-mediating proteins. The infection process and the interface with host cells are significantly influenced by the envelope proteins, commonly VP28, VP26, and VP24. In this study, we have used the immediate early structural proteins VP28, VP91, and VP108 to see the *in silico* impact of antiviral compounds extracted from the *Halomonas salifodinae* bacterium ethyl acetate extract. In the previous study, the compound's antiviral activity had already been confirmed in shrimp (*Fenneropenaeus indicus*) *in vivo*. Molecular dynamics and simulation studies validate the impact of ligand binding on proteins. Combined, these *in silico* methods show how well the ligand inhibits the trimers' ability to perform physiological functions. Consequently, employing perfluorotributylamine as an antiviral and deciphering the intricate relationship between VP28, VP91, VP108, and perfluorotributylamine can pave the way for the development of antivirals against white spot disease.

Keywords: WSSV, Docking, Simulation, RMSD, RMSF

Project: Application of Recombinant Protein of Early Expressing Gene to Combat White Spot Syndrome Virus of Penaeid Shrimp

Funding: Savitribai Jyotirao Phule Fellowship for Single Girl Child (SJSGC), University Grants Commission, Department of Higher Education, Government of India

In silico design of a noval multi-epitope vaccine candidate against Infectious Spleen and Kidney Necrosis Virus

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Infectious spleen and kidney necrosis virus (ISKNV), a megalocytivirus, is a major viral pathogen in Asian seabass (*Lates calcarifer*) and Pearlspot (*Etroplus suratensis*), responsible for up to 40 - 50% mortality in affected aquaculture farms, leading to significant economic losses. Despite its impact, no approved vaccine is currently available to control ISKNV in seabass, highlighting a crucial gap in disease prevention strategies. This study was aimed to design a multi-epitope subunit vaccine candidate against ISKNV using immune informatics and structural vaccinology tools. Conserved viral proteins, including the major capsid protein, threonine kinase, and membrane glycoprotein, were retrieved from NCBI GenBank and screened for antigenicity using VaxiJen. High-affinity cytotoxic T lymphocyte (CTL), helper T lymphocyte (HTL), and B-cell epitopes were predicted using the IEDB resource. Epitopes were selected based on antigenicity, non-allergenicity, and toxicity. Selected epitopes were linked using suitable peptide linkers and fused to a toll-like receptor (TLR) agonist adjuvant (e.g., flagellin or β-defensin) to form a chimeric vaccine construct. The 3D structure of the vaccine was modelled using SWISS-MODEL and validated through Ramachandran plots and ProSA-web. Molecular docking using ClusPro predicted stable and favourable interactions between the vaccine and innate immune receptors TLR3 and TLR5 followed by molecular dynamics simulations (GROMACS), and immune response profiling using C-ImmSim indicated strong immunogenic responses with high IgM titres and memory cell activation. Results of this study suggest that, designed construct can potentially stimulate both humoral and cellular immunity in seabass. The proposed approach is cost-effective and uses only freely available resources, making it ideal for rapid vaccine development in low-resource settings. Overall, this study demonstrates the promise of in silico methodologies for accelerating fish vaccine design and provides a strong groundwork for future in vivo validation against ISKNV in Asian seabass aquaculture.

Keywords: Aquatic Immunoinformatics, Computational Vaccinology, Aquaculture Health, Viral Pathogenesis

Project: Development of recombinant microalgae expressing Nervous necrosis virus capsid protein for vaccinating finfish against viral nervous necrosis (CRP on Vaccines and Diagnostics)
Funding: ICAR-Central Institute of Brackishwater Aquaculture, Chennai

Preliminary *in vivo* and *in silico* evaluation of portoamides a and b against white spot syndrome virus in freshwater crabs (*Paratelphusa hydrodomous*)

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White spot syndrome, caused by the white spot syndrome virus (WSSV) is a devastating viral disease responsible for significant economic losses in the shrimp farming industry. In the search for natural therapeutic alternatives against WSSV, this study explored the antiviral potential of portoamides, natural peptides produced by the cyanobacterium *Phormidium* sp. LEGE 05292. Given that the structural proteins of the viral envelope, specifically VP28, VP26, and VP24, are the primary mediators of host cell attachment, they serve as promising targets for antiviral drug development. Our approach combined *in vivo* post-infection histopathological analysis with *in silico* molecular docking to assess the antiviral efficacy of portoamides. In the *in vivo* study, crabs were injected with portoamides alongside WSSV and monitored for 30 days post-infection. The antiviral activity of portoamides was evaluated through survival rates and histopathological observations. The results revealed that crabs treated with portoamides showed improved survival and reduced signs of viral infection compared to the control group. In parallel, *in silico* molecular docking analysis was conducted to assess the binding affinity between portoamides and the viral envelope proteins VP28, VP26, and VP24. The docking results demonstrated that these proteins exhibited the highest binding energies with portoamides, indicating a strong interaction that could potentially inhibit viral attachment and replication. Our findings suggest that portoamides effectively inhibit WSSV replication by interacting with the viral envelope proteins, thereby preventing the virus from establishing infection in crabs. Moreover, it is hypothesized that portoamides may stimulate the immune system in crabs, further enhancing resistance to WSSV infection. However, additional studies are needed to fully understand the immunomodulatory mechanisms involved. These preliminary results highlight the potential of portoamides as natural antiviral agents for combating WSSV in aquaculture settings, paving the way for future research on their application in disease management strategies.

Keywords: White Spot Syndrome Virus, VP28, VP26, VP24, Portoamides, Cyanobacteria

Back to Basics: locally produced heat-killed vaccine as a practical solution for preventing Streptococcosis in farmed tilapia (*Oreochromis* spp.)

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Streptococcus agalactiae is a major etiological agent responsible for streptococcosis in tilapia aquaculture. Although commercial vaccines exist to prevent the disease, accessibility can be limited for many farms, and the vaccines may fail to cover the locally prevalent serotypes and strains, conferring suboptimal protection. In this study, we developed a local solution employing a heat-killed vaccine targeting local serotypes (Ia and III) to prevent streptococcosis in tilapia (*Oreochromis* spp.) in the field. A controlled laboratory experiment was conducted to compare the potential application of heat-killed and formalin-killed vaccines (HKV and FKV). Antibody assay (IgM) by ELISA revealed a robust and consistent humoral immune response of vaccinated fish over 84 days. Moreover, the injection challenge with lethal doses of individual serotypes demonstrated high vaccine efficacies, with relative percent survival (RPS) ranging from 97.4% - 100% and 86.8% - 97.4% against serotype Ia and III, respectively. HKV and FKV exhibited similar efficacy. Due to its production simplicity, safety, effectiveness, and adaptability to diverse laboratory settings, HKV was further implemented in cage culture of tilapia. In the farm, vaccinated group showed a significant increase in specific serum IgM production over 4 months, especially against serotype Ia. At harvest after six months, the vaccine achieved an effectiveness at 83.4% with productivity revenue increased by approximately 45%. The results demonstrate that the basic bivalent HKV is highly effective in mitigating streptococcosis in tilapia cage culture, presenting a promising, practical, and locally available approach for safeguarding tilapia production.

Keywords: *Streptococcus agalactiae*, Vaccine, Autogenous, Tilapia

Project: Applications of nanobubbles to reduce antibiotic use in aquaculture

Funding: The International Development Research Center (IDRC), Ottawa, Canada

Isolation and characterization of lytic bacteriophages against *Photobacterium damselae* from aquatic sources

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Photobacterium damselae is an emerging pathogen causing 60 –80% mortality in marine finfish culture. Antibiotics are widely used to treat bacterial infection, which has resulted in the emergence of resistant bacteria. Alternative strategies involving bacteriophages, exempts the risk of emerging antibiotic resistance. Isolation of *Photobacterium damselae* was attempted from the liver, kidney brain, eye and spleen tissues of marine fish samples collected from markets, landing centers and cage culture systems. Following biochemical and molecular confirmation, 66 out 189 presumptive isolates were confirmed to be *P. damselae* subsp., *damsalae* (Pdd) and 2 as *P. damselae* subsp., *piscicida* (Pdp). The isolates showed haemolytic activity (89.7%), DNase activity (97.05%), lecithinase activity (13.23%) and gelatinase activity (23.52%). Regarding virulence genes, 85.29% of Pdd (58/66) and 50% of Pdp (1/2) were positive for *Hly A* gene and 4 Pdd (5.88%) harbored *Dly* gene. 50 Pdp showed resistance to β-lactams, glycopeptides and aminoglycosides, while susceptible to cephalosporins, quinolones and chloramphenicol. Of 50 Pdd, 22 had bla_{SHV} gene (44%), 1 had *tet (a)* gene (2%),14 with *aac (3)* gene (28%), 2 with *ere (A)* gene (4%) and 4 with *str (B)* gene (8%). To combat *P. damselae* infection, lytic bacteriophages were isolated and characterized. Screening of phage activity in various water and fish samples has led to the isolation of five different lytic phages. Morphological characterization under transmission electron microscopy (TEM) revealed distinct virion structures, belonging to the *Siphoviridae* family. Further, the host range and lytic activity analysis revealed their specificity and infectivity against *P. damselae*. Survival under different environmental conditions indicates the ability of the bacteriophages to withstand adverse environmental conditions. These newly identified lytic phages represent a viable and sustainable alternative to antibiotics, offering a targeted and effective solution for the prevention and treatment of *Photobacterium damselae* infections in aquaculture.

Keywords: *Photobacterium damselae*, Characterization, Bacteriophage

Development of an effective management protocol to control *Octolasmis* sp. infestation in mud crab broodstocks

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Octolasmis spp., a genus of pedunculate barnacles, are generally considered as ectocommensals infesting the gills of mud crabs (*Scylla* spp.) significantly compromising the broodstock health and reproductive success in hatchery. The present study was aimed at disease investigation and development of therapeutic protocol for the effective management of *Octolasmis* spp. in mud crab broodstocks. In this study, a 33.33% mortality rate was observed among female mud crabs weighing 648 –1175 g (n=12) at the mud crab hatchery of ICAR-Central Institute of Brackishwater Aquaculture, Chennai during April, 2024 to March 2025. The disease investigation revealed the severe infestations of *Octolasmis* spp. on the gills of affected crabs (mean intensity of 312±99.6 parasites per crab; n=12), resulting in respiratory distress and mortality. To address this challenge, a targeted therapeutic protocol was developed, involving freshwater immersion and formalin bath. Brooders were exposed to freshwater treatments (1, 2, 3, 4 and 5 hours) and formalin concentrations (50, 100, 150, 200 and 250 ppm) over night, and monitored for spawning latency and hatching rate. The occurrence of first spawning showed significant difference between 2-hour freshwater (17±2 days) and 150 ppm formalin (18.33±2.7 days) treatments compared to others respectively. The hatching rate was also high in 2-hour freshwater treatment (91.67±3.5%) and in 150 ppm formalin treatment (95.33±3.06%), which is significantly different to the other treatments respectively. The second order polynomial regression showed that the optimum duration for freshwater treatment and dose for formalin treatment are 2.33 hrs and 151 ppm respectively for higher hatching rate and 2.3 hrs and 140.9 ppm for shorter spawning latency. The results revealed that significantly lower spawning latency and higher hatching rate was observed in the treatment group involving a 2-hour freshwater immersion and an overnight 150 ppm formalin bath. Freshwater immersion induced osmotic stress in ectoparasites, while formalin provided effective disinfection without compromising the broodstock viability. These findings demonstrate that routine freshwater and formalin baths serve as effective and practical interventions for managing *Octolasmis* infestations in hatchery-reared mud crab broodstock, thereby enhancing both animal welfare and production outcomes in commercial mud crab aquaculture.

Keywords: *Octolasmis* sp., Barnacle, Mud Crab, Parasite, Broodstock

Project: Evaluation of aquaculture potential of diversified crustacean species: *Penaeus japonicus*, *Scylla* spp. and ornamental crustaceans

Funding: ICAR-Central Institute of Brackishwater Aquaculture, Chennai

A bivalent vaccine against Tilapia lake virus (TiLV) and *Aeromonas hydrophila* provides immuno-protective effects in Nile tilapia (*Oreochromis niloticus*)

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Fish diseases are a significant constraint to aquaculture production worldwide. Particularly, tilapia has become susceptible to Tilapia lake virus and *Aeromonas hydrophila* infections, resulting in massive mortality. This study aimed to develop an inactivated bivalent vaccine targeting both TiLV and *A. hydrophila* and evaluate its protective, haematological and immunological effects in Nile tilapia (*Oreochromis niloticus*). Thus, the inactivated bivalent vaccine prepared using 0.1% formalin against TiLV and *A. hydrophila* was administered to healthy juvenile tilapia via intraperitoneal injection. On 28 days post-immunization, the respective groups were challenged with TiLV and *A. hydrophila* intraperitoneally. Vaccinated groups exhibited significant (p <0.05) alterations in the levels of haematological (RBC, HCT, Hb, PLT, WBC, MCV, MCH, MPV and MCHC) and immunological (RBA, SOD, CAT and MPO activity) parameters at 2nd, 4th and 6th days post-infection. Vaccinated tilapia, upon challenge with TiLV and *A. hydrophila*, exhibited relative percent survival (RPS) of 71.43% and 80%, respectively. Further, notable clinical symptoms such as tail and fin rot, abdominal swelling, enlarged spleen, hemorrhages on the body surface, discoloration, loss of scales and sluggish movement were observed prominently in unvaccinated groups, indicating severe systemic infection. According to RPS, haematological and immunological parameters, the inactivated bivalent vaccine conferred effective dual protection against TiLV and *A. hydrophila* infection, thereby offering a promising strategy for the integrated management of viral and bacterial diseases in tilapia aquaculture.

Keywords: *A. hydrophila*, Immunity, TiLV, Tilapia, Vaccine

Project: National Surveillance Programme for aquatic animal diseases —PhaseII

Funding: PMMSY-ICAR-NBFGR —NSPAAD, Phase II

One-pot RPA -CRISPR/Cas12a assay for visual detection of White spot syndrome virus (WSSV) in shrimp

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White spot syndrome virus (WSSV) is a lethal pathogen causing white spot disease (WSD) in shrimp. Since 1997, the World Organization for Animal Health (WOAH) has classified WSD as a notifiable disease. The frequent occurrences of WSD in shrimp aquaculture result in considerable production and economic setbacks, posing significant challenges to the health of aquatic animals globally. Current diagnostic methods for WSSV mainly depend on molecular techniques, which can be unfeasible in resource-limited areas. Furthermore, conventional detection approaches often fall short of meeting the increasing need for rapid, simple, user-friendly and accurate screening. To address these limitations, we have developed a simple, fast, and effective diagnostic method for WSSV by integrating Recombinase Polymerase Amplification (RPA) assay and the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) and associated (Cas) protein 12a (CRISPR/Cas12a) system in a single reaction. This innovative approach offers remarkable sensitivity and specificity for WSSV detection, completing the entire process in just 30 minutes without the need for sophisticated equipment. The limit of detection (LOD) was determined to be 10 copies/μl. In terms of specificity, the assay effectively detected WSSV-infected positive samples while showing no cross-reactivity with infectious *hypodermal and hematopoietic necrosis virus* (IHHNV) or *Enterocytozoon hepatopenaei* (EHP). This represents the first report of one pot RPA -CRISPR/Cas12a assay for WSSV detection from India. These findings indicate that the RPA-CRISPR/Cas12a assay has significant potential for on-site testing. Given the serious economic implications of WSSV in cultured shrimp, continuous monitoring of infection levels is crucial, especially in field conditions. The established one-pot RPA-CRISPR/Cas12a detection assay can thus be employed for the pathogen screening and swift identification of WSSV infections in the field, making it well-suited for practical applications owing to its sensitivity, specificity, and visual results.

Keywords: CRISPR/Cas12a, Onsite, Detection, RPA, Shrimp, WSSV

Project: Application of gene editing technologies (CRISPR/Cas) for disease diagnosis

Funding: ICAR-Central Institute of Brackishwater Aquaculture, Chennai

Freeze-dried bacteriophage-based biocontrol formulation for mitigating Aeromonas infections in *Labeo rohita* aquaculture

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Aeromonas infections present a significant threat to freshwater aquaculture, leading to high mortality and economic loss in *Labeo rohita* (rohu) farming. The present study addresses this concern by developing a novel bacteriophage-based biocontrol formulation utilizing a lytic phage strain, AvP-2, with broad-spectrum activity against *A. hydrophila, A. salmonicida,* and *A. veronii*. The phage was isolated from aquaculture water sources and characterized using transmission electron microscopy and whole-genome sequencing, classifying it under the *Myoviridae* family. To enhance its therapeutic utility and storage stability, AvP-2 was formulated into a freeze-dried product using sixteen combinations of protective agents including glucose (0.5 M, 1.0 M), sucrose (0.5 M, 1.0 M), maltodextrin (10%, 15%), and trehalose (2%, 4%). Among them, the combination of sucrose (0.5 M) and maltodextrin (10%) offered optimal protection, resulting in minimal $\log_{10}$ viability reduction immediately after freeze-drying and after 180 days at both room temperature (30±3°C) and 4°C. The formulation showed enhanced phage stability under thermal (40-100°C), pH (2 and 10), and bile salt (2.5%, 5%) stress. *In vitro* lytic activity against *A. hydrophila* was retained across MOIs 0.01-10, with MOI 10 being most effective. *In vivo* trials demonstrated significantly improved survival in *L. rohita* following dietary phage administration, with survival rates increasing from 36.67% to 63.33% against *A. hydrophila,* and up to 73.33% against *A. veronii*. The formulation offers a stable, eco-friendly, and effective alternative to antibiotics for controlling *Aeromonas* infections in freshwater aquaculture.

Keywords: Phage Therapy, *Aeromonas*, *Labeo rohita*, Freeze-drying, Protective Agents

Project: Biocontrol of *Aeromonas hydrophila* and *Flavobacterium columnare* infection in *Labeo rohita* through phage therapy and paraprobiotics

Funding: Department of Biotechnology, Government of India

Molecular identification, pathogenesis, and functional genomics of *Aeromonas veronii* as a basis for design of next generation live attenuated vaccine

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Aeromonas species are economically important bacterial pathogens affecting diverse freshwater fish, which leads to Motile *Aeromonas* Septicemia (MAS) disease in fish. *Aeromonas* veronii has recently been identified as a virulent infection linked to significant mortality in farmed freshwater food fishes. In this study, a series of molecular-level identifications including 16S rRNA amplification was accomplished along with whole genome sequencing by using Illumina (Hi-seq) platform for its characterization. Further, the virulent potential and Koch's postulate was confirmed by carrying out selected bioassay studies. The antibiotic susceptibility patterns were also measured by adopting the Kirby-Bauer method. In addition, Type VI secretion system (T6SS) was validated (*in silico*), and associated genes were screened through semi-quantitative PCR for further downstream application for the construction of mutant strain which will be used as a potential live attenuated vaccine (LAV) in fish against *A. veronii* infection or MAS. The colony morphology, 16S ribosomal RNA and whole genome analysis confirmed *A. veronii* (strain: WSLR01) and PGAP analysis further corroborated the same and assigned NCBI accession No. JBNPPA000000000. The lethal dose (LD_{50}) was estimated as 1.2×10^6 CFU/mL and the presence of selected virulent genes, and expressions of selected cytokines which are involved in inflammation and immunity affirms that the *A. veronii* WSLR01 is a potentially virulent strain. The T6SS components and genes (Tss-A to TssM) have been screened for suitable in-frame deletion approaches through a two-step allele exchange protocol by using an appropriate suicide plasmid (pDM4) and *Escherichia coli* strains (S17-1 λpir) to act as a suitable competent cell for raising the deleted mutant strain in the laboratory. This investigation will substantially contribute towards the standardization of in-frame deletion mutation protocol and construction of mutant strain of *A. veronii* for the design of effective LAV

Keywords: Next-generation Sequencing, Type VI Secretion System, Suicide Vector, Vaccines, Aquaculture

Project: Development of mutant constructs through in-frame deletion mutation approaches to generate live attenuated vaccines against bacterial fish pathogen *Aeromonas veronii*

Funding: ICAR-Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar, Odisha, India

Pharmacokinetics study and tissue distribution of single-dose Oxolinic acid in rohu, *Labeo rohita* administered through feed

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Oxolinic acid (OXO) is a quinolone antibiotic used for the control of bacterial fish diseases in several countries. The present study was undertaken to understand the tissue distribution and pharmacokinetics properties of OXO in an Indian major carp, rohu, *Labeo rohita*. Rohu juveniles (98.35±2.46 g) were fed a single dose of OXO 60 mg/kg in the feed, and 6 different tissues, *viz.*, liver, kidney, muscle, intestine, bile, and plasma, were analysed for the drug concentration at different time points up to 128 h using LC-MS/MS. It was found that the drug concentration was highest in the intestine (18,839.01 mg kg^{-1}) and liver (13,756.66 mg kg^{-1}), while the kidney, bile, and plasma had lower concentrations. The absorption rate constant (ka) was highest in muscle (9.85 h^{-1}) and intestine (8.98 h^{-1}) indicating faster absorption in these tissues, while plasma and bile showed slower absorption rates. The liver shows the highest elimination rate (K_{10}) at 0.09 h^{-1}, while bile has the slowest at 0.02 h^{-1}, indicating prolonged retention in the bile. The intestine showed the highest C_{max} at 17,500 mg kg^{-1}, indicating a high peak level of the compound at T_{max} of 2 h in the gut followed by the liver and bile with C_{max} of 11,100 mg kg^{-1} at 3 h and 6 h, respectively while muscle and plasma had C_{max} values of 4,050 mg kg^{-1} at 2 h and 3,100 mg kg^{-1} at 8 h. Bile shows the longest MRT at 44.15 h, showing prolonged retention, while muscle shows the shortest MRT of 14.10 h. Overall, the study identified the tissue distribution and elimination of oxolinic acid administered orally. These findings will help in optimizing the antimicrobial therapy for better health management in aquaculture.

Keywords: Antibiotics, Oxolinic Acid, Pharmacokinetics, Drug Distribution

Project: All India Network Project on Fish Health

Funding: Indian Council of Agricultural Research, New Delhi

Control of co-infecting bacterial pathogens in Oscar fish (*Astronotus ocellatus*): Genomics, host-pathogen interaction, and autogenous vaccination

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A severe disease outbreak with high mortality was reported in Oscar fish (*Astronotus ocellatus*) at an ornamental fish hatchery in Perumbavoor, Kerala, India, leading to significant economic losses and prompting urgent investigation. Two Gram-negative bacterial pathogens, *Aeromonas jandaei* and *Edwardsiella tarda*, were isolated from diseased fish. Identification was confirmed through biochemical characterization and whole genome sequencing, with genome sizes of 4.42 Mb for *A. jandaei* and 4.11 Mb for *E. tarda*. LD_{50} values were calculated as 5.31×10^8 CFU/mL and 7.49×10^8 CFU/mL, respectively. Co-infection trials revealed a synergistic effect, with 90% mortality in co-infected fish compared to 65% and 50% in fish infected with *A. jandaei* and *E. tarda* individually. To address the disease challenge, autogenous inactivated bivalent vaccines were developed using formalin and heat inactivation methods. Optimal inactivation conditions were standardized as 0.3% formalin and heat treatment at 70°C for 1 hour. Bivalent vaccines were formulated by combining inactivated *A. jandaei* and *E. tarda* (1×10^{10} CFU/mL) and emulsified in Freund's adjuvants. Ninety healthy Oscar fish were randomly assigned to three groups: bivalent formalin-inactivated (VFI), bivalent heat-inactivated (VHI), and a PBS-injected control. Fish received one primary and three booster intraperitoneal injections at 14-day intervals. Post-immunization, fish were challenged with a mixed bacterial suspension based on LD_{50} values. Over a 20 days observation period, vaccinated groups showed significantly higher survival. The relative percentage survival (RPS) was 92.6% for the VFI group and 82.5% for the VHI group. This study highlights the successful development of an autogenous bivalent vaccine to control co-infections by *A. jandaei* and *E. tarda* in Oscar fish, offering a practical disease management strategy for ornamental aquaculture farms.

Keywords: Vaccine, Prophylaxis, Co-infection, Aquaculture, Ornamental Fish

Project: Development and evaluation of autogenous inactivated vaccine against *Aeromonas* spp. and *Edwardsiella tarda* in Oscar fish (*Astronotus ocellatus*)

Funding: ICAR-National Bureau of Fish Genetic Resources, Lucknow

Protective efficacy of bivalent nano vaccines against *Aeromonas hydrophila*, and *Aeromonas caviae* in Red Tilapia (*Oreochromis aureus* x *Oreochromis mossambicus*)

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Multiple bacterial infections are frequently seen in natural farm environments and often result in significantly greater losses than infections caused by a single bacterial infection. Vaccination remains the most reliable and effective strategy for preventing infectious diseases in aquaculture. This study aims to develop and evaluate a potential bivalent vaccine for Red Tilapia (*Oreochromis aureus* × *Oreochromis mossambicus*) to protect against two major bacterial pathogens: *Aeromonas hydrophila* and *Aeromonas caviae*. The bivalent vaccine was formulated by combining formalin-killed strains of *A. hydrophila* and *A. caviae*. The vaccine was subsequently encapsulated in chitosan nanoparticles, and the particle size and morphology were characterized using FTIR and SEM analysis. One hundred Red Tilapia (10 ± 0.5g) were randomly assigned to three groups in triplicate. Group I served as the control and received no vaccine. Group II was fed an experimental diet coated with the bivalent vaccine, while Group III was orally immunized with the chitosan-conjugated bivalent vaccine via a feed-based method. The experiment was conducted over a period of 30 days. Blood and serum samples were collected from all groups on days 15 and 30 for hematological and immunological analyses. Following the 30-day treatment period, all groups were challenged with *A. hydrophila* and *A. caviae* to evaluate the protective efficacy of the bivalent vaccine. The results demonstrated that fish fed with the bivalent vaccine encapsulated in chitosan nanoparticles exhibited significantly enhanced hematological and immunological parameters, along with a higher survival rate against *A. hydrophila* and *A. caviae* compared to the control. The chitosan-conjugated bivalent vaccine demonstrated protective efficacy in tilapia, with relative percent survival values of 78% against *A. hydrophila* and 75% against *A. caviae*. These results suggest that the bivalent vaccine is a promising candidate for the prevention of Aeromoniasis caused by *A. hydrophila* and *A. caviae* in a tilapia fish model.

Keywords: Bivalent Vaccine, *Aeromonas hydrophila*, *Aeromonas caviae*, Red Tilapia, Chitosan Nanoparticles

Funding: Sathyabama Institute of Science and Technology, Chennai

Selection of non-hemolytic, quorum-quenching probiotics from inland saline shrimp for pathogen control in aquaculture

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A major challenge in aquaculture is to maintain the gut microbiota and overcome disease problems. One of the promising strategies involves the use of native quorum-quenching probiotics (QQPs), which target the pathogenic bacteria through quorum sensing (QS) systems, thereby inhibiting the virulence of pathogenic bacteria without contributing to antimicrobial resistance (AMR). In the present study, shrimp samples were collected from inland saline environments to isolate and identify potential QQP bacteria. A total of 50 bacterial isolates belonging to the *Lactobacillaceae* and *Enterococcaceae* families were identified through a molecular approach. Among them, *Bacillus cereus*, *Oceanobacillus kimchii*, *Bacillus marisflavi*, *Staphylococcus hominis*, *Enterococcus avium*, *Weissella paramesenteroides*, *Enterococcus faecium*, and *Pediococcus acidilactici* were selected for further probiotic evaluation. Most of the bacterial isolates showed tolerance to bile salt, phenol, and pH; these properties are essential in maintaining the gut microbiota. Further, prominent antagonistic activity against *Vibrio* pathogen was exhibited by *B. cereus* and *B. marisflavi*. Importantly, bacterial isolates such as *B. cereus*, *O. kimchii*, and *B. marisflavi* proved to be safe by showing the non-hemolytic activity; *E. faecium*, *W. paramesenteroides*, and *P. acidilactici* showed complete inhibition of biofilm formation. Overall, quorum-quenching and antagonistic properties were recorded in the bacterial isolates from the *Bacillaceae* family, which are confirming the probiotic properties. The present study highlights the importance of stress tolerance, antibiofilm activity, and non-hemolytic behavior in selecting beneficial QQP isolates. Based on these criteria, *O. kimchii*, *B. cereus*, and *B. marisflavi* were identified as promising candidates for further investigation as effective probiotics in aquaculture.

Keywords: Bacteria, Probiotic, Quorum-quenching, Shrimp, Inland Saline Aquaculture

Project: National Surveillance Programme for Aquatic Animal Diseases

Funding: Department of Fisheries, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India, & National Fisheries Development Board (NFDB) &, ICAR-National Bureau of Fish Genetic Resources (ICAR-NBFGR), Lucknow

Antibacterial and antibiofilm potential of ascorbic acid against shrimp-pathogenic, *Vibrio parahaemolyticus* isolate

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Biofilm is the aggregate of microbial cells attached to the surface of biotic or abiotic substances. It plays a crucial role in bacterial survival and persistence. Among biofilm-forming bacteria, *Vibrio parahaemolyticus*, a gram-negative, halophilic pathogen, thrives in aquatic environments. This study aimed to identify the antivibrio activity of ascorbic acid against *V. parahaemolyticus* from moribund shrimp larvae of *P. indicus*. The identification of *V. parahaemolyticus* isolate was confirmed through biochemical and molecular characterization of virulence genes. Growth patterns and biofilm formation were assessed at different temperatures (27°C and 32°C) over 24 hours. Additionally, the antibacterial and antibiofilm effects of ascorbic acid were evaluated at varying concentrations. Results showed that the bacterial growth and biofilm formation increased at 32°C compared to 27°C. PCR amplification yielded positive results for *tox-R*, *tlh*, *Apha*, *Mot-X*, while negative for *trh* and *tdh* encoding genes in *V. parahaemolyticus*. Ascorbic acid exhibited over 50% inhibition the biofilm formation within 24 hrs. This study demonstrates that ascorbic acid inhibits the biofilm formation ability of *V. parahaemolyticus* and downregulates the expression of biofilm-related genes. In silico analysis indicated favourable ADME&T properties and high binding affinity of ascorbic acid with targeted virulence proteins, including Flagellar Biosynthesis protein FlhA, Thermostable direct hemolysin-2, *AphA* family protein, TOX-R Cholera toxin homolog transcriptional activator, LPS-assembly protein LptD involved in *V. parahaemolyticus* pathogenesis. These findings underscore the biofilm-forming ability of *V. parahaemolyticus* and the significant inhibitory effect of ascorbic acid on biofilm formation and virulence gene expression. Therefore, ascorbic acid emerges as a promising candidate for mitigating *Vibrio* infections in shrimp aquaculture, offering a sustainable approach to enhancing shrimp health and productivity.

Keywords: *Penaeus indicus*, *Vibrio parahaemolyticus*, Ascorbic Acid; Antivibrio Activity, Molecular Docking

Project: PMMSY-Genetic Improvement Programme of *Penaeus indicus* Phase-1

Funding: Pradhan Mantri Matsya Sampada Yojana (PMMSY), Government of India

Identification of potential molecular targets and compounds against *Caligus* spp. through *in silico* docking approaches

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Caligus spp., commonly known as sea lice, an ectoparasitic copepod poses significant threat to aquaculture species, including *Etroplus suratensis*. It causes mortality to the fish host, subsequently resulting in additional costs for treatment and control. Anti-parasitic agents such as emamectin benzoate are among the current effective treatment strategies. However, there is a concern for growing resistance to these conventional agents thereby highlighting the need for alternative treatment approaches. This study aims to identify potential drugs and molecular targets through *in silico* molecular docking tools, to decrease the viability of *Caligus* spp. Among the identified targets, compounds inhibiting 'chitin synthase 1' enzyme showed promise. The protein receptor i.e., chitin synthase 1 structure of *Caligus rogercresseyi* with the highest average pLDDT score was downloaded from AlphaFold database. The active sites of this enzyme were predicted through PrankWeb tool & the site with the highest pocket score was selected for docking. Lead compounds (ligands) identified from literature, were downloaded from PubChem or related drug databases. Further, site specific docking was performed through AutoDock MGL and AutoDock Vina. The protein receptor-ligand interaction was visualized through BIOVIA Discovery Studio or PyMOL. The compounds were ranked based on their binding energy. Among them, plant-based compound C-2 holds potential as a chitin synthase inhibitor on the basis of its binding energy (-6.9 kcal/mol). This work offers a foundation for alternative anti-parasitic agents aimed at disrupting parasite viability, representing a significant step towards parasitic control in fishes.

Keywords: *Caligus* spp., *Etroplus suratensis*, Anti-Parasitic Agents, Molecular Docking, Binding Energy

Project: Genome editing approaches for improving growth and reproduction of brackishwater teleosts and Indian white shrimp, *Penaeus indicus*

Funding: ICAR-Central Institute of Brackishwater Aquaculture, Chennai

In Silico evaluation of Garlic-derived phytochemicals for potential inhibition of *Enterocytozoon hepatopenaei* essential proteins

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Global aquaculture productivity is greatly impacted by *Enterocytozoon hepatopenaei* (EHP), an emerging microsporidian disease that causes hepatopancreatic microsporidiosis in shirmp. The inhibitory potential of 28 bioactive compounds derived from garlic (*Allium sativum*) against five essential EHP proteins Aquaporin (AQP), CTP Synthase (CTP), Dihydrofolate Reductase (DHFR), Thymidine Kinase (TDK), and Methionine Aminopeptidase 2 (MetAP2) was assessed in this study using a thorough *in silico* approach. With the use of AI-based homology modelling methods, the target proteins 3D structures were produced, allowing for precise structural predictions even in the absence of crystal structures. To evaluate binding affinities and ligand-protein interactions, molecular docking was carried out using AutoDock Vina integrated in PyRx version 0.9.8 revealed that Apigenin exhibited the strongest binding interactions overall, with the highest affinity for AQP (-7.4 kcal/mol) and consistently strong interactions with all targets. Quercetin showed the best binding with CTP Synthase (-9.2 kcal/mol), while Kaempferol demonstrated moderate but significant inhibition across all targets. The binding energies for the tested ligands ranged from -6.3 to -9.2 kcal/mol, suggesting potential multitarget inhibition. The majority of substances demonstrated minimal projected toxicity, no blood-brain barrier penetration, high gastrointestinal absorption, and conformity with Lipinski's Rule of Five, according to ADME/Tox profiles generated using SwissADME, ProTox-II, and ADMETlab 2.0. Oral bioavailability and safety were validated using BOILED-Egg study. Overall, our study suggests that allicin and other bioactives produced from garlic are interesting options for natural, feed-based treatment approaches against EHP. In order to verify these findings for sustainable shrimp health management, experimental assays will be conducted after MD simulations.

Keywords: *Enterocytozoon hepatopenaei* (EHP), Garlic Bioactive Compounds, Molecular Docking, ADMET/Toxicity Prediction

Project: enetic Improvement Programme of *Penaeus indicus* Phase-1

Funding: Pradhan Mantri Matsya Sampada Yojana (PMMSY), Government of India

Evaluating the break and protect 2 (bp2) device as a non-chemical control for marine leech infestations in hybrid groupers

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Marine leech, *Pterobdella arugamensis*, infestations represent a persistent challenge to grouper aquaculture across Southeast Asia. In Brunei Darussalam, infestation rates as high as 100% have been reported, with individual fish hosting over 1000 leeches. Conventional chemical treatments pose risks to both human health and the aquatic environment, particularly in open-water cage systems. These concerns have prompted investigations into alternative, environmentally friendly approaches, with mechanical control showing promising potential. This study evaluated the effectiveness of the Break and Protect 2 (BP2) marine leech trap as a non-chemical intervention for managing *P. arugamensis* infestations in hybrid groupers under both controlled and uncontrolled culture conditions. The trials were conducted in six tanks (three with BP2 and three controls) and three marine cages (two with BP2 and one control). Infestation prevalence, mean intensity, and leech population removal were monitored over 10 weeks in tanks and seven months in cages. In controlled tank conditions, a significant ($p < 0.05$) reduction in prevalence of up to 89% was observed in BP2-treated groups. Although the device was less effective in uncontrolled cage environments, likely due to environmental variability and continuous re-infestation from adjacent waters, it demonstrated substantial potential for reducing leech populations. Weekly leech removals were quantified by collecting the device and enumerating attached adult leeches and cocoons. Based on cocoon hatching rate estimates, the BP2 device removed up to 1920 potential leeches per collection in the cage trials, indicating disruption of the parasite's life cycle and reduced infestation pressure. While controlled conditions results are encouraging, further refinements in deployment strategy and integration with other management practices are needed to enhance its efficacy in field environments. These findings highlight the BP2 device as a promising, environmentally responsible tool for managing marine leech infestations without the use of chemicals, which contributes to sustainable aquaculture practices.

Keywords: Aquaculture Health, Cage Culture, Fish Ectoparasite, Mechanical Control, Sustainable Practice

Determination of minimum effective exposure time of common aquaculture disinfectants against *Streptococcus agalactiae* isolates from fish disease outbreaks

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Aquaculture plays a pivotal role in ensuring global nutritional security. However, its long-term sustainability is increasingly threatened by bacterial diseases *Streptococcus agalactiae*. Effective disinfection strategies are essential to control this pathogen. This study was undertaken to evaluate the minimum exposure time required to achieve the recommended effect ($\geq$5 $\log_{10}$ reduction) in *S. agalactiae* for eight commonly used aquaculture disinfectants, *viz.* chloramine-T, benzalkonium chloride (BKC), iodophor, formalin, Clorox, 50% glutaraldehyde, 30% hydrogen peroxide (H_2O_2), and potassium permanganate ($KMnO_4$). Each disinfectant was tested against *S. agalactiae* isolates obtained from five distinct fish disease outbreaks. Initially, exposure times were assessed at the predetermined minimum inhibitory concentration (MIC) for each disinfectant, which were as follows (in ppm): 9.37 (chloramine-T), 7.8 (BKC), 4.8 (iodophor), 31.25 (formalin), 281.2 (Clorox), 37.5 (glutaraldehyde), 37.5 (H_2O_2), and 15.62 ($KMnO_4$). Chloramine-T and iodophor achieved a $\geq$5 $\log_{10}$ reduction within 10 minutes. For the remaining disinfectants, higher concentrations were then evaluated. Significant variability was observed in bactericidal efficacy with increasing exposure time. BKC (31.25 ppm), Clorox (2250 ppm), and glutaraldehyde (150 ppm) also achieved $\geq$5 $\log_{10}$ reduction within 10 minutes at higher concentrations. In contrast, formalin required 60 min for effective reduction even at tenfold its MIC. Similarly, H_2O_2 required 60 min at MIC and 30 min at 20-fold higher concentrations. $KMnO_4$ required 24 hours at MIC and 60 min at fourfold higher concentrations, indicating the necessity for prolonged exposure for these agents. Based on the criterion of achieving $\geq$5 $\log_{10}$ reduction within 10 min, only five disinfectants (chloramine-T, BKC, iodophor, Clorox, and 50% glutaraldehyde) were categorized as efficacious against *S. agalactiae*. The findings provide evidence-based recommendations for developing practical and time-efficient disinfection protocols, ultimately contributing to improved aquatic animal health and reduced disease outbreaks in aquaculture systems.

Keywords: Aquaculture Biosecurity, Bacterial Disease Control, Aquatic Animal Health, Streptococcosis

Project: All India Network Project- Fish Health

Funding: ICAR-Central Institute of Brackishwater Aquaculture, Chennai

Comparative efficacy evaluation of commonly used aquaculture disinfectants against *Photobacterium damselae* isolates from fish disease outbreaks

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Aquaculture is crucial for ensuring global food security and economic growth. However, its continued expansion is increasingly hindered by the prevalence of bacterial diseases. One such disease, photobacteriosis, caused by *Photobacterium damselae*, results in high mortality rates among marine fish populations. To mitigate such outbreaks, disinfectants are widely employed for equipment sterilization, tank cleaning, and prophylaxis. This study assessed the efficacy of eight commonly used aquaculture disinfectants, chloramine-T, benzalkonium chloride (BKC), iodophor, formalin, Clorox (sodium hypochlorite), 50% glutaraldehyde, 30% hydrogen peroxide (H_2O_2), and potassium permanganate ($KMnO_4$) against *P. damselae* isolates obtained from five distinct disease outbreaks. The evaluation included determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), along with the minimum exposure time required to achieve a $\geq$5 $\log_{10}$ reduction in viable bacterial counts at MIC levels. The MIC values (ppm) recorded were: chloramine-T (30 $\pm$ 10.27), BKC (13.28 $\pm$ 10.91), iodophor (17.58 $\pm$ 12.73), formalin (118.75 $\pm$ 83.85), Clorox (1350 $\pm$ 853.07), glutaraldehyde (13.13 $\pm$ 5.14), H_2O_2 (54 $\pm$ 34.12), and $KMnO_4$ (21.88 $\pm$ 8.56). Furthermore, an MBC to MIC ratio of 1 for all disinfectants confirmed their bactericidal activity. Among the disinfectants tested, BKC, chloramine-T, and $KMnO_4$ exhibited the highest efficacy at their MIC, resulting in a $\geq$5 $\log_{10}$ reduction in pathogen counts within 10 minutes. Notably, BKC and chloramine-T achieved this bactericidal effect within just 1 minute, whereas $KMnO_4$ required 10 minutes. In contrast, iodophor and Clorox took 30 and 60 minutes, respectively, while formalin, glutaraldehyde and H_2O_2 failed to achieve the target reduction even after 24 hours. These findings identify BKC and chloramine-T as effective for rapid disinfection, and $KMnO_4$ as a promising alternative where extended contact times are acceptable. The study offers valuable insights for selecting efficient and sustainable disinfectants against *P. damselae* to enhance biosecurity and disease control in aquaculture systems.

Keywords: Aquaculture Biosecurity, Bacterial Disease Control, Aquatic Animal Health, Photobacteriosis, Minimum Inhibitory Concentration (MIC)

Project: All India Network Project- Fish Health (AINP-FH)

Funding: ICAR-Central Institute of Brackishwater Aquaculture, Chennai

Prophylactic measures to prevent parasitic infection in pearlspot (*Etroplus suratensis*) brooders for enhanced egg production in modular RAS

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Pearlspot *Etroplus suratensis* (Bloch, 1790) belongs to the family *Cichlidae* is distributed in peninsular India, Sri Lanka and declared as state fish of Kerala. Pearlspot is one of the most preferred fish species for brackishwater aquaculture for its wider salinity tolerance, omnivorous nature, excellent meat quality and ornamental value. ICAR-CIBA has standardized pair breeding of pearlspot in temperature-controlled RAS for continuous egg production. Pearlspot broodstocks are collected from saline pond, open water and prone to infections from various pathogens. However, several adult pearlspot being reared in the tank and hapa system infested with ectoparasites such as *Caligus* sp. in gill, caudal fin, snout and operculum areas. To overcome these challenges, wild collected brooders from saline ponds (25-28 ppt) were acclimatized in 10-12 ppt water in 3 t FRP tanks @ 10 fishes/tank and treated with 100 ppm formalin (37%) for 1 h. Fishes were kept in quarantine tanks for five days and afterwards stocked in RAS in equal sex ratio having body weight of 150-220 g for female, 78-140 g for males. RAS water temperature of 32-33°C, Photoperiod 14 L: 10 D and salinity 5 ppt were continuously maintained. In second stage disinfection measures fishes were fortnightly treated with 50 ppm formalin (37%) for 1 h. Commercially available beneficial bacteria were enriched in RAS biofilters @ 10g/1t of water. Average TAN and nitrite value found to be 0.07 ppm and 0.03 ppm respectively. Following these prophylactic measures it has been observed that brooders maintained in low saline RAS were free from ectoparasite infections. All these measures resulted in continuous spawning @ three spawning/tank/month with average fecundity of 1500 eggs. Healthy attached eggs were separated from stalk and given 2-3 ppm methylene blue to prevent fungal infections during incubation for better hatchability. Lower spawning rate and chronic parasite infestation were observed in brooders maintained in pond-based cages. This prophylactic protocol can help to achieve tank based year-round seed production of pearlspot.

Keywords: Pearlspot, Ectoparasite, Prophylaxis, RAS, Breeding

Project: Genome editing approaches for improving growth and reproductive performance of Asian seabass (*Lates calcarifer*) and Indian white shrimp (*Penaeus indicus*)

Funding: Indian Council of Agricultural Research

Factors influencing the efficacy of disinfectants against bacterial pathogens in brackishwater aquaculture

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Disease outbreaks continue to threaten profitable and viable aquaculture operations throughout the world. Predominant bacterial pathogens in shrimp and fish aquaculture facilities require application of disinfectants to control diseases. The present study assessed the effectiveness of four disinfectants commonly used in brackishwater aquaculture, against important bacterial pathogens belonging to the genus *Vibrio harveyi*, *V. campbelli* and *V. parahaemolyticus*. Among the disinfectants studied, benzalkonium chloride (BKC) was most effective showing antibacterial activity at 1 ppm. All the disinfectants tested showed reduced efficacy when the organic load was >2%. Contact time of 20 minutes was optimum for BKC and formaldehyde while potassium permanganate ($KMnO_4$) and iodophor were effective at 30 minutes. The acute toxicity test (LD_{50}) showed 50% mortality with 10 ppm BKC, 120 ppm $KMnO_4$, 200 ppm formaldehyde and 180 ppm iodophor in shrimp and shrimp post larvae. The study identifies the effective concentration, contact time, acute toxicity and the water quality parameters influencing the efficacy against the pathogenic bacterial pathogens in various brackishwater aquaculture farming systems.

Keywords: BKC, Aquaculture Disinfectant, Formaldehyde, Iodophor, Potassium Permanganate

Project: All India Network Project on Fish Health

Funding: ICAR-Central Institute of Brackishwater Aquaculture, Chennai

Comparison of TaqMan-based real-time PCR assays for diagnosis of Translucent Postlarvae Disease (TPD) in *Penaeus vannamei*

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Translucent Post larvae Disease (TPD), caused by *Vibrio parahaemolyticus*, has emerged as a critical disease impacting shrimp hatcheries in Asian countries. This disease severely affects the larval stages and can lead up to 100% mortality. Thus, controlling TPD is essential to reduce losses in shrimp hatcheries. PCR-based assays have recently been reported for the detection of TPD-causing *V. parahaemolyticus* (VpTPD), but these methods have not been thoroughly validated following the Aquatic Animal Health Code of the World Organization for Animal Health (WOAH, Paris, France). We describe here TaqMan real-time PCR assays targeting three candidate virulence genes of VpTPD, *vhvp-1*, *-2*, and *-3*. The diagnostic accuracy (DSp & DSe) of all three assays was 100% and all assays specifically detected TPD with analytical sensitivity (i.e., limit of detection, LOD) of 10 copies per reaction. We performed experimental challenges using VpTPD and Specific Pathogen Free (SPF) *Penaeus vannamei* shrimp and compared the assay performance in detecting VpTPD following a published protocol to three newly developed protocols. The results showed that both the previously published method as well as the currently described methods specifically detected VpTPD in experimentally challenged *P. vannamei* shrimp. The availability of a validated real-time PCR assay for detecting VpTPD will be immensely beneficial in screening *P. vannamei* stocks before the movement of live animals within and outside country, and prevent the spread of disease beyond its current geographic boundary.

Keywords: Translucent Postlarvae Disease (TPD), VpTPD, Post-larvae, VpTPD Detection Methods

Genome-wide documentation of antimicrobial peptides from penaeid shrimps

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Though crustaceans lack a true adaptive immune system, they possess a potent innate immune system. Antimicrobial peptides are a crucial part of crustacean immune system. Crustins and Anti-lipopolysacccaride Factors are present widely in most crustaceans while Penaeidins are specific to penaeid shrimps. Considering the biggest threat of antibiotic resistance caused by conventional antibiotics, rendering the benefits of antimicrobial peptides can be an alternate solution for countering pathogens. In this study, antimicrobial peptide sequences were collected from the database, APD3 (https://aps.unmc.edu/) and similar sequences were mined from five shrimp full genome available namely, *Penaeus chinensis*, *Penaeus japonicus*, *Penaeus monodon*, *Penaeus vannamei*, and *Penaeus indicus* by performing Blastp. A total of 20 common peptide sequences were identified across all the five species of shrimp. Antimicrobial peptides unique to crustaceans like Crustins and Anti-lipopolysaccaride factors and penaeidins which are exclusively present in penaeid shrimps were documented. Other novel peptides obtained through genome search were also documented. To further demonstrate the peptides have antimicrobial properties, *Penaeus vannamei* (host) genome and *Enterocytozoon hepatopenaei* (pathogen) genomes were utilized to document 1198 protein-protein interactions between host and pathogen following orthology-based and domain-based approaches. Nineteen host proteins containing antimicrobial peptide domains were found to interact with 3 proteins of pathogen

Keywords: Antimicrobial Peptides, *Penaeus* sp., *Enterocytozoon hepatopenaei*

Project: Genomics and bioinformatics for identifying candidate solutions for economically important diseases of brackishwater aquaculture

Funding: ICAR- Central Institute of Brackishwater Aquaculture, Chennai

Rapid field-deployable detection of tilapia parvovirus using CRISPR-Cas12a integrated RPA-LFA

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Tilapia aquaculture plays a critical role in global food security but the viral pathogens like Tilapia parvovirus (TiPV) pose significant threats to production and sustainability. The diagnostic techniques used today are labour-intensive, time-consuming, and frequently impractical for field use. To address this gap, a quick and extremely precise lateral flow assay (LFA) for TiPV detection based on CRISPR-Cas12a, is being developed. Recombinase Polymerase Amplification (RPA) is used in the assay design to amplify viral genomic targets. Three sets of RPA primers have been developed: VP1-RPA (139 bp), which targets the capsid protein (VP1) gene; NS1-RPA (178 bp); and NS1-RPA2 (104 bp), which targets the non-structural (NS1) region. In order to guide Cas12a to the appropriate amplicons, two guide RNAs, gRNA-NS1 and gRNA-VP1, were designed. Upon specific attachment to its target, the Cas12a enzyme indiscriminately cleaves adjacent single-stranded DNA reporters, exhibiting collateral trans-cleavage activity. This principle is harnessed to detect the positive amplicon. This characteristic was initially used to confirm target recognition and gRNA efficiency in a fluorescence-based detection experiment. The HybriDetect Milenia LFA platform-specific reporter degradation approach was combined with collateral cleavage for translation to a point-of-care format. In particular, the incorporation of labelled ssDNA reporters (FAM-biotin) enabled the visual detection upon Cas12a-mediated cleavage. Crucially, the CRISPR-LFA method offers a low-cost, field-deployable option for early TiPV diagnosis by avoiding the drawbacks of traditional PCR and serological techniques. This platform could lower the danger of viral outbreaks, increase productivity, and improve surveillance and management techniques in tilapia farming. The developed CRISPR-Cas12a LFA is an excellent candidate for a point-of-care detection of viral TiPV pathogen.

Keywords: CRISPR-Cas12a, Lateral Flow Assay (LFA), Tilapia Parvovirus (TiPV), Point-of-Care Diagnostics, Recombinase Polymerase Amplification (RPA)

Project: Applications of crisper/cas system in molecular detection of fish and shrimp diseases
Funding: Indian Council of Agricultural Research (ICAR)

Comparative assessment and optimization of a CRISPR/Cas-based diagnostic assay for rapid detection of *Tilapinevirus tilapiae* (TiLV) in Indian isolates: Validation against conventional nucleic acid detection techniques

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In this study, we developed and evaluated the Cr-TiLV assay, a rapid and sensitive diagnostic platform based on the CRISPR-Cas12a system, integrated with reverse transcriptase-recombinase polymerase amplification (RT-RPA) for the detection of *Tilapinevirus tilapiae* (TiLV) in Indian isolates. The assay was specifically designed to target segment 6 of the TiLV genome, selected for its high sequence conservation across various Indian strains, ensuring broader detection coverage. Two candidate CRISPR RNAs (crRNA1 and crRNA2) were designed and assessed based on their predicted secondary structure stability, with crRNA2 demonstrating superior performance due to a lower minimum free energy of -2.20 kcal/mol compared to -3.50 kcal/mol for crRNA1. Integration of the RPA step with the CRISPR-Cas12a detection system significantly enhanced assay sensitivity, reducing the detection threshold from 6×10^8 viral copies per reaction (CRISPR alone) to as low as 100 copies with crRNA1 and 10 copies with crRNA2 when using fluorescence-based readout. Validation with field-collected clinical samples further confirmed the reliability of the Cr-TiLV assay, demonstrating diagnostic sensitivities of 82.29% and 86.67% and overall accuracies of 85% and 90% when benchmarked against conventional RT-qPCR and nested RT-PCR methods, respectively. The comparable diagnostic performance of the CRISPR-based assay to these standard nucleic acid detection techniques, combined with its rapid turnaround time and minimal equipment requirements, highlights its potential as a valuable point-of-care tool for TiLV surveillance in resource-limited settings

Keywords: *Tilapinevirus tilapiae*, CRISPR-Cas12a, Cr-TiLV, Early Detection, Point-of-care

Project: Consortia Research Platform for Vaccine and Diagnostics

Funding: Indian Council of Agricultural Research (ICAR), Government of India, under the project CRPVD

Isolation and characterization of bacteriophage against planktonic and biofilm forming Multidrug-Resistant *Vibrio parahaemolyticus*

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The increasing prevalence of multidrug-resistant (MDR) *Vibrio parahaemolyticus* in aquaculture presents a serious threat to aquatic animal health and human food safety. This pathogen is the causative agent of vibriosis, acute hepatopancreatic necrosis disease (AHPND) and translucent post-larval disease (TPLD), which are some of the most severe diseases affecting shrimp farming, leading to substantial economic losses. Traditional antibiotic treatments are becoming less effective and poses adverse problems to human, necessitating alternative antimicrobial strategies. One of the key challenges in treating *V. parahaemolyticus* infections is its ability to form biofilms, which act as a protective barrier against antibiotics and enhance bacterial persistence in the environment. Therefore, alternative strategies are essential to control *V. parahaemolyticus*. Lytic bacteriophages represent a promising option, as they are viruses that specifically infect and destroy pathogenic bacteria, thereby reducing their populations. In this study, we evaluated the potential of lytic phages as biocontrol agents targeting multidrug-resistant (MDR) *V. parahaemolyticus* and its biofilms. Bacterial isolates were obtained from aquaculture environments and tested for resistance to commonly used antibiotics. Phages were isolated from environmental samples using the double-layer agar technique and were subsequently characterized for their host range, lytic capability, environmental tolerance, chloroform sensitivity, and one-step growth dynamics. The effectiveness of phages in inhibiting and disrupting biofilms was assessed through crystal violet staining and microscopy. Optimal conditions for phage stability were observed at temperatures between 25–50°C, pH levels from 2.0 to 12.0, and salinity ranging from 1.5% to 5.0%. The selected phages showed potent lytic effects on MDR strains and significantly reduced established biofilms. These results support the bacteriophage-based therapy as a safe, targeted, and environmentally friendly alternative to antibiotics for managing *V. parahaemolyticus* infections in aquaculture.

Keywords: Aquaculture, Lytic Bacteriophage, Antibiotic Resistance, Biofilm, Shrimp Disease, Antimicrobial Alternative

Project: All India Network Project on Antimicrobial Resistance (AINP-AMR) in Fisheries and Livestock

Funding: Indian Council of Agricultural Research (ICAR)

Comparison of non-lethal sampling methods for screening Asian seabass, *Lates calcarifer*, for viral nervous necrosis

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Nervous Necrosis Virus (NNV), an important viral pathogen of brackishwater fishes, leads to substantial economic losses and high mortality rates, particularly in larvae and juvenile developmental stages. NNV causes viral nervous necrosis, leading to significant threats to fish health and aquaculture management. This virus belongs to the *Betanodavirus* genus under the family of *Nodaviridae*. NNV is a 25 nm non-enveloped virus, featuring a bi-segmented, single-stranded, positive-sense RNA genome. The RNA-1 segment, around 3.1 kb in length, encodes the RNA-dependent RNA polymerase. The RNA-2 segment, 1.4 kb long, encodes the capsid protein around 42 kDa. NNV is a predominantly waterborne disease that affects mostly young fish and is carried by brooders and adult fish. The NNV-affected brain and retina are primary sites of infection, which causes neuronal vacuolation and degradation during virus multiplication. Adult fish, when infected, become carriers for the virus and transmits the virus vertically to offspring, resulting in high mortality in the newly hatched larvae. Hence, it is essential to screen the broodstock in Asian seabass hatcheries using non-lethal sampling methods and use only virus-free stock for breeding. Non-lethal sampling of blood, caudal fin, and ovarian fluid, was carried out in Asian seabass brooders. RNA was isolated from the samples and converted into cDNA. The cDNA was used to quantify the virus load in different samples. The results and the best method for sampling are discussed.

Keywords: NNV, VNN, RT-PCR, Nested PCR, *Betanodavirus*, Non-lethal Sampling.

Project: Consortium Research Platform on Vaccines and Diagnostics (CRP on V&D)
Funding: Indian Council of Agricultural Research (ICAR)

Comparative pharmacokinetics and tissue distribution of emamectin benzoate, lufenuron, and praziquantel in Rainbow Trout, *Oncorhynchus mykiss*

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Ectoparasites pose a persistent challenge to salmonid aquaculture in India, contributing to significant production losses and impacting fish welfare. The study comparatively evaluated the pharmacokinetics and tissue distribution of three commonly used antiparasitic agents emamectin benzoate, lufenuron, and praziquantel in juvenile rainbow trout (*Oncorhynchus mykiss*). Emamectin benzoate was administered orally at therapeutic doses of 50 μg kg^{-1}. At the recommended dose, the drug showed rapid absorption, extensive distribution, and minimal muscle accumulation (C_{max}: 13.07 µg kg^{-1}), with the kidney exhibiting the highest exposure ($AUC_{0-\infty}$: 71,136.84 $\mu g \cdot h$ kg^{-1}) and the intestine retaining the drug longest (MRT: 916.75 h). Lufenuron was evaluated following a single 5 mg kg^{-1} oral dose. The compound was absorbed rapidly (T_{max}: 16 h) and concentrated primarily in the liver (C_{max}: 1,840 mg kg^{-1}; AUC_{0-t}: 89,660.50 mg $\cdot$ h kg^{-1}) and intestine (C_{max}: 1,630 mg kg^{-1}), with prolonged gill retention (MRT: 73.79 h). Plasma concentrations remained comparatively low, indicating limited systemic exposure. Praziquantel, administered as a single 50 mg kg^{-1} oral dose, showed the fastest uptake in the liver (C_{max}: 205 μg kg^{-1} at 2 h; k_a = 1.03 h^{-1}) and intestine (k_a = 0.958 h^{-1}). Muscle exhibited delayed absorption (k_a = 0.134 h^{-1}) and rapid elimination ($t_{1/2}$: 13.38 h), while the intestine retained the drug longest ($t_{1/2}$: 70.18 h; MRT: 102.29 h). Across all agents, tissue distribution patterns reflected differences in physicochemical properties and mechanisms of action. Emamectin benzoate at 50 μg kg^{-1} and lufenuron at 5 mg kg^{-1} demonstrated optimal efficacy and safety with acceptable residue profiles, while praziquantel required consideration of prolonged intestinal retention when establishing withdrawal intervals.

Keywords: Fish Health Management, Pharmacokinetic Profiling, Tissue Residue Depletion, Histopathological Evaluation, Aquaculture Therapeutics

Project: All India Network Project on Fish Health (AINP-FH)

Funding: Indian Council of Agricultural Research (ICAR)

Pharmacokinetic disposition and safety evaluation of emamectin benzoate in Rainbow Trout under therapeutic and elevated dosing regimens

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India is the world's second-largest aquaculture producer and experiences considerable production losses due to ectoparasites, including sea lice in salmonids. Emamectin benzoate, a semi-synthetic avermectin derivative, is known for its high antiparasitic efficacy and low potential for tissue bioaccumulation. This study assessed the pharmacokinetics, withdrawal period, safety, and histopathological changes associated with oral administration of emamectin benzoate to rainbow trout (*Oncorhynchus mykiss*) over 21 days at therapeutic (50 μg kg^{-1}; 1X) and elevated (250 μg kg^{-1}; 5X and 500 μg kg^{-1}; 10X) doses. At the therapeutic dose, absorption was rapid with broad tissue distribution. The kidney exhibited the highest maximum concentration (C_{max}: 346.29 μg kg^{-1}) and greatest exposure ($AUC_{0-\infty}$: 71,136.84 μg $\cdot$ h kg^{-1}), while the intestine showed the longest mean residence time (MRT: 916.75 h), indicating delayed clearance. Muscle tissue demonstrated low accumulation (C_{max}: 13.07 μg kg^{-1}) and rapid elimination, suggesting minimal residue risk. Histopathology revealed no significant alterations at the therapeutic level. In contrast, the 5X and 10X groups developed progressive, dose-dependent lesions, including muscle fiber necrosis, hepatocellular vacuolation and hypertrophy, renal glomerular damage with Bowman's space dilation, gill epithelial hyperplasia, and intestinal mucosal disruption with inflammatory infiltration. Behavioral signs in the highest dose group included reduced feeding, skin darkening, and occasional mortality. Findings indicate that 50 μg kg^{-1} body weight achieves effective parasite control with no adverse tissue changes while maintaining compliance with withdrawal regulations and ensuring food safety in aquaculture production.

Keywords: Fish Therapeutics, Ectoparasite Management, Tissue Pharmacokinetics, Histological Safety, Aquaculture Residue Compliance

Project: All India Network Project on Fish Health (AINP-FH)

Funding: Indian Council of Agricultural Research (ICAR)

Efficacy and residue profile of emamectin benzoate in managing argulus infestations in juvenile Rainbow Trout, *Oncorhynchus mykiss* under coldwater conditions

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Rainbow trout (*Oncorhynchus mykiss*) is an important coldwater aquaculture species in India, especially in the Himalayan region. However, ectoparasitic infestations caused by *Argulus* species remain a major constraint, resulting in reduced productivity, compromised fish health, and economic losses. The study aimed to evaluate the efficacy and tissue residue profile of emamectin benzoate, an anti-parasitic agent approved by the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA), for controlling natural *Argulus* infestations in juvenile rainbow trout under Indian coldwater conditions. Fish were orally administered emamectin benzoate at doses of 25, 50, and 150 μg kg^{-1} body weight for 7 consecutive days. Post-treatment observations included behavioural assessment, histopathological examination, and tissue residue analysis. Histopathology focused on gill, liver, kidney, intestine, and muscle tissues. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to quantify emamectin benzoate residues, and results were compared with the international Maximum Residue Limit (MRL) of 100 μg kg^{-1}. The 50 μg kg^{-1} treatment group showed prominent improvement in feeding behaviour and reduced signs of stress related to parasitic infection. Histopathological analysis revealed telangiectasia in the secondary lamellae of the gills, while no pathological changes were observed in other examined organs. The liver exhibited the highest drug accumulation and the fastest elimination rate. Residue analysis confirmed that emamectin benzoate levels in edible tissues remained below the MRL of 100 μg kg^{-1} throughout the trial period. Emamectin benzoate was effective in reducing *Argulus* infestations in rainbow trout without causing significant tissue damage or exceeding food safety limits. The absence of residue levels above the MRL of 100 μg kg^{-1} suggests no withdrawal period is necessary, making it a safe and efficient treatment option for parasite management in Indian coldwater aquaculture.

Keywords: Ectoparasitic Control, Drug Residue Analysis, Telangiectasia, Aquaculture Therapeutics, Food Safety Standards

Project: All India Network Project on Fish Health

Funding: Indian Council of Agricultural Research (ICAR)

In silico approach to identify differentially expressed lncRNAs and mRNAs in Asian Seabass (*Lates calcarifer*) infected with Nervous Necrosis Virus

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Long noncoding RNAs (lncRNAs) are a category of functional RNA molecules that do not code for proteins and have a transcript length exceeding 200 nucleotides. Emerging evidences suggest that lncRNA play a role in gene expression regulation, acting as a signaling molecule involved in host-pathogen interactions. In this study, we investigated the expression profiles of lncRNA in the brain tissue of Asian seabass (*Lates calcarifer*) at 20 days post-infection (dpi), compared to mock-treated controls. Publicly available RNA-Seq datasets were retrieved from the NCBI SRA database and processed through a comprehensive bioinformatics pipeline. Raw read quality was assessed using FastQC, followed by adapter trimming and filtering with fastp. High-quality reads were aligned to the reference genome using HISAT2, and alignment files were processed with SAM tools to obtain sorted and indexed BAM files. Transcript assembly and quantification were performed with StringTie, and gene-level and transcript count matrices were generated using prepDE.py. Differential expression analysis was performed with edgeR, leading to the identification of dysregulated lncRNAs and mRNAs between infected and control samples. To further characterize these potential lncRNAs, FEELnc pipeline was used to predict the cis-regulatory interactions with neighbouring protein-coding genes. This integrative analysis revealed potential regulatory roles of lncRNAs and mRNAs in immune-related pathways, especially those involved in innate antiviral immune responses, including pcnx1, GPCR, IFN alpha or beta receptors. These findings suggest that they may act as key modulators of host-virus interactions, influencing neuronal survival, antiviral responses, and disease progression. Understanding their functions could aid in developing molecular biomarkers for early diagnosis and identify targets for genetic selection or therapeutic strategies, thereby contributing to improved resistance against viral nervous necrosis (VNN) in aquaculture species.

Keywords: *Lates calcarifer*, lncRNAs, VNN, EdgeR, HISAT2, Differential Expression

Project: Genome editing approaches for improving growth and reproduction of brackishwater teleosts and Indian white shrimp, *Penaeus indicus*

Funding: Indian Council of Agricultural Research (ICAR)

Isolation and characterization of pathogenic *Vibrio* spp. and evaluation of herbal extracts for disease management in *Penaeus vannamei*

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Shrimp aquaculture is one of the fastest-growing sectors of global seafood production but continues to face substantial economic losses due to bacterial diseases, particularly those caused by *Vibrio parahaemolyticus* and *Vibrio alginolyticus*. This study focused on isolating and characterizing pathogenic *Vibrio* strains from diseased *Penaeus vannamei* and assessing the antibacterial efficacy of a plant-based extract as a sustainable alternative to conventional antibiotics. A total of 22 bacterial isolates were recovered, of which five highly virulent strains were identified through 16S rRNA gene sequencing and subsequently deposited in the NCBI GenBank database, enriching the reference library for future research. Pathogenicity assays and LD_{50} determinations confirmed the severe virulence of selected isolates, emphasizing their role in shrimp mortality and the urgent need for sustainable disease management strategies. The herbal extract, prepared using methanol: chloroform solvent extraction, exhibited strong antibacterial activity, producing inhibition zones of 18–23 mm against both *V. parahaemolyticus* and *V. alginolyticus*. The minimum inhibitory concentration (MIC) was determined to be 32 μg/mL, demonstrating potent antimicrobial efficacy. Comprehensive gas chromatography-mass spectrometry (GC-MS) analysis identified several key bioactive compounds, including pyrogallol, bakuchiol acetate, and furan derivatives, all widely recognized for their antimicrobial and therapeutic properties. Feeding trials revealed that shrimp challenged with pathogenic *Vibrio* strains achieved survival rates of 83% (*V. parahaemolyticus*) and 80% (*V. alginolyticus*) when fed extract-supplemented diets, whereas control groups suffered high mortality. These findings underscore the promise of plant-derived compounds as safe, sustainable, and effective tools for disease control in shrimp aquaculture. By reducing dependency on synthetic antibiotics, this approach offers a viable strategy to mitigate antimicrobial resistance, improve animal welfare, and enhance farm productivity. Overall, this research contributes a commercially scalable, an eco-friendly environment in managing *Vibrio*-associated diseases, supporting the long-term sustainability and growth of the global shrimp farming industry.

Keywords: Pathogenicity, Antimicrobial, Eco-friendly, Sustainability, Environment

Project: Common Vibrio species in shrimp culture, prevention and remedial measures

Funding: Avanti Feeds Limited, Visakhapatnam, Andhra Pradesh, India

Immuno-protection in GIFT tilapia against ISKNV using recombinant major capsid protein: A transcriptomic and survival-based study

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Infectious spleen and kidney necrosis virus (ISKNV), classified under the genus *Megalocytivirus* in the family *Iridoviridae*, has recently emerged as a significant pathogen in Indian freshwater aquaculture and ornamental fisheries. Its rapid dissemination and high virulence have led to severe economic losses due to widespread mortality in susceptible species. Current control strategies, mainly reliant on biosecurity, early diagnostics, and supportive management, remain inadequate. Consequently, development of effective vaccines is a critical priority. This study evaluates the immune-protective efficacy of a recombinant major capsid protein (rMCP) subunit vaccine against ISKNV in all-male genetically improved farmed tilapia (GIFT). The ISKNV isolate demonstrated distinct cytopathic effects (CPE) in susceptible fish cell lines, including cellular rounding, detachment, and lysis. Transmission electron microscopy revealed icosahedral virions measuring approximately 120–146 nm, enclosed by a double-layered proteinaceous envelope. As a conserved structural component critical for host cell entry and immune activation, the MCP was selected as the antigenic target for recombinant vaccine design based on its high immunogenicity potency. The major capsid protein (MCP) gene was successfully cloned into the pET-32a (+) expression vector via restriction enzyme digestion and subsequently transformed into *Escherichia coli* BL21 (DE3) cells for heterologous expression. Protein induction was achieved using isopropyl β-D-thiogalactopyranoside (IPTG) induction, and SDS-PAGE analysis confirmed expression of MCP as a prominent band at approximately 67.31 kDa. Tilapia immunized with purified rMCP exhibited a relative percent survival (RPS) of 71.44%, significantly exceeding the adjuvant-only control group (35.72%). Transcriptomic analysis of spleen and kidney samples at sequential time points post-vaccination indicated elevated expression of immune-related genes. Notably, MHC I and MHC II genes peaked toward the study's conclusion, while cytokines IL-1β and IL-4 showed early and sustained upregulation. These patterns reflect a robust activation of both innate and adaptive immune pathways, affirming the vaccine's potential efficacy.

Keywords: ISKNV, Megalocytivirus, Major Capsid Protein, Tilapia, Immunization

Project: Phase 2 of National Surveillance Programme for Aquatic Animal Diseases (NSPAAD): Implementation of surveillance in Freshwater and Brackishwater sectors in the state of Karnataka

Funding: Department of Fisheries, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India, Phase 2 of National Surveillance Programme for Aquatic Animal Diseases (NSPAAD) under Pradhan Mantri Matsya Sampada Yojana (PMMSY)

05

Technical Session V

Mollusc and Seaweed Health

Field-based insights for an integrated pest management system for mollusk diseases

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In recent years, efforts to increase mollusk production have led to more active seed transfers across regions and the expansion of high-density aquaculture systems. While the former increases the risk of pathogen introduction, the latter raises the likelihood of disease transmission and outbreaks. As a result, effective disease control has become essential for the sustainable development of mollusk aquaculture. Nonetheless, disease management in mollusks remains challenging due to their limited feeding behavior, which makes medication impractical, and their lack of acquired immunity, which renders vaccination unfeasible. Despite these difficulties, we have frequently obtained highly valuable information for disease control through labor-intensive seasonal epidemiological surveys and close communication with local stakeholders, including producers in farming areas. For example, during a recent outbreak of ovarian enlargement disease in Pacific oysters in Hokkaido, Japan, information from producers indicated that the disease occurred exclusively in oysters derived from externally sourced seed. Based on this, we identified that the pathogen had already invaded one of Japan's most important seed production areas. This finding allowed us to issue an early warning to help prevent further spread. In the case of *Francisella halioticida* infection in scallops, producers reported that earlier density adjustment during the intermediate culture period helped reduce production losses. Rearing experiments confirmed that lower stocking densities led to reduced mortality and lower infection rates. For viral amyotrophy in abalones, larger individuals from older year classes appeared to be more resistant to mortality, suggesting that early seed production—allowing for growth before the onset of outbreaks—may be an effective mitigation strategy. To date, no comprehensive control methods have been established for mollusk diseases. Moving forward, we aim to develop disease-specific strategies and work toward establishing an integrated pest management system that combines multiple approaches.

Keywords: Infection, Pathogens, *Marteilioides chungmuensis*, AbALV

The diversity and eco-anthropology of algal pathogens in a rapidly changing world

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For the last two decades, omics-enabled studies in particular have unveiled a diversity and prevalence of algal pathogens far greater than had previously been appreciated. In parallel, algal cultivation has been growing steadily, in the wider context of intensifying use and increasing dependency of mankind on the oceans. We know from land-based agriculture that the health of crops dictates agricultural yields and is intimately linked with ecosystem health; that plant pathogens co-evolve with domesticated species, and that intensive farming favours outbreaks as well as the emergence of novel pathogens. Aside from natural disasters, disease outbreaks and pest infestations have become major hurdles to sustaining seaweed yields; alarmingly, reports of diseased or declining wild macroalgal populations accumulate worldwide. This disease pressure is compounded by a faster rate of climate change in the sea compared to terrestrial systems. This suggests that man-driven changes potentially occur at a vastly higher pace in marine ecosystems than they did historically on land when agriculture arose, calling for the inception of suitable mitigation strategies to underpin the ecological sustainability of seaweed (and more generally algal) cultivation. In this talk, I will brush upon my work towards accelerating the discovery of algal pathogens, as well as their phylogenetic and physiological characterization. This will lead to some pilot work on disease monitoring and management in algal production facilities, as well as developing proof of concepts of enhancing resistance through microbiome engineering or breeding. Finally, I will report on capacity and capability building initiatives to support disease management, as well as inform the development of conservation and biosecurity policies worldwide.

Keywords: Diversity, Domestication, Disease Management, Biosecurity, Seaweed

Project: PHABB: PatHogens of Algae for Biocontrol and Biosecurity

Funding: European Union HORIZON-MSCA-2022-DN-01-01

06

Technical Session VI

New emerging technologies

Genome engineering in aquaculture: From genetic modification to gene editing

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Genome engineering technologies are transforming aquaculture by enabling precise and efficient genetic improvements to improve aquatic animal health, productivity, and sustainability. Traditional genetic modification (GM) involves inserting foreign DNA, such as growth hormone genes from Pacific salmon into Atlantic salmon, using recombinant DNA (rDNA) techniques. In contrast, gene editing (GE), especially CRISPR-based methods, allows targeted modifications without introducing foreign DNA, offering a more refined and broadly accepted approach. Regulatory frameworks for GE vary worldwide, impacting its adoption. For instance, a gene-edited organism is considered a genetically modified organism (GMO) in New Zealand but not in Australia or Japan. Public acceptance of GE is generally higher than GM, with Japan having already approved three gene-edited fish for human consumption. In Australia, the government has deregulated the use of GE technologies in animals, including fish, when no new genetic material is added, known as Site-Directed Nuclease 1 (SDN-1) techniques, as not GMOs. Utilising DNA-free gene editing methods that avoid inserting foreign genes enables compliance with these regulations, thereby bypassing GMO classification. Despite these advancements, significant challenges remain in the GE workflow, especially in identifying gene targets and delivering editing tools at high throughput. While microinjection of fertilized eggs is a common practice of gene editing in fish, it is labour-intensive, inefficient, and often results in mosaicism, requiring breeding strategies to develop stable gene-edited lines. Therefore, developing more efficient, scalable delivery systems is crucial to fully harness the potential of GE technologies for sustainable and resilient aquaculture.

Keywords: Genome Engineering, Genetic Modification, Gene Editing, CRISPR, Aquaculture

Project: SIP PGE

Funding: CSIRO

Innovative technologies on alternatives to antimicrobials towards mitigation of antimicrobial resistance (AMR) in aquaculture

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Antimicrobial resistance (AMR) is a growing concern in aquaculture, where antibiotics are commonly used for disease prevention and control. The Asia-Pacific region is the largest aquaculture producer, contributing around 92% of the world's farmed aquatic foods. In Southeast Asia, in particular, aquaculture underpins food security and livelihoods, particularly in low- and middle-income countries (LMICs). However, the misuse and overuse of antimicrobials in aquaculture have accelerated the development of resistant strains of bacterial pathogens, posing risks to cultured aquatic organisms, public health and threatening the long-term sustainability of the industry. As such, current innovations focus on alternatives to antimicrobials which are aimed at reducing antimicrobial use (AMU) and AMR in aquaculture. Two promising innovations in reducing AMU in aquaculture are the use of nanobubble technology and novel bacteriophages. Nanobubbles can stay suspended in the water column for a long time (several days or even months) because they are negatively charged and with high internal pressure and low buoyancy. Nanobubble has been proven effective in water disinfection and reduction of bacterial loads (using ozone nanobubble) and improving water quality (oxygen nanobubble), which can help significantly in preventing any disease outbreak through better water quality management. Recently, nanobubble has also been explored for vaccine delivery (nanovaccine), addressing the issues on vaccine delivery by injection (labor intensive and costly) and immersion (low uptake of antigen). On the other hand, novel bacteriophages (which literally means "bacteria eater") is a type of viral particle that infects and destroy/kill their host bacterial cells (e.g. pathogenic bacteria). In some countries, particularly Thailand, phage concoctions have been pilot tested in some shrimp farms for prevention of diseases caused by pathogenic *Vibrio* species, with the aim of reducing the use of antimicrobials. Although bacteriophage products were shown to effectively control important bacterial pathogens, farmers are still reluctant to adopt the technology because of lack of awareness, which should go hand in hand in bringing the product into the farm.

Keywords: Antimicrobial Use, Nanobubble, Nanovaccine, Novel Bacteriophages, Disease Prevention

Project: Supporting the monitoring, evaluation and learning in addition to knowledge dissemination of the InnoVet 2.0 program

Funding: IDRC

Baci-VAC, a novel RNAi vaccine from a non-pathogenic *Bacillus subtilis* cell expressing dsRNA-EHP to protect microsporidian infection in shrimp

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Shrimp aquaculture is a vital pillar of global food security, yet it continues to face persistent threats from infectious pathogens. One of the most serious is *Enterocytozoon hepatopenaei* (EHP), a microsporidian parasite that causes growth retardation and significant economic losses in penaeid shrimp. Despite advancements in disease management, there is still no effective vaccine available against EHP. RNA interference (RNAi) has emerged as a promising strategy, as double-stranded RNA (dsRNA) can silence specific genes and stimulate innate immune responses in shrimp. However, the lack of a scalable, safe, and cost-effective dsRNA delivery system remains a major limitation for practical implementation in aquaculture. To address this challenge, we present Baci-VAC, an early-stage RNAi vaccine platform that explores the use of *Bacillus subtilis*, a non-pathogenic probiotic bacterium, as a living cell factory for producing dsRNA targeting EHP. In this study, dsRNA was designed to silence *EhPTP2*, a gene encoding polar tube protein 2, which plays a critical role in EHP spore extrusion and host cell invasion. A strain of *B. subtilis* deficient in RNase III was employed to promote intracellular dsRNA accumulation. The quality and yield of dsRNA were assessed using RNase digestion assays. Shrimp were injected with the dsRNA and subsequently challenged with EHP to assess transcriptional suppression of *EhPTP2*. In addition, transcriptomic analyses were conducted to explore the molecular responses in shrimp. This study aims to lay the groundwork for a novel RNAi-based strategy to mitigate microsporidian infections in shrimp. Future work will focus on developing a modular *B. subtilis* based platform adaptable to other aquatic pathogens, with the long-term goal of enabling feed-based delivery of dsRNA for disease prevention in farm environments. The Baci-VAC concept highlights a promising direction in microbial biotechnology and aquatic health, aligning with sustainable and One Health approaches for the future of aquaculture.

Keywords: Baci-VAC, RNAi, *Bacillus subtilis*, EHP, Shrimp

Funding: International Veterinary Vaccinology Network (IVVN)

Vaccine target discovery in a fish parasite using an *in vivo* model

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Tetracapsuloides bryosalmonae is an endoparasitic myxozoan that completes its life cycle between an invertebrate (bryozoan) and a vertebrate (salmonid fish) host. It is the causative agent of proliferative kidney disease (PKD), a significant disease affecting wild and farmed salmonids. Although PKD is not reported in Asian aquaculture, the salmonid-*T. bryosalmonae* system could serve as a valuable model for understanding host-parasite interactions and discovering potential vaccine targets against fish parasitic diseases. During infection, host-pathogen interactions trigger the expression of parasite virulence factors in *T. bryosalmonae*. The parasite genes expressed in vivo are likely crucial for fish pathogenesis. Identifying such in vivo induced genes of *T. bryosalmonae* could improve our understanding of pathogenesis and facilitate the development of novel therapeutic targets. Therefore, we used in vivo induced antigen technology (IVIAT) to identify antigens of *T. bryosalmonae* that are specifically expressed during infection in brown trout. Brown trout were experimentally exposed to the spores of *T. bryosalmonae* under controlled laboratory conditions, and blood and tissue samples were collected at different time points. Fish sera were first pre-adsorbed with antigens and cDNA phage expression library was screened using the adsorbed sera. This immune-screening identified 136 in vivo induced antigens. Functional annotation using gene ontology revealed that these genes are involved in key biological processes such as signal transduction, actin cytoskeleton organization, transport, metal ion binding, protein folding, and metabolism. These findings provide new insights into the molecular mechanisms of *T. bryosalmonae* pathogenesis in salmonids. Notably, the identification of molecules such as Rab-35 and calmodulin suggests their potential as candidate targets for therapeutic or preventive interventions against PKD in salmonid aquaculture.

Keywords: Cnidarian Endoparasite, *Tetracapsuloides bryosalmonae*, Proliferative Kidney Disease, *In vivo* Induced Antigens

Engineering 2D nanosheets for point-of-use biosensors: Selective identification of volatile organic compounds emitted by stressed shrimps

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India, producing ∼17% of global farmed shrimp in 2024, plays a key role in global aquaculture but suffers heavy losses from disease outbreaks, costing the industry over USD 10 billion in the past decade—80% in Asia, with India losing ∼₹. 7,400 crores annually. Current diagnostics are often reactive, leading to delayed detection and mass mortalities. Detecting volatile organic compounds (VOCs) from stressed shrimp offers a non-invasive and rapid alternative. This study employs high-performance computing and algorithmic workflows to design and screen novel nanoscale chemiresistive sensing materials for real-time, point-of-use monitoring, aiming to prevent catastrophic losses in shrimp farming. Using first-principles density functional theory (DFT) calculations, we examined the adsorption and sensing properties of pristine, vacancy-induced, and single-atom-decorated two-dimensional (2D) materials specifically tungsten diselenide (WSe_2), a transition metal dichalcogenide (TMD), and $MoSiN_4$, a recently discovered MXene-like material towards VOCs associated with shrimp disease: dimethylamine, trimethylamine, dopamine, histamine, and glucose. Pristine WSe_2 and $MoSiN_4$ exhibited weak adsorption, with energies ranging from -0.01 eV to -0.54 eV, indicating limited sensing capability. In contrast, defect engineering, via vacancy creation or single-atom doping, significantly enhanced VOC binding, improving chemical sensitivity and selectivity. Charge transfer analysis, electrostatic potential mapping, and work function calculations confirmed the superior sensing performance of defect-engineered systems. Thermodynamic behaviour was further explored using the Langmuir adsorption model to assess stability under varying temperature and pressure conditions relevant to aquaculture environments. Additionally, Non-Equilibrium Green Function (NEGF) simulations revealed distinct current-voltage (I-V) responses upon VOC exposure, suggesting measurable electrical signatures for detection. These results establish a theoretical foundation for developing next-generation TMD and MXene based chemiresistive nano-sensors tailored for aquaculture. The optimized nanosheets identified here could be integrated into real-time, on-site monitoring devices, enabling early detection of shrimp stress and disease.

Keywords: 2D Nanosheets, Chemiresistive Biosensors, VOCs, Shrimp Aquaculture, Tungsten

Diselenide, Density Functional Theory

Project: ICAR-CIBA / HITS Collaborative Research
Funding: DBT / HIET

Markerless expression of nervous necrosis virus capsid protein in microalgae

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Viral nervous disease is a lethal disease affecting larvae and juveniles of many marine, brackishwater and some freshwater fish species. The disease is caused by nervous necrosis virus (NNV), a betanodavirus of *Nodaviridae* family. Four genotypes of this virus have been reported viz., striped jack NNV, barfin flounder NNV, tiger puffer NNV and red spotted grouper NNV (RGNNV). RGNNV is the most widely prevalent genotype and the only genotype reported in India. The disease causes up to 100% mortality in the early life stages. Adult fish when infected do not exhibit clinical signs but become carrier of the virus. The virus is transmitted both horizontally and vertically. Hence, the practical way to prevent the spread of the virus is to vaccinate the larval stages at the earliest age possible. Oral and immersion vaccination are the ideal routes for vaccination of larval stages. The capsid protein gene of RGNNV was codon optimized for expression in microalgae, *Chlamydomonas reinhardtii*. The codon optimized gene was synthesized, PCR amplified and cloned into expression vectors pSRSapI and pASapI. The recombinant plasmids were sequenced to verify the presence of the insert in correct orientation. The recombinant plasmids containing the insert were transformed into *C. reinhardtii* strains TN-72 and HT-72 by electroporation. The microalgal mutant strains lack psbH gene and require acetate for its growth. After homologous recombination with the plasmid insert, the transformants regain PsbH activity and when grown on acetate free medium in the presence of high intensity light, only the recombinant clones grow. The presence of the insert in the recombinant colonies was verified by PCR. The recombinant clones do not have any antibiotic resistance marker and can be used to vaccinate finfish larval stages by oral and immersion routes against VNN.

Keywords: Red-Spotted Grouper Nervous Necrosis Virus, Viral Nervous Necrosis, Capsid Protein, Microalgae

Project: Consortium Research Platform on Vaccines and Diagnostics

Funding: Indian Council of Agricultural Research, India

How tilapia macrophages adapt: functional and epigenetic insights into polarization and trained immunity

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Macrophages, central to the innate immune system, exhibit functional plasticity and the capacity for trained immunity. Understanding these processes in a significant aquaculture species such as *Oreochromis niloticus* is vital for developing next-generation prophylactic strategies. Head kidney-derived mononuclear cells were isolated and cultured in L-15 medium with 10% FBS. Macrophage identity was confirmed via IBA-1 immunolocalization, morphology (Field/Giemsa stains, SEM), and functional assays (NO, ROS, phagocytosis). Phenotypic polarization and priming were assessed through targeted strategies of ligand stimulation. Stimulation with LPS, β-glucan, and PGN induced M1 polarization, characterized by enhanced ROS, NO, phagocytosis, and iNOS/IL-1β expression. cAMP triggered M2 phenotype, evident from altered morphology, increased arginase activity, and upregulation of *arg-2, tgm2b, and timp2b*. Sequential stimulations demonstrated plasticity: M1 (LPS/PGN) macrophages repolarized to M2 under cAMP, whereas β-glucan-primed cells exhibited resistance to reprogramming. Macrophages were primed with β-glucan $\pm$ metformin, a training inhibitor, followed by LPS restimulation following appropriate resting period. Trained groups displayed elevated TNF-α, IFN-γ, IL-1β levels; increased HIF-1α and mTOR expression; and enrichment of activating histone marks (H3K27ac, H3K4me1, H3K4me3). Enhanced LDH activity and the histone marks supported metabolic reprogramming and epigenetic modifications indicative of trained immunity. Responses of macrophages depending on the stimuli indicates the varying mechanisms adopted by the immune cells in immunoprotection of the host. The study presents a unified *in vitro* platform for studying macrophage polarization and trained immunity in tilapia. The observed functional plasticity and innate memory responses offer novel avenues for immune modulation, laying the groundwork for epigenetic and metabolic-based interventions in fish health management. Further, the understanding on macrophage polarization and plasticity unravels innovative strategies to sustainable aquaculture health management.

Keywords: Tilapia, Macrophage, Polarization, Trained Immunity, Plasticity

Project: Development of heterologous prime and boost strategies for augmentation of immunoprophylaxis in Nile Tilapia, *Oreochromis niloticus*

Funding: ICAR-Central Institute of Fisheries Education, India

Enhanced nucleic acid extraction of white spot syndrome virus by employing a novel protocol

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Penaeid shrimp and other aquatic crustaceans are highly susceptible to infection by the White Spot Syndrome Virus (WSSV). In recent years, genomic DNA extraction techniques have become central to many molecular diagnostics and technologies. DNA extraction kits were not included in the comparison because they often lack the flexibility and cost-effectiveness needed for large-scale applications in aquaculture research. This study introduces a novel Dimethyl Sulfoxide (DMSO)-based DNA extraction method that is simple, rapid, and equally sensitive, marking a unique advancement in aquaculture research. We used identical sample volumes to compare the DMSO-based method with the traditional phenol-chloroform and Guanidium Hydrochloride techniques. In the study, the DNA yield obtained using DMSO was 378.4 ng/µL, which was significantly higher compared to the other two extraction methods. The phenol-chloroform method yielded 267.8 ng/µL, while the GHCL method produced 151.2 ng/µL. The DMSO method has a lower detection limit and can detect DNA at concentrations as low as 10^{-9}, whereas the GHCL and Phenol-Chloroform methods have detection limits of 10^{-7}. These results suggest that DMSO provides a higher DNA yield and has a lower detection limit than the phenol-chloroform and GHCL methods, indicating its potential for more efficient DNA extraction. Therefore, our study introduces a high-quality genomic DNA extraction protocol applicable to the diagnosis of diseases in other marine organisms.

Keywords: DNA Extraction, White Spot Syndrome Virus, Shrimp Pathogen, PCR, Copy Number

Project: Application of recombinant protein of early expressing gene to combat white spot syndrome virus of Penaeid shrimp

Funding: Savitribai Jyoti Rao Phule single Girl child fellowship, University Grants Commission

Pan-genome analysis of potentially probiotic and halo-tolerant bacteria

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Aquaculture production is largely influenced by various biotic and abiotic factors such as disease outbreaks, sudden changes in environmental conditions, and water quality management. Conventionally, these challenges are handled by using chemotherapeutics, supplements and antibiotics, these preventive and curative measures cause various side effects such as residue accumulation, antibiotic resistance, immunosuppression and reduced consumer preference. In recent days, beneficial non-pathogenic bacteria that are predominantly present in the aquaculture and marine environment are being explored as probiotics and biocontrol agents. For instance, strains of *Bacillus paralicheniformis, B. licheniformis*, and *B. amyloliquefaciens* isolated from salt environments have demonstrated antagonistic activity against common pathogens in laboratory studies. Given their halotolerance and potential probiotic properties, we investigated the genomic diversity of the three species through pangenome analysis. A total of 189 complete genome assemblies were retrieved from the NCBI RefSeq repository, annotated using PGAP and subjected to pangenome analysis using PPANGGOLin software. An open pangenome comprising 901, 7714, 7407 persistent, shell and cloud genes respectively was constructed. Notably, genes associated with antimicrobial compounds were predominantly identified in the shell and cloud partitions, signifying the presence of these compounds in more than one strain. These genes are involved in producing plantaricin C family lantibiotic, lactococcin 972 family bacteriocin, mersacidin/lichenicidin type II lantibiotic, and gallidermin/nisin family lantibiotic. Out of 189 strains, both plantaricin C and lactococcin 972 were found to be present in 59 strains, all belonging to *B. licheniformis*. While, genes coding for mersacidin/lichenicidin and gallidermin/nisin were observed only in two and six strains respectively. Among these halo-tolerant species tested, *B. licheniformis* was found to be the most suitable species for aquaculture due to its inherent probiotic potential.

Keywords: Pangenome, *Bacillus*, Probiotic, Antimicrobial Peptides, Lantibiotics

Project: Network Project on Agricultural Bioinformatics and Computational Biology

Funding: Indian Council of Agricultural Research, India

Complete genomic sequence of snakehead rhabdovirus-Indian strain (SHRV-In)

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Rhabdoviruses pose significant threats to economically important farmed and wild fish populations, and are a large and ecologically diverse group of viruses. This study aimed to determine the complete genome sequence of Snakehead rhabdovirus - Indian strain (SHRV-In) and compare it with other rhabdoviruses. The complete nucleotide sequence of SHRV-In has been determined following cDNA cloning of the viral genomic RNA. The genome comprises 11550 bases and contains six long open reading frames (ORFs) encoding the structural proteins such as nucleoprotein [N], phosphoprotein [P], matrix protein [M], glycoprotein [G], non-structural viral protein [NV], and polymerase [L]. The structural protein genes are organized in the canonical order 3'-N-P-M-G-NV-L-5'. Multiple sequence alignment, phylogenetic analysis, and mutation identification were performed against other related fish rhabdovirus genomes, representing the evolutionary lineage among SHRV-In and other rhabdovirus isolates. Using the complete genome, ORFs encoding structural protein genes were annotated with a closely related fish rhabdovirus genome, highlighting genetic divergence from SHRV-In and other SHRV genome. Comparison of the complete genome and ORFs encoding structural proteins exhibit significant homology to corresponding sequences in the related fish rhabdovirus and confirm the assignment of SHRV-In to the genus *Novirhabdovirus*.

Keywords: Genome, Genetic Variance, ORFs SHRV-In, Structural Protein

Project: National Surveillance Programme for Aquatic Animal Diseases

Funding: PMMSY - ICAR-NBFGR - NSPAAD

Discovery of three *Perkinsus* species in the Philippines: A potential biogeographic link in the Indo-Pacific

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Perkinsus are protozoan parasites that infect marine molluscs, with some species posing serious threats to global shellfish aquaculture and biodiversity. Five *Perkinsus* species have been reported in the Asia-Oceania region and were previously thought to share common origins and similar biological traits. However, recent studies suggest a more complicated situation. For instance, *P. olseni* from Oceania and Japan differ in genome structure and biochemistry, while *P. beihaiensis* found in Japan is genetically closer to South American strains than to those from India or China. These findings suggest local evolution and transregional introductions, highlighting the need to better understand *Perkinsus* diversity and spread. Despite these insights, most studies on *Perkinsus* have been largely focused on the region's northern and southern extremes, leaving Southeast Asia understudied. Here, we report the first detection of *P. olseni, P. beihaiensis*, and *P. chesapeaki* in molluscan hosts from the Philippines. The discovery of three *Perkinsus* species where none were previously known fills a long-standing surveillance gap and points to the potential for wider, undetected distributions in the region. These findings also position the Philippines, and Southeast Asia more broadly, as a biogeographic link in the species' Indo-Pacific dispersal and evolutionary pathway. In addition, we successfully established cultured isolates of *P. olseni*, representing the first from Southeast Asia, opening new opportunities for comparative and functional studies. Ongoing phylogenetic and biochemical analyses of these samples aim to clarify the evolutionary relationships and regional spread of *Perkinsus* species across the Asia-Oceania region.

Keywords: Protozoan Parasites, Marine Molluscs, Transregional Introductions, Pathogen Diversity

Project: Fundamental studies on the factors determining host range of *Perkinsus* protozoans parasitizing bivalves

Funding: JSPS Kakenhi 23K27000

Novel direct acyclic graph-based approach provides new insights on microbial interactions

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The gut microbiota plays a vital role in the health and disease resistance of *Penaeus vannamei* (Pacific white shrimp), a cornerstone species in Asian aquaculture. While 16S rRNA gene sequencing is widely used to profile microbial communities, its compositional nature and the lack of temporal resolution limit inference of directional ecological interactions among taxa. To address this, we developed a novel approach to infer directional and effect-based microbial interaction networks using CLR (Centered Log Ratio) normalization, linear regression slopes, and local sensitivity analysis. This method captures both the directionality and pairwise effect polarity (+/+, +/-, -/+, -/-) at genus/species levels. We applied this approach to a public dataset on *P. vannamei* (BioProject: PRJNA921960) under pathogenic (*Vibrio parahaemolyticus*), non-pathogenic and control treatments. This yielded 21 condition and time-specific directed networks, spanning 168 samples (7 time points x 3 treatments x 8 replicates). Directionality and interaction effects were computed across genus pairs, with edges filtered by weighted correlation scores. Consistent directed acyclic graphs (DAGs) were tracked across conditions to study dynamic interaction changes. In healthy (non-pathogenic/control) conditions, networks showed high temporal and conditional stability, with consistent positive interactions among core species like *Photobacterium damselae* and *Motilimonas cestriensis*. In contrast, pathogenic conditions display frequent reversals in interaction direction and slope, indicating dysbiosis. Notably, network centrality measures align with microbial abundance patterns: *Vibrio* dominates central nodes in high-mortality samples, while *Photobacterium* maintains dynamic yet interpretable shifts across all conditions. This shows that network-derived insights reflect underlying abundance trends, adding a functional layer to standard taxonomic analysis. Moreover, species like (e.g., *Corynebacterium* sp., *Lawsonella clevelandensis_A*) exhibit context-dependent regulation, suggesting targets for microbiome modulation. This framework bridges a critical gap in amplicon-based metagenomic by enabling a mechanistic, interaction-driven view of community dynamics. It enables informed probiotic or intervention strategies, with wide applicability across aquaculture systems.

Keywords: Metagenomics, Microbiome, Directed Networks, Network Predictions

Project: Unravelling signatures of dietary protein sparing and fiber tolerance in *Penaeus vannamei* for development of cost effective feeds through omics approaches

Funding: Department of Biotechnology, Government of India

Bioprospecting seaweed-associated endophytic bacteria in a multiomics approach identifies potential antibacterial compounds from a novel *Vibrio* sp.

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In addition to their role as a novel food source, seaweeds play a crucial role in maintaining the health of marine ecosystems. These macroalgae harbor diverse symbiotic bacterial communities responsible for producing a variety of secondary metabolites essential to the vitality of adjacent fishery habitats. Evidence is increasingly supporting the antimicrobial properties of these seaweed-associated bacterial isolates, highlighting their potential applications in fish health management. Utilizing a multiomics approach facilitates the high-throughput identification of these endophytic bacteria along with their bioactive secondary metabolites. Insights derived from these integrative methodologies hold the potential to significantly improve fish health management practices within aquaculture systems. An optimized protocol for isolating endophytic bacteria from seaweed was developed and applied to 12 seaweed samples collected from the southeast coast of India, resulting in the recovery of 305 bacterial isolates. Following whole genome sequencing, one notable isolate exhibiting strong antimicrobial activity was characterized as a novel species within the *Vibrio* genus. Identification of a biosynthetic gene cluster (BGC) responsible for synthesizing prodigiosin, a known antimicrobial compound, was achieved. The ethyl acetate extract from the fermentation broth of this isolate demonstrated significant antibacterial activity against a range of foodborne pathogens. Furthermore, high-throughput metabolomics and lipidomics analyses performed using liquid chromatography-high resolution mass spectrometry confirmed the presence of prodigiosin. Molecular networking of the metabolomics data also revealed additional promising antibacterial compound classes, including diketopiperazines, buibui lactones, and indoles. The lipidomics profile further confirmed that the isolate is a novel species under the genus *Vibrio*. This study establishes a comprehensive high-throughput workflow for bioprospecting antimicrobial compounds derived from seaweed-associated endophytes, paving the way for innovative applications in aquaculture health management.

Keywords: Metabolomics, Lipidomics, Biosynthetic Gene Cluster, LC-HRMS, Aquaculture

Computational insights into probiotic-pathogen interactions in biofloc-based brackishwater aquaculture

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Brackishwater aquaculture plays a vital role in global food security and livelihoods. However, the industry faces persistent challenges from bacterial diseases, particularly those caused by *Aeromonas* species. These pathogens adversely affect fish survival and growth, often leading to the excessive use of antibiotics, which contributes to antimicrobial resistance, drug residues, and environmental degradation. Eco-friendly disease management strategies such as biofloc technology and probiotics have emerged as promising alternatives, though the molecular mechanisms underlying their protective effects against pathogens remain poorly understood. This study aimed to identify specific natural metabolites from biofloc and probiotic systems capable of mitigating bacterial infections in fish and further to elucidate the interaction between these microbial metabolites with virulence-associated proteins of *Aeromonas* spp. Using molecular modelling approaches, we examined the binding affinities between key virulence proteins of *Aeromonas* spp. (eg. aerolysin and other toxins), and a range of microbial metabolites commonly present in biofloc and probiotic systems including *Bacillus*-derived lipopeptides, siderophores (e.g., bacillibactin), polyhydroxybutyrate (PHB) oligomers, and short-chain fatty acids (SCFAs). Top-performing compounds were further evaluated through molecular dynamics (MD) simulations to assess the stability of their interactions. Additionally, ADMET (absorption, distribution, metabolism, excretion, and toxicity) analyses were also performed to evaluate their potential safety and bioavailability in aquaculture applications. Among the tested compounds, bacillibactin demonstrated strong binding affinity (docking score $\leq$ -7 kcal/mol) to the active site of virulence protein aerolysin, forming stable hydrogen bonds. MD simulations confirmed the sustained stability of these interactions. SCFAs and PHB oligomers also exhibited favourable drug-like properties with low predicted toxicity. These findings suggest that such metabolites may suppress bacterial virulence by targeting key pathogenic mechanisms, rather than directly killing the bacteria. This study provides evidence supporting the use of probiotic- and biofloc-derived metabolites as natural anti-virulence agents, offering a sustainable strategy to enhance biosecurity in brackishwater aquaculture.

Keywords: Probiotic metabolites, Biofloc-integrated immunity, Antibiotic alternatives, In-silico pathogen inhibition

Project: Genome editing approaches for improving growth and reproduction of brackishwater teleost and Indian white shrimp (*Penaeus indicus*)

Funding: Indian Council of Agricultural Research, India

Assessing the biochemical benefits of integrating *Gracilaria salicornia* in *Penaeus vannamei* aquaculture systems

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A novel seaweed-shrimp integrated farming system was developed to enhance the productivity and sustainability of shrimp aquaculture. The trial was conducted in 500 m^2 lined ponds stocked with 22,000 PL10 Penaeus vannamei per pond. Two treatments—control (without seaweed) and seaweed-integrated (with *Gracilaria salicornia*)—were maintained in duplicate. Seaweed was stocked at 7.5 kg per monoline tube net across 30 lines. Over a 110-day culture period, the integrated system achieved shrimp production with an average shrimp weight of 23 g and a survival rate exceeding 98%, whereas the control system yielded an average shrimp weight of 21 g with a survival rate of 97%. Concurrently, 1.2 tons of *G. salicornia* were harvested in two cycles from the same pond without the use of supplementary feed or fertilizers. Biochemical parameters such as amylase, cholesterol, triglycerides, total protein, and glucose were analysed in the shrimp serum. Among these, amylase, cholesterol, and glucose showed no significant difference ($p \geq 0.05$) between shrimp groups. However, triglycerides showed a significant increase ($p \geq 0.05$) in the seaweed-integrated group, while total protein was significantly higher ($p \geq 0.05$) in the control group. The results suggest that while seaweed integration does not alter all serum biochemical parameters, it significantly affects triglyceride and total protein levels, indicating potential metabolic or physiological impacts of seaweed inclusion in shrimp culture systems

Keywords: Brackishwater, *Gracilaria salicornia, Peneus vannamei*, Biochemical Analysis

Project: Demonstration of viable farming protocols for Indigenous brackishwater seaweed species for income generation among coastal folks

Funding: National Fisheries Development Board, India

Influence of *Priestia megaterium* strain PCLi4 on flocculation and performance of *Hypselobarbus pulchellus* and *Labeo fimbriatus* fry in bio-floc tanks

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The present study was designed to establish the role of *Priestia megaterium* strain PCLi4 supplementation on flocculation in bio-floc tanks and performance of *Labeo fimbriatus* and *Hypselobarbus pulchellus* fry. *L. Fimbriatus* (0.69±0.07g) and *H. pulchellus* (0.81±0.07g) fry were stocked in 1000 L FRP tanks at a density of 200 each. Fish were fed with formulated feed crumbles (28%CP) @ 10% of biomass thrice a day. Jaggery and dextrose were added as carbon sources in different treatments with or without the probiotic *Priestia megaterium* PCLi4. Inoculation of the probiotic enhanced carbon and nitrogen utilisation in the tanks forming flocs. The floc volume was the highest in Jaggery+Probiotic treatment (8.5ml/L) and the least in Control (0.75ml/L). It was observed that the ammonia level was the lowest with Jaggery+ Probiotic treatment (0.04±0.01 ppm) and highest with Control group (1.67±0.09). The growth data indicated that *H. pulchellus* did not respond to probiotics treatment or addition of carbon source in terms of final length, weight, survival and condition factor. However, the final weight of *Labeo fimbriatus* was the highest with Jaggery+Probiotic treatment and condition factor with Dextrose+Probiotic treatment indicating the positive effects. It looks like *H. pulchellus* being a macro-vegetation feeder does not respond positively to biofloc feeding. The fringe-lipped carp, *L. fimbriatus* seems to utilize biofloc better than *H. pulchellus*.

Keywords: *Priestia megaterium* PCLi4, Bio-floc, Growth, *Hypselobarbus pulchellus, Labeo fimbriatus*

Funding: Indian Council of Agricultural Research, India

Long noncoding RNA-mediated *cis* and *trans* regulation of mRNA expression in response to white spot syndrome virus infection in *Penaeus vannamei*

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lncRNAs are long non coding RNAs usually above 200 nucleotides in length and are increasingly recognized as regulators of various metabolic processes. While various transcriptome studies in response to WSSV infection in *Penaeus vannamei* have been studied, the specific regulatory roles of lncRNA in this context remains unclear. Therefore, this study aims to identify and predict the regulatory mechanisms of lncRNAs involved in WSSV infection in *P. vannamei*. RNA sequencing data from healthy and WSSV-infected shrimps were collected from five distinct tissues - gills, hemocytes, intestine, muscle and pancreas. Genome-guided differential expression analysis using DESeq2 revealed, respectively in gill, hemocyte, intestine, muscle and pancreas, a total of 36, 57, 21, 12 and 30 upregulated lncRNAs and 41, 26, 30, 6 and 28 downregulated lncRNAs. Additionally, the analysis also identified several significant differentially expressed mRNAs. Correlation analysis on normalized expression data, together with FEELnc and LncTar predictions, were used to identify and characterize cis- and trans-acting lncRNA-mRNA regulatory pairs respectively. A total of 9 intergenic and 1 genic cis acting lncRNA were identified which included 3 intergenic cis acting lncRNAs in hemocytes and pancreas, 2 in gills, 1 in muscle and 1 genic cis acting lncRNA in hemocytes. Furthermore 65, 7, 20, 24 and 11 trans regulatory lncRNA - mRNA pairs were also identified across the tissues. Notably, differentially expressed lncRNAs were predicted to regulate key host immune and cell death pathway-related mRNAs, including those encoding Crustacean cardioactive peptide (CCAP), Ankyrin-repeat, SH3-domain, and Proline-rich-region containing protein, zinc finger CCCH domain-containing protein 13-like, penaeidin-3b-like, and pro-resilin-like proteins. In summary, this study provides a novel comprehensive analysis of lncRNA-mediated regulatory networks in the WSSV response of *P. vannamei*, highlighting the potential of targeting specific lncRNAs to enhance disease resistance in shrimp aquaculture.

Keywords: lncRNAs, *Penaeus vannamei*, WSSV, Cis-acting, Trans-regulatory

Project: Whole genome sequencing of brackishwater aquaculture candidate species and development of genomic resources

Funding: Indian Council of Agricultural Research, India

Development and validation of a multiplex polymerase chain reaction (PCR) assay for the simultaneous detection of three species of *Perkinsus*

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Species of the genus *Perkinsus* are protistan parasites that cause substantial mortality in bivalve molluscs, posing a serious threat to global bivalve aquaculture. Accurate and rapid diagnosis is crucial for robust disease surveillance and effective management. This study details the development and validation of a novel multiplex polymerase chain reaction (PCR) assay for the simultaneous detection of three major pathogenic species of *Perkinsus* - *P. olseni, P. beihaiensis*, and *P. marinus* already reported from Indian waters. Additionally, a fourth genus-specific primer set was incorporated to detect all members of the genus *Perkinsus*, allowing identification of unreported taxa and their differentiation from known species. The assay was developed using four sets of species- and genus-specific primers targeting the ribosomal RNA gene regions. To assess the assay's performance, its sensitivity was determined using plasmid controls containing the target sequences, which were serially diluted to establish the limit of detection (LOD). The LOD for all four targets was found to be in the range of 10 to 100 copies of the target DNA. Specificity was evaluated by amplifying genomic DNA from tissue samples of three bivalve species (*Perna viridis, Mytella strigata*, and *Magallana bilineata*), which were independently verified as *Perkinsus*-negative. No cross-reactivity was detected in any of the samples. The optimized multiplex PCR assay successfully amplified distinct products of the expected sizes for each target simultaneously in a single reaction. This newly developed assay is a sensitive, specific, and efficient diagnostic tool that can greatly enhance disease surveillance and management in bivalve aquaculture, providing a more comprehensive and cost-effective alternative to the traditional Ray's Fluid Thioglycollate Medium (RFTM) culture method and single-plex assays.

Keywords: Bivalve aquaculture, Diagnostic assay, Disease surveillance, Multiplex PCR, *Perkinsus*

Project: National surveillance programme for aquatic animal diseases (NSPAAD)

Funding: PMSSY, Government of India; ICAR-Central Marine Fisheries Research Institute, India

DAA'12

Travel Awards

Development of autogenous vaccine against warm water Lactococcosis caused by bacterium *Lactococcus garvieae* in farmed rainbow trout of northern Himalayan region of India: An alternative to antibiotics

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Lactococcus garvieae (KG+ phenotype, strain RTCLI10) poses a significant threat to rainbow trout (*Oncorhynchus mykiss*) farming globally and in India. To counter this, we developed an inactivated immersion vaccine against this pathogen. Its biosafety was confirmed *in vitro* using epithelioma papulosum cyprini (EPC) cell lines, where cell survivability was over 90% after 120 h of exposure to various vaccine dilutions, and in vivo in rainbow trout (0.5 ± 0.01 g) immersed at 2.4 × 10^9 cells mL^{-1}. In vivo assessments showed no adverse effects on survival, behavior, or general health, and no histological changes were observed in key organs. For efficacy, rainbow trout (1.26 ± 0.43 g) received a primary vaccination and a booster vaccination seven days later, each at 1.2×10^9 cells mL^{-1}. Fish were then challenged with the homologous *L. garvieae* RTCLI10 strain (LD_{50}: 2.6 × 10^5CFU mL^{-1}). The vaccinated and challenged (VC) group demonstrated a remarkable 96.15% survival, compared to 76.28% in the non-vaccinated and challenged (NVC) group, resulting in relative percentage survival (RPS) of 83.78%. Furthermore, the VC group showed significantly elevated bactericidal activity, lysozyme activity, and serum antibody titers between 3- and 5- days post-challenge. Quantitative PCR analysis of 12 immunogenic genes in the spleen and anterior kidney also revealed significantly increased inflammatory gene expression in vaccinated trout. These findings conclusively demonstrate developed vaccine biosafety, and significant protective efficacy against *L. garvieae* infection, offering a promising, sustainable solution for Indian rainbow trout aquaculture.

Keywords: Biosafety, Efficacy, Fish vaccine, Immunogenic genes, Antimicrobial resistance

Project: Development of autogenous vaccine against *Lactococcus garvieae* infection in farmed rainbow trout: An alternative to antibiotics

Funding: Department of Biotechnology, Government of India

Identification and Genomic Investigation of a Novel Fish Poxvirus in Hatchery-Reared Red Seabream *Pagrus major*

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Red seabream (*Pagrus major*) is an economically important fish species in Japan, accounting for approximately 25% of the total fish aquaculture production. Since around 2020, unexplained mortalities have been observed at a red seabream hatchery in Japan. Affected juveniles exhibit skin darkening and sleep-like symptoms, which occasionally cause mass mortality. In this study, we identified a novel poxvirus genome in diseased fish using next-generation sequencing. Transcriptome analysis of moribund juveniles revealed several contigs with sequence similarity to salmon gill poxvirus (SGPV), suggesting the presence of a related virus. Subsequent metagenomic analysis led to the assembly of a 308-kbp genome of a novel fish poxvirus, designated Japanese seabream poxvirus (JSPV). This genome comprises 338 predicted open reading frames (ORFs). The DNA polymerase of JSPV shared 62% and 44% amino acid identity with those of SGPV and carp edema virus, respectively. Genomic comparison among fish poxviruses showed high conservation of synteny, particularly in the central genome region. A PCR assay was developed based on the I7L gene, which encodes the virion core cysteine protease, and JSPV was detected in 16 production batches between 2020 and 2024. Phylogenetic analysis based on partial sequences of the I7L gene revealed three distinct genetic groups: Ia, Ib, and II. In addition, a multiplex qPCR assay was developed to distinguish between genogroups I and II. These findings suggest that this novel poxvirus is likely associated with disease outbreaks in red seabream aquaculture, and the developed molecular tools will support future epidemiological surveillance and risk assessment efforts in the industry.

Keywords: Red seabream, fish poxvirus, next-generation sequencing, molecular epidemiology, PCR-based diagnostics

Project: Establishment of an emergency biosecurity framework for stabilizing the rearing of healthy red seabream juveniles

Funding: Japan Society for the Promotion of Science

First report of Red sea bream iridovirus (RSIV) genotype I and II recombinant strain in Japan

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Red seabream iridovirus (RSIV) is a fish pathogen that causes severe damage in aquaculture. In Japan, RSIV genotype II is mainly detected, while genotype I has not been detected for two decades. However, in 2024, two different genotypes of RSIV were detected. In this study, we conducted a phylogenetic analysis of the RSIV strains detected last year to investigate how these strains differ from those previously reported. This study analyzed three RSIV-positive samples (GT1-Oita2024, GT2-Oita2024, and Ehime2024) from Japanese aquaculture farms. After total DNA was extracted, in the case of GT2-Oita2024, it was sequenced using an Illumina sequencer. In the case of GT1-Oita2024 and Ehime2024, some genes were amplified by PCR, then sequenced using an ONT-nanopore sequencer. Phylogenetic analyses showed that GT2-Oita2024 and Ehime2024 were classified into RSIV genotype II. GT1-Oita2024 showed high identities with genotype I for major capsid protein, *SNP1,2* regions, and laminin-type growth factor-like domain. On the other hand, the ATPase sequences showed high identities with genotype II. These results suggest that GT1-Oita2024 was a genotype I and II recombinant strain. This is the first report of the genotype I and II recombinant strain in Japan. In recent years, the import and export of fish and the mixed aquaculture of multiple species have become common practices. It is possible that unknown strains different from previously reported strains may be found in the future. Early detection through regular monitoring of RSIV in aquaculture, as emphasized in this study and previous ones, is necessary.

Keywords: Red sea bream iridovirus, Viral recombination, Genotype I and II recombinant strain, Phylogenetic analyses

Characterization and Investigating the Dietary Effects of Co-Administration of *Shewanella putrefaciens* and *Rhodovulum sulfidophilum* on Gut microbiome changes in four-finger threadfin *Eleutheronema tetradactylum*

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The modern practices in aquaculture have led to significant challenges, particularly concerning disease outbreaks. Over the past two decades, the reliance on various medications, including antibiotics, has escalated, resulting in severe consequences such as the emergence of antibiotic-resistant microorganisms and disruptions in the gut microbiome of aquatic species. In response to these issues, the use of probiotics has gained considerable traction as a viable alternative. This study investigates the characterisation and dietary effects of co-administering two autochthonous probiotic strains, *Shewanella putrefaciens* S5 and *Rhodovulum sulfidophilum* FA120, on the gut microbiome of four-finger threadfin (*Eleutheronema tetradactylum*). The molecular identification of these strains was achieved through 16S rRNA gene amplification and Sanger sequencing. We evaluated their probiotic potential by assessing bile salt and acid tolerance, antibacterial activity against pathogenic fish bacteria, and antimicrobial susceptibility. Notably, S5 demonstrated significant inhibition of fish pathogens. Preliminary in-vivo evaluations, supported by metagenomic analyses, revealed a synergistic effect of the co-administration of these strains, enhancing gut colonization compared to monostrain treatments. This suggests that the combination of *S. putrefaciens* and *R. sulfidophilum* may effectively improve gut microbiome dynamics, thereby enhancing disease resistance and promoting sustainable aquaculture practices. The findings warrant further investigation to validate the potential applications of these strains in aquaculture, particularly in enhancing fish health and reducing reliance on antibiotics.

Keywords: Probiotic Strains, Immune modulation, Gut Microbiome, Sustainable aquaculture, Disease resistance

Project: Role of Gut Microbiome and Probiotics in Four-finger Threadfin

Funding: National Science and Technology Council under grant no. NSTC 112-2313-B-020 -007 -MY3 from the Ministry of Science and Technology, Taiwan.

WSSV proteins drive glutamine metabolism through glutamate dehydrogenase activation in *Litopenaeus vannamei*

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White spot syndrome virus (WSSV), the causative agent of the white spot syndrome (WSD), inflicts substantial economic losses in shrimp production. To facilitate virus replication, WSSV hijacks several metabolism pathways, including glutamate-driven anaplerosis; this enhances production of α-ketoglutarate via glutamate dehydrogenase (GDH), replenishing the tricarboxylic acid (TCA) cycle and providing essential carbon sources and ATP for virus replication. Although viruses often modulate enzyme activity through viral interactions with host metabolic enzymes, GDH regulation in WSSV remains unclear. The objective was to identify WSSV proteins that interact with LvGDH (Pacific white shrimp), using the yeast two-hybrid screening and the Spodoptera frugiperda (Sf9) cell interaction system. Two viral proteins, WSSV079 and WSSV164 were identified, and their interactions with LvGDH confirmed via co-immunoprecipitation. In addition, co-expression of LvGDH with either WSSV079 or WSSV164 significantly enhanced its enzymatic activity compared to controls. Conversely, in vivo dsRNA-mediated gene silencing of WSSV079 and WSSV164 reduced viral replication and LvGDH mRNA expression. To further explore the roles of WSSV079 and WSSV164, a proteomics interaction network was constructed between the protein interactors of WSSV079 and WSSV164 identified through an Affinity purification-mass spectrometry (AP-MS) analysis and our in-house proteomics databases. Based on correlation networks, these viral proteins interacted with host proteins involved in key biological pathways during WSSV infection, including the ESCRT-III (endosomal sorting complex required for transport) complex, actin cytoskeleton dynamics, and PCNA (proliferating cell nuclear antigen) implying a multifunctional modulator role to enhance viral replication. We concluded that both WSSV079 and WSSV164 are crucial for GDH activity regulation, serving as a GDH enzyme activator and reprogramming host glutamine metabolism to support WSSV replication in shrimp.

Keywords: AP-MS, glutamate dehydrogenase, WSSV079, WSSV164, yeast two-hybrid system

Project: Integrative omics strategy for Shrimp-WSSV interactome to elucidate viral pathogenesis and host responses

Funding: National Science and Technology Council, Taiwan

Glutamate-pyruvate transaminase (GPT1) as a key metabolic regulator during WSSV infection in *Litopenaeus vannamei*

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White spot syndrome virus (WSSV) causes high mortality in shrimp aquaculture, leading to severe economic losses. WSSV hijacks host metabolism, inducing Warburg-like metabolic reprogramming, including glycolysis and glutamine metabolism. Due to a reduced carbon flux from the Warburg effect, host cells rely on glutamine to replenish the TCA cycle. Glutamate-pyruvate transaminase (GPT) catalyzes the reversible transamination of alanine and α-ketoglutarate, producing pyruvate and glutamate. Our objectives were to characterize GPT1 (LvGPT1) and investigate its role during WSSV infection. In vertebrates, GPT1 and GPT2 are cytosolic and mitochondrial isoforms, respectively. However, only LvGPT1 was identified in our in-house shrimp transcriptomic database. Immunofluorescence localized LvGPT1 to mitochondria. During WSSV infection, LvGPT1 gene expression and enzymatic activity increased at the viral replication stage, but decreased at the late stage. To assess LvGPT1's function, dsRNA-mediated gene silencing was performed in WSSV-infected shrimp. Knockdown of LvGPT1 significantly reduced viral gene expression and genome copy number, highlighting its role in viral replication. Furthermore, metabolite replenishment (glutamine and α-ketoglutarate) partially rescued viral gene expression after LvGPT1 silencing, suggesting their importance in viral metabolism. Based on specific inhibitors of the PI3K-Akt-mTOR signaling pathway, mTORC2 positively regulated LvGPT1 expression, whereas Ras negatively regulated it during infection. Additionally, yeast two-hybrid screening revealed potential interactions between LvGPT1 and viral proteins. These findings underscored the essential roles of LvGPT1 in WSSV infection, with potential as a target for antiviral strategies in shrimp aquaculture.

Keywords: White spot syndrome virus, glutamate-pyruvate transaminase, glutamine metabolism, shrimp metabolism

Project: Integrative omics strategy for Shrimp-WSSV interactome to elucidate viral pathogenesis and host responses

Funding: National Science and Technology Council, Taiwan

Dietary perilla leaf extract supplementation enhances shrimp immunity and disease resistance

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The shrimp farming industry seeks natural and sustainable solutions to enhance shrimp health, immunity, and productivity while improving disease resistance against major pathogens. Acute Hepatopancreatic Necrosis Disease (AHPND), caused by *Vibrio parahaemolyticus* secreting PirAB toxins, led to severe shrimp mortality and economic losses globally. Perilla leaf extract (PLE), derived from *Perilla frutescens*, is rich in bioactive compounds with reported antioxidant and antimicrobial properties, but its application as a functional feed additive for shrimp and its efficacy against AHPND remain largely unexplored. The objective was to evaluate impacts of dietary PLE supplementation on shrimp growth, disease resistance, and immune response. Based on *in vitro* tests, including minimum inhibitory concentration (MIC) analysis and biofilm formation assays using *Vibrio* and non-*Vibrio* strains, PLE did not directly inhibit bacterial growth nor prevent biofilm formation. Additionally, western blot analysis detected no effect of PLE on inhibiting PirAB toxin secretion from AHPND-causing *V. parahaemolyticus*. In an *in vivo* feeding trial, shrimp were allocated into a control group and experimental groups receiving 0.5 or 1% PLE for 14 days. Post-feeding, shrimp were challenged with AHPND-causing *V. parahaemolyticus*. Shrimp fed PLE diets exhibited significantly reduced mortality after immersion challenge and effectively reduced AHPND-causing *Vibrio* loads and suppressed PirAB toxin production *in vivo*. These findings highlighted PLE's potential in mitigating AHPND pathogenesis *in vivo* despite its limited *in vitro* antimicrobial activity. Moreover, 1% PLE significantly upregulated key immune-related genes, including prophenoloxidase, serine proteinase 7, superoxide dismutase, and controlled ROS modulator-1 gene levels in hemocytes, suggesting that PLE's protective effects may be mediated through immune modulation and antioxidant responses. Furthermore, PLE supplementation improved hepatopancreas histology, with 1% PLE yielding the highest lipid content and best structural integrity. In conclusion, PLE-supplemented diets enhanced immune function, promoted disease resistance, and improved shrimp health.

Keywords: Perilla leaf extract, AHPND, disease resistance, sustainable aquaculture

Project: Integrative omics strategy for Shrimp-WSSV interactome to elucidate viral pathogenesis and host responses

Funding: National Science and Technology Council, Taiwan

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Technical Session VII

One Health and Aquatic Animal Biosecurity

Antimicrobial resistance and emergence of new pathogens in aquaculture in one health context

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The concept of one health recognizes that human, animal, plant and environmental health are interconnected and one health approach is characterized by collaborative multisectoral transdisciplinary principle. Mitigation of issues like zoonotic diseases, antimicrobial resistance and food safety are important concerns requiring one health approach. Unlike the homeothermic animals which may carry zoonotic pathogens, there are very few such pathogens associated with poikilothermic aquatic animals. But as aquaculture practices are changing, new pathogens are emerging. Till recently, the common estuarine organism, *Vibrio parahaemolyticus* was known as an organism of food safety concern, but during the last decade, this organism is emerging as an important shrimp pathogen and this is due to the acquisition of new mobile genetic elements like plasmids. Acute Hepatopancreatic Necrosis Disease (AHPND) of shrimp is caused by *V. parahaemolyticus* that has acquired a 69-70 kb plasmid carrying genes encoding the binary toxin, Photorhabdus Insect Related (pir) toxin. This disease affects shrimp during early larval stages. Another pathogen that has emerged recently is hypervirulent *V. parahaemolyticus* carrying a 187 kb plasmid carrying the virulence gene *vhvp-2*. This strain is known to cause translucent larval disease. The aquatic environment is a reservoir of bacteria resistant to antimicrobial agents with resistance determinants that have emerged in agriculture, veterinary sector or in hospitals. Biofilms of bacteria in the aquatic environment provide conducive niche for horizontal transfer of genes and emergence of multi-drug-resistant pathogens that may affect aquatic animals or humans. Thus, mitigation of problems like zoonoses, food safety and aquatic animal health requires a one health approach and collaboration with all the sectors.

Keywords: One Health, Antimicrobial Resistance, Zoonoses, Horizontal Gene Transfer

A call for further research on epigenetics: Examples from the field

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Diseases have proven to be one of the major threats to the sustainability of the aquaculture industry. They have caused severe economic impact at all levels, starting with small farmers, corporate companies up to national economies. Diseases have a wide range of expression. They may range from severe and acute mortality to low chronic mortality or slow growth. In addition to the diseases caused by primary pathogens, there are also the ones caused by opportunistic pathogens which can easily contribute to significant losses. Aquaculture is an economic activity and biosecurity measures need to be defined to contribute to the profitability of the industry in the long term. Effective biosecurity requires a multilayer approach: international, national and farm levels, each of them have different objectives and actors. Farm level biosecurity has a very broad spectrum of action covering different aspects related to the animal, to the pathogen and the environment. Biosecurity plans are specific to the facility, the species, the endemic and emerging pathogens, culture system etc. Even if the biosecurity plans are tailor made, there are a series of principles that will guide the definition of these plans. Such principles cover the economic, pathological, epidemiological and production aspects. The presentation will discuss the principles and their implementation.

Keywords: Biosecurity, Aquaculture, Cost-effective

Occurrence of foodborne pathogens in retail catfish from Bangkok, Thailand

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The rise of foodborne pathogens poses a significant threat to public health across Southeast Asia. This study evaluated the distribution of *Escherichia coli, Salmonella enterica*, and *Vibrio cholerae* in catfish sold at fresh markets in Bangkok, Thailand. A total of 390 tissue samples (gills, mucus, and intestines) were collected from 130 fish across seven markets. Standards of International Organization for Standardization (ISO) and Bacteriological Analytical Manual (BAM) microbiological protocols were followed for bacterial identification and confirmation. The sample showed positive for *E. coli* (62.3%), *S. enterica* (56.2%), and *V. cholerae* (11.0%). Significant associations were observed between sample types and pathogen detections. However, there is no significant difference between market locations, with gills showing the highest contamination over the sample types. These findings reveal a differential pattern of *S. enterica* contamination across tissue types, with gills showing the highest levels. *V. cholerae* was also more frequently found in gills than in intestines, indicating a higher risk of contamination in external tissues. This contamination level highlights the need for improved hygiene practices during fish handling and retail to reduce pathogens spread to other food commodities that may be sold together in retail market.

Keywords: Bacterial contamination, Catfish, Retail fish market, Thailand

Project: Emergence of antimicrobial resistance in *Escherichia coli, Salmonella enterica* and *Aeromonas hydrophila* isolated from catfish aquaculture and environment

Funding: The Thailand Science Research and Innovation Fund, Chulalongkorn University, The National Research Council of Thailand (NRCT) (Project ID N43A660897) and by LPDP, The Indonesia Endowment Fund for Education, Ministry of Finance, the Republic of Indonesia.

Evaluation of antimicrobial and disinfectant resistance and biofilm-forming ability of *S. agalactiae* pathogens from the aquaculture farms, Kerala

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Streptococcus agalactiae, a Gram-positive bacterium commonly associated with neonatal infections in humans, has emerged as a significant pathogen in aquaculture, particularly warm water fish species Tilapia (*Oreochromis* spp,). It causes streptococcosis, characterized by meningoencephalitis, septicemia, ocular lesions, and high mortality in fishes, which lead significant mortality, high transmission and resulting severe economic losses. A total of 206 samples consisting of fish, collected from the aquaculture farms in Thrissur (92) and Alappuzha (114) districts in Kerala for the isolation, identification, and evaluation of its antimicrobial and disinfectant resistance and biofilm-forming ability. Six number of *S. agalactiae* isolates were isolated from these samples with an overall incidence of 2.91% and it was high from Alappuzha (5) as compared to Thrissur (1) district. *S. agalactiae* are Grams positive bacilli, non-motile, beta-haemolytic, grow up to 30°C, grow in 6.5% NaCl, utilize pyruvate, production of acids from sugars and further confirmed by species specific STRA-AGI primers which target 16S to 23S rRNA intergenic space region. The antimicrobial susceptibility test by disk diffusion assay was carried out to nine numbers of antibiotics in Tryptic Soy Blood Agar and found resistance to cefotaxime (24mm), chloramphenicol (24.5mm), tetracycline (23mm), and levofloxacin (22.5mm). The genes such as emrE, mdfA, ydgE, ydgF, SugE(C), and qacF encode for efflux pumps for the quaternary ammonium compounds (QAC) were screened. One isolate was resistant to these all genes; three showed resistance to five genes except qacF and two isolates to emrE, mdfA, ydgE, and SugE(C). Three isolates were strong, and two were moderate biofilm producers. Through the lower incidence of *S. agalactiae* pathogen in the aquaculture farms in Kerala but showed higher resistance to antibiotics and disinfectants. It further suggested that the understanding of pathogenesis, host response, antibiotic and disinfectant resistance and also potential biofilm-formers, in aquaculture setting is critical for developing effective disease management strategies and promoting sustainable fish farming.

Keywords: Aquaculture farms, Infections, *Streptococcus agalactiae*, Antimicrobial Susceptibility, Disinfectant Resistance

Project: Diagnostic development for important pathogens, emerging AMR and other

pathogens in aquatic environments and seafood

Funding: ICAR funded Institute project

Setting epidemiological cut OFF values: *Vibrio parahaemolyticus* a one health case study

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Vibrio parahaemolyticus is a true One Health pathogen that causes significant economic loss in aquaculture business, is of concern in Northern Europe as clinical cases increase due to climate change and globally, the bacterium responsible for most food poisoning outbreaks in bivalve molluscs. The expert rules established by CLSI and EUCAST include only four aquatic pathogens to date. Here we address common errors in disk diffusion, what the expert rules require for an interlaboratory study, and the publicly available algorithms to help you analyse your data. Cefas is working with an international consortium of expert's that endeavours to bridge the data gap in setting epidemiological expert rules for aquatic pathogens in both Minimum Inhibition Concentration (MIC) and Disk Diffusion (DD).

Keywords: *Vibrio parahaemolyticus*, ECOFF, Expert Rules

Project: Setting ECOFF values for Aquatic pathogens

Funding: Fleming Fund

Risk of 'ESKAPE' Pathogens in aquaculture of Eastern India

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Antimicrobial resistance (AMR) is a growing menace in global health and food security. Chemotherapeutic agents are frequently used in aquaculture for disease management. Drug resistant bacteria have emerged due to entry of some of the antibiotics into aquatic environments. On analysis of fish and water samples from 897 freshwater fish farms, a total, 3961 bacteria were isolated which included *E. coli* (1337), *Staphylococcus* sp. (1344), and *Aeromonas* sp. (1280). Antibiotic susceptibility results were analyzed using WHONET 5.6 software following CLSI guidelines. *E. coli* isolates exhibited maximum resistance to ampicillin (20%), tetracycline (19.3%), nalidixic acid (19.1%), and cefpodoxime (16.7%). Whereas *Aeromonas* sp. showed high resistance to cefoxitin (44.2%), cefotaxime (22.7%), trimethoprim/sulfamethoxazole (18.3%). *Staphylococcus* sp. showed high resistance to penicillin (74.1%), erythromycin (32.3%) and cefoxitin (30.1%). Besides, the resistance pattern of other ESKAPE pathogens like *Klebsiella* sp., *Acinetobacter baumannii, Pseudomonas* sp., *Enterococcus* sp. were analyzed from fish and water samples collected from most urbanized parts of two districts of Odisha. The result revealed that *Enterococcus* sp. is resistant to erythromycin (14.75%) and tetracycline (9.83%). Klebsiella sp. was resistant to imipenem (19.2%), meropenem (6.85%) and ampicillin (100%). However, *A. baumannii* was found 100% resistant to many of the antibiotics like ceftazidime, trimethoprim/ sulfamethoxazole, ticarcillin/clavulanic acid, cefoperazone/sulbactam, gentamicin (88.9%), and amikacin (88.9%). Moreover, *Pseudomonas* spp. were resistant to minocycline (9.72%), piperacillin/tazobactam (9.86%), tigecycline (11.1%), aztreonam (12.3%), and trimethoprim /sulfamethoxazole (12.5%). However, these bacterial isolates demonstrated resistance to antibiotics not used in aquaculture practices, suggesting potential contamination from other sources.

Keywords: Aquaculture, Bacteria, ESKAPE pathogen, Antimicrobial resistance

Project: All India Network Project on Antimicrobial Resistance in Fisheries and Livestock
Funding: Indian Council of Agricultural Research, New Delhi

Overview on the national plan for the development of the aquatic animal health project

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Aquaculture in the Sultanate of Oman is one of the key pillars to diversify its national economy. The overall vision of the Ministry of Agriculture, Fisheries and Water Resources is to develop a sustainable, competitive and environment-friendly aquaculture sector that meets the needs of customers from the high-quality aqua products. The Ministry has been engaged in different research and development projects on fish and shellfish culture such as abalone hatchery, mussel and oyster culture, shrimp farming, cage and pond culture of finfish, hatchery development for finfish, sea cucumber aquaculture and development of freshwater integrated tilapia farms. The projects were run by the collaboration of multiple parties within the Fisheries Wealth sector, including the General Directorate of Fisheries Research, represented by the Aquaculture Center.

The "National Plan for the Development of the Aquatic Animal Health" project within the aquaculture Center consists of six programs; 1) The rehabilitation and development a specialized laboratory for aquatic Animal health, 2) Development of advanced detection method for Aquatic Animal pathogens wild and farmed species for research and diagnostic purposes, 3) Contribution to biosecurity implementation programs with collaboration with other parties in the Fisheries Wealth sector, 4) Contribution to the early warning programs by providing the technical support through participation in the filed inspection visits and responding to the mortalities reports in aquaculture facilities, 5) A program to enhance the economy and investment by supporting innovation and technology in the field of aquatic disease control through rapid screening or prevention and 6) Training program of Omani personnel in aquatic Animal disease diagnosis.

During the project, the inventory of aquaculture facilities was monitored, the implementation of biosecurity measures for some aquaculture facilities was inspected, the major challenges and health risks in farms were highlighted, and future research plans were proposed to combat the faced challenges.

Keywords: Aquaculture, Diagnostics, Biosecurity, Pathogen, Plan

Project: National Plan for the Development of the Aquatic Animal Health

Funding: Ministry of Agriculture, Fisheries Wealth, and Water Resources (MAFWR) and the National Program for Enhancing Economic Diversification (Tanfeedh)

Kinetic evaluation of formalin degradation under varying environmental conditions in aquaculture water

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Aquaculture contributes significantly to global food security providing protein-rich seafood. India is a major producer and exporter of seafood, with estimated shipments of 1,781,602 MT valued at USD 7.38 billion in FY 2023-24. Formalin, a crucial disinfectant in aquaculture, is typically administered in short-term bath treatments, allowing for targeted treatment with minimal stress to the aquatic organisms. It effectively treats bacterial, fungal and parasitic infections. To understand the influence of abiotic factors (pH, temperature, salinity and sunlight) on formalin degradation in water, an experiment was conducted with three different concentrations of formalin (2, 100 & 200 ppm) along with three different salinities (0.5, 15 & 25 ppt) and pH levels (5, 7 & 9) under two illuminations (sunlight & dark). During the experiment, the temperature and light intensity varied from 30 to 38°C and 8,150 to 118,354 lux with an average photoperiod of 12 hours and 5 minutes. Samples were collected periodically and formalin concentration was quantified using UV-spectrophotometry with the NASH method. Data fit was assessed and further analysis was performed using Computer Assisted Kinetic Evaluation. The degradation kinetics followed first-order reaction. Under sunlight at 2 ppm of formalin, the degradation was rapid, with the half-lives of 0.78, 6.1 and 9.0 days at 2, 100 and 200 ppm respectively. Salinity played a major role in degradation; low salinity (0.5 ppt) showed faster degradation (half-life 4.6 days), followed by 15 ppt (4.72 days) and 25 ppt (6.55 days). Faster degradation occurred at pH 9 (half-life 4.12 days) compared to pH 5 (4.49 days) and pH 7 (5.74 days). In contrast, under dark conditions, the half-life was 7.49, 19.46 and 28.83 days at 2, 100 and 200 ppm concentration respectively. These findings offer valuable insights into the environmental fate of formalin in sunlight-exposed aquatic systems and are essential for sustainable aquaculture management.

Keywords: Aquaculture, Disinfectant, Formalin, Kinetics, Photodegradation

Project: All India Network Project on Fish Health

Funding: Indian Council of Agricultural Research

Laboratory study on microbial degradation of antibiotic substances in sediment samples under controlled conditions

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Although antibiotics are known for their therapeutic value, excess application can result in the accumulation and resistance in native flora. Microorganisms can degrade antibiotic substances either through co-metabolism or utilization as growth substrates. The present study aimed to analyze the microbial changes in the sediment samples spiked with antibiotics under dark conditions, incubated in BOD at 25 °C. Sediment samples were subjected to an aging process with water of salinity 25 ppt for 30 days before being spiked with antibiotics. The sulphonamide group of antibiotics (n=11) was spiked at a concentration of 10 µg/kg. The sediment samples were placed in glass beakers and incubated under two different sediment conditions: moist and waterlogged. Microbial total plate count (TPC) was carried out at various time intervals (0, 1, 2, 5, 10, 20, 40, 80, and 160 days). Duplicate samples were maintained for residue degradation analysis using LC-MS/MS. Computer-Assisted Kinetic Evaluation (CAKE) software was used to determine the half-life of the antibiotics. TPC of sediment samples in control, moist, and water-logged sediment samples were found to be 5.38 and 4.93 Log_{10} CFU gm^{-1}, respectively. During the study, TPC decreases till day 10 in the sediment samples, and thereafter an increase in counts was recorded in moist (7.2%) and water-logged (0.46%) conditions on day 20. However, finally TPC showed a decreasing trend till day 160 in both moist (3.53%) and water-logged (-1.68%) conditions in comparison with the TPC of the control sediment. Among the antibiotics tested, in moist conditions, Sulfathiazole recorded the lowest half-life time of 16.8 days, while in waterlogged conditions, sulfamethoxazole recorded the lowest half-life time of 22.6 days. This study provides insights into the degradation kinetics of antibiotics in sediment under moist and waterlogged conditions by microorganisms through various degradation pathways.

Keywords: Sulphonamides, Microbial degradation, Moist and water-logged conditions, LC-MS/MS, Half-life-time

Project: AINP-FH project

Funding: Indian Council of Agricultural Research

Dietary impact of antibiotic oxolinic acid on intestinal microbiome, and antibiotic residue bioaccumulation in juvenile *Labeo rohita*: Implications for aquaculture and human Health

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Oxolinic acid (OA) is a broad-spectrum antibiotic recommended for use in aquaculture to control bacterial infections. While its use has been documented in various fish species, limited research has explored the potential physiological effects of oxolinic acid on freshwater fish. Dietary oxolinic acid (OA) on juvenile *Labeo rohita* (rohu carp), a species of significant economic importance in aquaculture, were investigated for extended period of 35-day with two different doses i.e. 1x (60 mg/kg body weight/day) and 3x (180 mg/kg body weight/day) and followed by a withdrawal period of 10 days. Results indicated that OA supplemented feeding led to insignificant/reduced growth performances and caused marked histopathological alterations in the liver and kidney tissues. OA exposure for extended period also induced intestinal dysbiosis, oxidative stress, and compromised immune functions, particularly at the 3x (180 mg/kg body weight/day) OA dose treatment. Further, in the 1x OA treatment group, significant (p>0.05) upregulation of proinflammatory cytokines, TLR pathway genes, innate and acquired immune genes, and antioxidative genes was observed in comparison to both the control and 3x OA treatment groups. Additionally, a significant (p>0.05) downregulation of anti-inflammatory genes was noted in the 1x, 3x OA group. After a 10-day withdrawal period, improvements in intestinal microbiome composition were observed in both the 1x and 3x OA treatment groups. However, the restoration of microbiota composition was most pronounced in the 1x treatment group when compared to the 3x treatment groups. Residue analysis revealed the highest OA concentrations in liver tissue, followed by intestine, plasma, kidney, and muscle, with a noteworthy decrease in residue levels following the withdrawal period. Notably, the residual OA levels in muscle tissue remained below the safety threshold after therapeutic recommended dose of OA supplementation, suggesting no health risk for human consumption.

Keywords: Oxolinic Acid, *Labeo rohita*, Antibiotic Residues, Intestinal Microbiome, Immune Function

Project: All India Network Project on Fish Health (AINPFH)
Funding: Indian Council of Agricultural Research

Detection of virulent and antimicrobial resistant genes in *Vibrio* species isolated from shrimp farms of Karnataka

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Shrimp aquaculture in Karnataka has witnessed rising incidences of bacterial infections, notably those caused by *Vibrio* species, posing threats to yield, economic and environmental stability. This study investigates the molecular profiles of *Vibrio* spp. isolated from multiple shrimp farms across 10 coastal sites across three districts in Karnataka, focusing on the detection of key virulence factors and antimicrobial resistance (AMR) genes. Samples including shrimp (44), water (22), and pond sediment (4) were used for the isolation of various *Vibrio* spp. through conventional methods. In the present study, a total of 160 *Vibrio* isolates were isolated including *Vibrio orientalis, V. parahaemolyticus, V. vulnificus, V. fluvialis, V. harveyi, V. campbellii*, and *Salinivibrio costicola*, among others. All *Vibrio* isolates were subjected to PCR-based screening targeting 11 virulence associated genes and 10 AMR gene markers. Most of the virulence genes showed high prevalence including *chiA* (83.3%), *flac* (68%), *tlh* (46.9%), and *luxR* (40%), while *tdh* (1.5%), *ctxA* (15.5%), *toxR* (16.6%), *Va.clg* (10.6%), and *vhhA* (5.3%) were less frequently detected. AMR profiling showed dominant presence of the *blaTEM* gene (74.24%) in most of the *Vibrio* isolates, followed by *sul1* (26.5%), *catII* (14.4%), *sulII* (12.8%), *tetC* (9.84%), and *qnrA* (7.5%). AMR gene markers like *tetM, mcr3, cmlB*, and *cmlA* were detected at lower frequencies. These findings reveal the widespread occurrence of pathogenic and multidrug-resistant *Vibrio* spp. in shrimp farm and its environment, underscoring the urgency for integrative health management and rational antibiotic use in aquaculture systems. The molecular insights provided here serve as a valuable reference for future epidemiological monitoring and targeted intervention strategies across India's coastal aquaculture zones.

Keywords: *Vibrio*, AMR genes, Virulence genes, Shrimp

Project: Phase 2 of National Surveillance Programme for Aquatic Animal Diseases (NSPAAD): Implementation of surveillance in Freshwater and Brackishwater sectors in the state of Karnataka

Funding: Department of Fisheries, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India Phase 2 of National Surveillance Programme for Aquatic Animal Diseases (NSPAAD) under Pradhan Mantri Matsya Sampada Yojana (PMMSY) vide letter no. 35028/05/2021-Fy (Trade)(E-3603) dated July 7, 2022

Assessment of microplastic contamination in commercially available fishes

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Plastics have widespread applications for human use, but their disposal poses a significant threat to living organisms and these plastics end up in the marine environment. They will be fragmented into small pieces as a result of ultraviolet exposure, climatic changes, and temperature changes; Microplastics (MPs) are plastics that are less than 5 mm in size. The level of MPs pollution in commercially harvested fish from different habitant in Vellore, India is currently unknown. Therefore, this study aimed to determine the presence and characteristics of ingested or inhaled MPs in marine and freshwater fishes highly consumed by the local population. Fish gills and gastrointestinal tracts were aseptically dissected and digested (30% hydrogen peroxide), then filtered and examined under a microscope for the presence of MPs. Further analysis was performed on the samples using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR) and Scanning Electron Microscopy (SEM) with Energy Dispersive X-Ray (EDAX). Of the samples analysed, a total of 875 MPs were recovered from 32 fishes, with 478 from marine fishes and 397 from freshwater fishes. The most common colours of the MPs were blue and black, while stereo microscopy analysis revealed that the majority of MPs were fibers (91%), followed by fragments (8%) and a small number of films. The ATR-FTIR analysis identified polyvinyl alcohol (39.76%), polyethylene (16.51%), methylcellulose (12.84%) and styrene (9.07%), as the predominant types of MPs in the fish samples. This study highlights the significant impact of MP pollution on marine ecosystems. The research provides insight into the nature and extent of MPs in fish from both marine and freshwater habitats, with an aim for policies and interventions aimed to reduce plastic pollution in the locality.

Keywords: Microplastics (MPs), Marine fishes, Lake fishes, Human health, Food contamination

Molecular identification of Anisakid nematodes from Indian waters

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The parasitic nematodes of Anisakidae family are notorious for posing risk to human health all across the globe owing to their zoonotic nature. The developmental stages of anisakid nematodes which are capable of parasitizing multiple hosts ranging from zooplanktons, teleost fishes, cephalopods and marine mammals. Along with the consumer dissatisfaction and food safety concern, the occurrence of these parasites incur huge economic losses in seafood industry. India being a major producer, exporter and consumer of seafood have poorly reported and documented the presence of these zoonotic parasites. In the study, 30 specimens of *Auxis thazard* collected from a local fish market in Kochi, Kerala were examined for the nematodes. A total of 10 worms were collected from the visceral cavity of the fish. The morphological features of the worms were studied under light microscopy. The characteristic morphology of the Anisakidae family like the boring tooth, ventriculus and mucron were observed in the worms. The prevalence and mean intensity of the anisakid worms were found to be 13.33% and 1.75 respectively. Of them, subsequent molecular analysis of the Internal Transcribed Spacer region (ITS) (ITS1-5.8s-ITS2) of the ribosomal RNA gene revealed that the representative worms belonged to *Anisakis typica* with identity value of 100%. The sequences were submitted to GenBank with accession numbers- PV892391 and PV892393. A member of a related family *Raphidascarididae* namely, *Hysterothylacium amoyense* (Accession no. PV892392) was also present in the fish specimens with a prevalence of 6.67% and mean intensity of 1.5. Further insights into the genetic diversity of these nematodes in Indian waters are essential in establishing proper surveillance strategies for countering zoonotic threats and for ensuring the seafood quality and safety, thereby safeguarding consumer rights in the country.

Keywords: Zoonotic parasite, Seafood industry, Consumer health, *Anisakis typica, Auxis thazard, Hysterothylacium amoyense*

Project: ASEAN-India Collaborative research project "Detection of zoonotic parasite Anisakis spp. through molecular tools: An emerging public health concern"

Funding: Anusandhan National Research Foundation (ANRF)

Whole genome analysis of *Chryseobacterium* sp. isolated from diseased goldfish (*Carassius auratus*): Insights into its antimicrobial susceptibility and pathology

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Chryseobacterium sp., a member of the family *Weeksellaceae*, is emerging as a significant opportunistic pathogen in aquaculture. In this study, a disease outbreak among goldfish (*Carassius auratus*) from an ornamental fish farm in Kozhikode, Kerala, was investigated. Bacteria isolated from the kidneys of clinically infected fish formed yellow pigmented colonies on nutrient agar after 24 hours of incubation at 28 °C. The isolates were characterized as Gram negative, rod shaped, catalase positive, and oxidase positive. Molecular identification through 16S rRNA gene sequencing confirmed the organism as *Chryseobacterium* sp., showing 97% sequence similarity with known strains. The pathogenicity of this bacterial pathogen was experimentally confirmed in goldfish by a challenge experiment and the LD_{50} was determined as 7.46 x 10^7 CFU/mL Histopathological analysis revealed necrosis and degenerative changes in the kidney and spleen, supporting the organism's virulent nature. To gain deeper insights into its genetic basis of virulence, whole genome sequencing was performed using the Illumina NovaSeq 6000 platform, generating 2.69 million paired-end reads (150 bp). High-quality reads were assembled de novo, resulting in 97 scaffolds with an average length of 47,290 bp. Genome annotation predicted 4,276 coding sequences, 77 tRNA genes, and 10 rRNA genes (16S, 23S, and 5S rRNA). Several putative virulence factors, including genes related to adhesion, iron acquisition, stress response, and antibiotic resistance, were identified. This work provides comprehensive genomic and pathological insights into *Chryseobacterium* sp., highlighting its potential as an emerging threat in ornamental fish health and the need for vigilant monitoring and appropriate biosecurity measures.

Keywords: *Chryseobacterium* sp., Goldfish, Pathogenicity, Genome analysis

Project: National Surveillance Programme for Aquatic Animal Diseases- Phase II

Funding: Pradhan Mantri Matsya Sampada Yojana

Monitoring AMR in aquatic animals and the environment: Developing methodology for AMR and antibiotic residue screening

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Antimicrobial resistance (AMR) poses significant risks to human, animal, and ecosystem health, particularly in aquatic environments where pollution from various sources can exacerbate the problem. This poster presents a comprehensive overview of the methodologies developed for environmental AMR and contaminant monitoring. It highlights the importance of understanding and mitigating pollution from agri-aquaculture, manufacturing industries, hospitals, and municipal settings. The methodologies include microbial and chemical recovery approaches, as well as the use of minimum inhibitory concentrations (MICs). The development of a multidisciplinary toolbox approach is fundamental to improving our knowledge of the implications of pollution on waterbodies, microbial ecosystem function, and the epidemiology of AMR. The proposed methodologies aim to generate baseline data that will support the development of robust environmental AMR surveillance frameworks for 'One Health' protection.

Keywords: AMR, One Health, Monitoring, Antibiotics

Project: AMR surveillance in aquatic animals and their environment

Funding: Fleming Fund, DEFRA

Aquatic environmental monitoring: A pillar for sustainable biosecurity in aquaculture

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As aquaculture becomes increasingly vital to global food security, ensuring the health of aquatic ecosystems through sustainable biosecurity practices is essential. The Department of Fisheries Brunei Darussalam, through the Aquatic Animal Health and Laboratory Services Centre (AAHLSC) and the Mobile Technical Unit (MTU), has undertaken proactive measures in environmental monitoring and shrimp health surveillance to address biosecurity risks within aquaculture systems. Environmental monitoring is crucial for the early detection of waterborne pathogens, harmful algal blooms, and chemical pollutants. By tracking water quality parameters, such as temperature, pH, dissolved oxygen, ammonia, and microbial presence, it provides actionable insights that enable timely interventions, reduce disease outbreaks, and ensure compliance with environmental standards. The integration of emerging technologies, such as environmental DNA analysis using PCR, plays a pivotal role in assessing water conditions, particularly in detecting microsporidian parasites like *Enterocytozoon hepatopenaei* (EHP), a significant pathogen in shrimp aquaculture. Collaboration among stakeholders such as farmers, government agencies, researchers, and neighbouring countries, is essential for developing effective monitoring protocols. The Department of Fisheries keeps on improving their staff capacities in using and mastering the advance diagnostic techniques and environmental monitoring to safeguard aquatic animal health, mitigate the impacts of environmental stressors, and reduce economic losses. Understanding how environmental conditions affect immune function and disease transmission is central to these efforts. Ultimately, integrating science-driven environmental monitoring into aquaculture operations shifts the industry from reactive crisis management to proactive, long-term biosecurity and environmental stewardship. This paper will discuss this approach as it is fundamental for sustaining both aquaculture productivity and the ecological balance of aquatic ecosystems, ensuring the industry's resilience in the face of growing global demand.

Keywords: Aquaculture, PCR, Shrimp Health, Surveillance, Water Quality

Degradation kinetics of sulfonamides: Insights from mesocosm studies

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Antibiotics are extensively used in animal husbandry, dairy, and aquaculture to enhance productivity and control bacterial infections. However, indiscriminate use leads to environmental contamination and promotes the development of antibiotic resistance. Sulfonamides, widely applied in aquaculture as prophylactic and therapeutic agents, often enter aquatic environments via animal waste. Their persistence in water and sediments poses ecological risks and disrupts microbial communities. To investigate the degradation dynamics of sulfonamides in aquatic systems and evaluate the roles of light, microbial activity, and sediment adsorption using a mesocosm experimental approach. A laboratory-scale mesocosm experiment was conducted using 10 L reactors filled with sediment and water (0.5 ppt salinity). Selected sulfonamides (n=11) were spiked (10 µg L^{-1}) and monitored for adsorption, biodegradation, hydrolysis, and photodegradation under both sterile and non-sterile conditions, with light and without light exposure. The half-lives of the analytes were determined using Computer-Assisted Kinetic Evaluation (CAKE) software, and all sulfonamides followed first-order kinetics. Under sterile conditions, where microbial activity was absent and only hydrolysis occurred, the half-lives of sulfonamides ranged from 21 to 104 days. Light exposure enhanced photodegradation and significantly reduced half-lives to 5.6-47 days in water-only reactors and 5.6-24 days in water-sediment reactors. In contrast, under dark conditions, sulfonamides exhibited prolonged persistence due to the absence of photodegradation, with half-lives of 2648.8 days in water-only reactors. Water-sediment reactors under both light and dark conditions showed similar half-life values for all sulfonamides tested, emphasizing the important role of sediment adsorption in their removal. Overall, microbial activity (biodegradation), light exposure (photodegradation), and sediment adsorption emerged as the primary processes driving sulfonamide degradation in mesocosm bioreactors, while hydrolysis contributed only minimally. These findings highlight the combined influence of biotic and abiotic factors on the environmental fate of antibiotics in the mesocosm model.

Keywords: Mesocosm, Sulfonamides, Degradation

Project: All India Network Project on Fish Health

Funding: Indian Council of Agricultural Research

Prevalence and antimicrobial resistance mapping of *Vibrio parahaemolyticus* from key shrimp farming zones of Kerala

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Vibrio parahaemolyticus is a significant pathogen of concern with dual faces, as it causes Acute Hepatopancreatic Necrosis Disease (AHPND) in aquaculture and food borne illness in human beings. In addition, the presence of antimicrobial resistance in V. parahaemolyticus poses more risk to aquaculture, human health and food safety. Therefore, the present study aimed to estimate the prevalence of *V. parahaemolyticus* in shrimp farms from key farming zones of Kerala Viz., Ernakulam (n=20), Thrissur (n=20), Alappuzha (20) and Kannur (n=18). A total of 156 samples which comprised of 78 animals [*Penaeus vannamei* (n=40); *P. monodon* (n=25); *P. indicus* (n=13)] and respective water were screened for the presence of *V. parahaemolyticus* according to BAM protocol. All the isolates were confirmed by PCR targeting *toxR* gene with amplicon size 360 bp. The antimicrobial susceptibility test was performed by disc diffusion assay against 12 antibiotics of different classes such as penicillins, cephems, carbapenems, aminoglycosides, tetracyclines, fluroquinolones, folate pathway inhibitors and phenicols. In this study, 61% (n=48) and 47% (n=37) isolates of *V. parahaemolyticus* were recovered from shrimp and water samples respectively and subjected for resistant profiling. Even though MDR was not identified by antibiogram profiling, resistance was noted against antibiotics such as ampicillin, cefotaxime and cefoxitin. Therefore, it is crucial to monitor the animal as well as its environment as reservoirs of AMR in order to control its rapid spread to develop better strategies to ensure safety and security for sustainable aquaculture practices.

Keywords: *Vibrio parahaemolyticus*, toxR Gene, Antimicrobial Resistance (AMR), Shrimp Farms, Kerala

Project: All India network project on antimicrobial resistance

Funding: Indian Council of Agricultural Research

Distinct distribution in AMR pattern found among *Escherichia coli* screened from shrimp farms of Kerala

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Extended spectrum beta lactamase producing *Escherichia coli* is considered as a dual threat in both aquaculture and clinical environments across the globe very recently. They pose significant risk in treating infections as they are resistant to many of the last resort antibiotics such as 3rd and 4th generation Cephalosporins which are widely used in modern healthcare settings. This study evaluates the resistance profiles of *E. coli* from the shrimp aquaculture environments in major farming zones of Kerala Viz., Ernakulam (n=20), Thrissur (n=20), Alappuzha (20) and Kannur (n=18). A total of 156 samples which comprised of 78 animals [*Penaeus vannamei* (n=40); *P. monodon* (n=25); *P. indicus* (n=13)] and respective water samples were screened for the presence of *E. coli* according to AINP-AMR standard protocol and further confirmed by PCR. The Antimicrobial susceptibility test was performed by Kirby bauer disc diffusion assay against 15 antibiotics which belongs to different Classes. All the resistant isolates were subjected to PCR for the respective antibiotic-resistant genes (ARGs) such as $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}2}$, $bla_{CTX\text{-}M\text{-}9}$, bla_{TEM}, bla_{SHV} (cephalosporins), *tetA*, and *tetB* (tetracyclines), *sul 1, sul 2* (Sulphonamide), *qnrA, qnrS*, (*quinolones*). In this study, it was found that 56% (n = 44) of the shrimps and 69% (n = 54) of the water samples were found positive for E. coli. Among these isolates 3% and 6% were identified as ESBLs respectively by antibiogram profiling. Maximum ESBL prevalence were noted in *P. vannamei* farms of Ernakulam district. MDR was noted among 12.3 % of isolates and high resistance was prominent against antibiotics such as ampicillin, tetracycline and nalidixic acid. Monitoring both aquatic animals and their surrounding environments as potential reservoirs of antimicrobial resistance (AMR) is essential to curbing its rapid spread and devising effective control strategies and ensuring the safety and sustainability of aquaculture practices.

Keywords: ESBL, *E. coli*, MDR, Shrimp Farms, Kerala

Project: All India network project on antimicrobial resistance

Funding: Indian Council of Agricultural Research, India

Isolation and characterization of sulfur-oxidizing *Rhodococcus* spp. for bioremediation in aquaculture systems

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Sulfur compounds, particularly hydrogen sulfide (H_2S), are recognized as critical environmental pollutants in aquaculture systems, often leading to significant economic and ecological damage. H_2S is highly toxic to aquatic organisms, disrupting respiratory and metabolic processes, thereby contributing to increased mortality and impaired growth. The use of sulfur-oxidizing bacteria (SOB) as probiotics has gained prominence as a sustainable strategy for mitigating sulfide toxicity and improving water quality. In the present study, *Rhodococcus* spp., known for their sulfur-oxidizing capability, were selected as candidate organisms. Twenty isolates (RC1-RC20) were obtained from various coastal environments. Initial screening was carried out based on acidification potential (pH reduction), from which ten promising isolates were selected for further characterization. These isolates were assessed for salt tolerance, growth on diverse sulfur compounds (both organic and inorganic), and their capacity to utilize these as energy sources. In addition, their potential for ammonia and nitrite removal was evaluated to explore their broader applicability in water quality enhancement. Molecular identification was performed through 16S rRNA gene sequencing. Four isolates demonstrating the highest sulfate ion production and multifunctional bioremediation potential were selected for consortium development. Future efforts will focus on the formulation of a stable and synergistic probiotic consortium using these strains, with the aim of developing an effective bioremediation strategy to combat sulfide toxicity and promote environmental sustainability in aquaculture systems.

Keywords: *Rhodococcus* spp., Sulfur-Oxidizing Bacteria, Bioremediation, Aquaculture, Sulfide Toxicity

Project: All India Network Project on Fish Health

Funding: Indian Council of Agricultural Research, India

Molecular characterization and antimicrobial resistance of enterococcus species from farmed carp and trout in Northern India

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The rapid global expansion of aquaculture, driven by the growing demand for animal-derived protein, faces increasing challenges from antimicrobial resistance in aquatic pathogens. *Enterococcus* species, naturally occurring in the gastrointestinal tract of humans, animals, and aquatic organisms, are of concern due to their ability to develop and disseminate resistance traits. The study explored the occurrence, molecular identity, and antimicrobial susceptibility of *Enterococcus* species isolated from cultured carp and trout obtained from aquaculture farms in Himachal Pradesh and Uttarakhand, India. A total of 110 fish were examined, and intestinal samples were processed on Slanetz-Bartley Agar for selective isolation. Colonies with characteristic morphology underwent biochemical testing, including catalase and DNase activity, aesculin hydrolysis, and methyl red reactions. Molecular confirmation and species-level identification were achieved through partial sequencing of the 16S ribosomal RNA gene, followed by phylogenetic analysis. Susceptibility to 29 antimicrobial agents was assessed using the disk diffusion method, following Clinical and Laboratory Standards Institute (2016) guidelines. Sixty-four isolates were recovered, indicating an overall prevalence of 58%. Sequence analysis identified four species: *Enterococcus faecalis, Enterococcus faecium, Enterococcus hirae*, and *Enterococcus durans*. Resistance to multiple commonly used antibiotics was observed, with clindamycin, kanamycin, penicillin-G, imipenem/cilastin, and lincomycin showing the highest resistance rates. The detection of multidrug-resistant *Enterococcus* species in farmed fish from these regions signals a potential risk of resistance transfer through the food chain. The findings highlight the need for targeted antimicrobial stewardship, regular monitoring, and improved biosecurity practices in aquaculture operations.

Keywords: Multidrug Resistance, Intestinal Microbiota, Phylogenetic Analysis, Aquaculture Biosecurity, Zoonotic Risk

Project: All India Project on Antimicrobial Resistance in Fisheries and Livestock (AINP-AMRFL)

Funding: Indian Council of Agricultural Research, India

Phenotypic and molecular identification of *Streptococcus agalactiae* isolates from veterinary, clinical, and aquatic sources in a one health context

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Streptococcus agalactiae is an important pathogen affecting humans, animals, and fishes and possesses potent virulence mechanisms that permit its colonization, invasion, and evasion in the host immune system. Ten serotypes have been identified among Group B *Streptococcus* (GBS) based on the capsular polysaccharides, viz., Ia, Ib, II, III, IV, V, VI, VII, VIII and IX. More recently, fish consumption has been associated with an increased risk of *S. agalactiae* serotypes Ia and Ib colonization in humans. Several gaps exist related to the occurrence of GBS, risk factors related to the infection, and the transboundary potential of the pathogen. The organism is recognized as a human pathogen causing neonatal infections and is increasingly being reported from urinary tract infections in adults. In the veterinary sector, it is a primary causative agent of bovine mastitis, leading to substantial economic loss due to decreased milk production coupled with contaminated milk.

Using modified conventional microbiological and molecular techniques were optimised for the isolation and identification of *S. agalactiae*. We evaluated the efficiency of available primers for the molecular detection of *S. agalactiae* and the results were further confirmed by 16 SrRNA sequencing. A total of 210 samples comprising fish, sediment, water, and sewage were obtained from aquatic environments across eleven districts in the west coast of India. Among the 63 isolates that showed amplification for polymerase chain reaction (PCR) with *S. agalactiae* specific 16SrRNA primers, only 20 isolates could be confirmed as GBS based on PCR with *cfb* (CAMP factor) and *atr* (alanine transferase) primers followed by 16SrRNA sequencing. The clinical and veterinary isolates were also validated using the modified phenotypic and molecular methods developed. The confirmation of presumptive isolates has presented ambiguity in all the studies reported, and this was resolved to a great extent.

Keywords: One Health, *Streptococcus agalactiae*, PCR, 16SrRNA, CAMP Factor

Project: Deciphering the pathogenicity of zoonotic pathogen *Streptococcus agalactiae* obtained from fish

Funding: VGST

Role of Aquatic Quarantine Facility (AQF) in disease mitigation in the Indian shrimp sector

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The Aquatic Quarantine Facility (AQF), established by the Marine Products Export Development Authority (MPEDA), serves as a centralized quarantine unit for the country, specifically set up to quarantine Specific Pathogen Free (SPF) shrimp stocks imported from overseas. Established in 2009 at the behest of the Ministry of Fisheries, Government of India, the AQF ensures that all shrimp stocks entering the country are free from specific pathogens before they are transported to hatcheries or broodstock multiplication centres. The facility plays a key role in disease mitigation through its rigorous screening protocols and stringent biosecurity measures. Although the stocks are certified or declared free from specific (listed) pathogens (SPF) by overseas suppliers, there have been instances where certain pathogens were detected due to unexplained reasons. To date, AQF has reported three such cases at different instances. In all instances, the results declared by AQF were validated by its referral laboratory. The infected stocks were subsequently destroyed within the AQF premises as per the direction of the Technical Committee. Thus, AQF demonstrated its commitment in upholding its main objective of disease prevention through transboundary shipments. This paper provides a comprehensive overview of the AQF, its operations, and its significant role in mitigating shrimp diseases.

Keywords: Quarantine, Shrimp Broodstock, Biosecurity, Specific Pathogen Free, Disease Mitigation

Funding: NFDB and MoCI

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Technical Session VIII

Aquatic Animal Epidemiology, Disease Surveillance and Reporting

Improving early detection of aquatic animal disease: More sophistication or back to basics?

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Early detection of introduced, new and emerging aquatic animal diseases is a fundamental to an aquatic animal health system. Members of the World Organisation for Animal Health declaring freedom from disease need to demonstrate that their disease early detection system (EDS) is effective. An EDS is critical of the successful disease control. Key requirements of an EDS are that farmers know the signs associated with listed and emerging diseases and about their obligation to report suspicion and unexplained mortality. As farmer reporting is at the centre of an effective EDS its improvement requires that barriers to reporting (fear, lack of awareness) are overcome. Under-reporting may be technological or behavioural. Building trust between the Competent Authority (CA) and the farming community is crucial to an EDS. Work with farmers should focus on syndromic surveillance, monitoring mortality, feeding and clinical signs. The widespread access to the internet and mobile phone technology provides the tools for rapid reporting. Natural language processing, new statistical methods, and artificial intelligence can all increase our capacity to transform data to information. Information generated must of use to Government, but farmers must also benefit. In some systems farms can act as sentinels for disease in wild aquatic animals. The efficacy of active surveillance in wild populations not epidemiologically lined to farms, can be improved by focusing efforts are farms and regions at highest risk of disease introduction and the use of environmental DNA (eDNA). Inter-agency collaboration, and especially data sharing, between CA for AAD, governmental and non-governmental environmental agencies working in aquatic ecosystems is critical to early detection of disease in wild populations. There are limits to which technological advances can operate independently of the human element of an early detection system. The design of an EDA needs to start with the farmer, their perspectives and needs.

Keywords: Surveillance, Detection, Disease, Pathogen, Reporting

Funding: Department of Environment, Fisheries and Rural Affairs (Defra), United Kingdom

Aquatic animal disease surveillance in India

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Diseases are a serious constraint to the growth of aquaculture, and early detection is considered important in controlling the diseases, which can be achieved through a structured surveillance programme. Recognizing the importance, Government of India has been implementing National Surveillance Programme for Aquatic Animal Diseases (NSPAAD) since 2013. Phase I of the programme was initiated in 14 states involving 24 collaborating centres and was coordinated by ICAR-National Bureau of Fish Genetic Resources. To further strengthen the aquatic animal disease surveillance in the country, the Phase II of NSPAAD is being implemented by Government of India with pan-India coverage involving the State Fisheries departments and Marine Products Export Development Authority. Some of the important highlights of the NSPAAD include; a strong network of aquatic animal health laboratories in the country, diagnostic capability for detection of WOAH-listed/emerging aquatic animal pathogens, detection of 14 new pathogens from the country for the first time, mechanisms for first-time confirmation of transboundary and emerging diseases, and sending alerts/advisories to stakeholders. Under the programme to strengthen the farmer-based reporting "ReportFishDisease" App has been developed and it is available in Hindi, English and 11 regional languages. Using the app, the farmers can report disease cases in finfish, shrimps and molluscs to the fish health experts and get scientific advice for addressing the disease problem on their farms. NSPAAD is strengthening the farmer-based reporting of aquatic animal diseases and providing timely scientific advice. Besides, it is also informing the Competent Authority about the disease situation in the country, allowing efforts to control, reduce the risk of spread, and providing early warning of disease emergencies. Importantly, NSPAAD has led to establishment of a transparent reporting system and increasing the credibility of the country in terms of reporting aquatic animal diseases to international organizations (WOAH & NACA).

Keywords: Aquatic Animals, Disease, Surveillance, NSPAAD

Project: National Surveillance Programme for Aquatic Animal Diseases

Funding: Indian Council of Agricultural Research, India

Challenges in implementing WOAH aquatic animal disease surveillance standards

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Aquaculture is crucial for global food security, yet diseases cause over 6 billion USD in annual losses, a challenge exacerbated by production intensification, climate change, and global trade. The World Organisation for Animal Health (WOAH) establishes international standards to prevent and control aquatic animal diseases and ensure safe trade. However, the effective implementation of these standards faces significant challenges. Surveys conducted under the WOAH Aquatic Animal Health Strategy (2021-2025) have identified key barriers. Primary obstacles to disease surveillance and data collection include a lack of material, financial, and human resources, insufficient expertise, and inadequate laboratory diagnostic capacity. More than 75% of WOAH Members report deficient initial and continuing education for Aquatic Animal Health Services personnel, with a strong statistical association linking poor education to a greater number of implementation barriers. Furthermore, concerns regarding the unjustified impact of disease notification on international trade, a lack of government prioritization, and insufficient knowledge of reporting obligations hinder timely and comprehensive disease reporting. Many countries also lack national legislation aligned with WOAH standards, particularly for trade measures and disease prevention. To address these issues, WOAH and STAR-IDAZ have prioritized research areas for finfish health, including the validation of diagnostic tools, understanding host-pathogen-environment interactions, developing disease spread models, and optimizing biosecurity. Diagnostic research specifically focuses on AI tools, effective sampling, and point-of-care tests. Capacity building in disease surveillance, detection, diagnosis, and reporting is identified as a top priority. The Strategy emphasizes collaborative research, targeted funding, regulatory harmonization, and public-private partnerships to foster innovation and strengthen global aquatic animal health systems.

Keywords: Aquatic Health, Disease Surveillance, WOAH Standards, Implementation Barriers, Capacity Building

The quantitative assessment of the sensitivity of surveillance using scenario tree modelling for Australia's prawn and barramundi farming industries

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Australian aquaculture industries face challenges against infection with white spot syndrome virus (WSSV; a crustacean disease) and infection with megalocytiviruses (MCV; a finfish disease). Convincing out trading partners on exotic or officially controlled disease freedom without testing has been difficult. We are often requested to provide objective evidence to prove Australia's (or parts of Australia's) disease-free status. A project to quantitatively assess the sensitivity of our surveillance systems using an epidemiological tool, scenario tree modelling, has been initiated to address those key challenges as a national priority activity. WSSV for Australia's prawn farming industry and MCV for Australia's barramundi farming industry was used as case studies. For both cases, we conducted stakeholder interviews or sent a questionnaire to producers, to gather key data around the target population and testing activities. Following that, we developed a stochastic scenario tree model of the WSSV and MCV surveillance systems separately. The models were evaluated to determine the component (CSe) and system (SSe) sensitivity for demonstration of freedom in the farming sectors. For the WSSV case study, the SSe for freedom surveillance was extremely high (98.3%, 95% credible intervals (CI) 97.4-99.1%), driven primarily by a very high CSe for passive surveillance. Subsequently, the probability of freedom was also very high (98.1%, 95% CI 97.2-99.0%). Current surveillance activities, particularly passive surveillance, are highly effective for detecting WSSV in farmed prawns and farmed barramundi. For the MCV case study, as megalocytiviruses are exotic pathogens to Australia, there was a limited active surveillance activity via molecular testing. Most of barramundi fingerlings were examined by histopathology at the age of approximately 20 days for translocation and other health monitoring purposes. We used that data as disease investigation testing to develop a scenario tree modelling for each farm and combined to determine the SSe and then CSe.

Keywords: Surveillance, Freedom, Scenario Tree Modelling, Megalocytiviruses, White Spot Syndrome Virus

Project: The assessment of the sensitivity of Australia's surveillance system for aquatic animal diseases

Funding: Fisheries Research and Development Corporation, Australia

Modelling the transmission dynamics and reproduction number (R_0) of Tilapia Lake Virus (TiLV) across varying stocking densities and biomass

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Tilapia Lake Virus (TiLV) is an emerging viral pathogen causing severe disease and economic losses in tilapia aquaculture. Understanding its transmission dynamics is essential for effective disease control. A key parameter in disease modeling is the basic reproduction number (R_0), representing the average number of secondary infections caused by one infected individual in a fully susceptible population. Estimating R_0 helps quantify outbreak potential and informs targeted biosecurity and quarantine strategies. In this study experimentally assessed TiLV transmission in fingerlings stocked at an intermediate density of 100 fish/m^3 (average weight 4-5 g), exposed via immersion challenge using a known $TCID_{50}$ dose. Infection outcomes were evaluated using PCR diagnostics and clinical signs to estimate transmission probability (β). R_0 was calculated using a standard compartmental epidemic model (Anderson & May, 1991) and refined via the Next Generation Matrix (NGM) method (Diekmann et al., 2010), incorporating β, estimated contact rates (c), and infectious period (D = 7 days). The R_0 values for other densities were extrapolated using this experiment and adjusted contact rates based on biomass and spatial behaviour. The estimated R_0 at 100 fish/m^3 was approximately 37. In high-density fry culture (∼3000 fish/m^3, ∼1 g), the extrapolated R_0 exceeded 1300, reflecting intense waterborne transmission. For market-size fish (∼800g, 40-60 fish/m^3), R_0 ranged between 23 and 46, depending on probable contact rates. These results demonstrate that stocking density and fish biomass are major drivers of TiLV transmission. All modelled scenarios indicate high outbreak potential ($R_0 > 1$), underscoring the need for stage-specific biosecurity. Quantitative R_0 modelling provides a predictive framework to design effective interventions across production stages. Hatcheries must implement strict screening and quarantine, while grow-out systems require controlled movement, isolation, and ongoing surveillance.

Keywords: Tilapia Lake Virus (TiLV), Basic Reproduction Number (R_0), Transmission Modeling, Stocking Density, Epidemiology

Project: National Surveillance Programme for Aquatic Animal Diseases (NSPAAD II)
Funding: Department of Fisheries, Government of India

Prevalence of *Edwardsiella tarda* and antimicrobial resistance in Indian freshwater fish farms

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Edwardsiella tarda is a bacterial pathogen that causes Edwardsiellosis in fish. This study estimated the prevalence of *E. tarda* in 227 freshwater fish farms across three major aquaculture regions of India: Northern region (Uttar Pradesh), Central region (Madhya Pradesh & Chhattisgarh), and Southern region (Andhra Pradesh). The overall prevalence of *E. tarda* was 15.41% (95% CI: 10.68-20.15). Farms in the Southern region had a significantly higher ($p < 0.05$) prevalence than those in the Northern region. The prevalence of *E. tarda* was notably higher in pangasius farms (24.71%) compared to carp farms (9.85%). A significantly higher prevalence of *E. tarda* was observed in farms affected by disease, larger farms (> 2 hectares), those with high stocking densities (> 7,000 fingerlings per acre), and during the summer months. The antimicrobial resistance (AMR) profile of *E. tarda* isolates showed resistance to only three classes of antimicrobials: fluoroquinolones (38.23%), tetracyclines (35.29%), and sulphonamides (29.41%). Nearly 14.70% of isolates were multidrug-resistant. Higher AMR was particularly observed in pangasius farms located in the southern region and in farms affected by disease. All tetracycline-resistant isolates carried the *tetA* gene, most fluoroquinolone-resistant isolates harboured the *qnrB* gene, and some carried *qnrS*. Approximately 60% of sulphonamide-resistant isolates carried the *sul1* gene. Our findings suggest that pangasius farms in India have a significantly higher prevalence of *E. tarda* with higher AMR than carp farms.

Keywords: Prevalence, *E. tarda*, Pangasius, Carp, Risk Factors, Antimicrobial Resistance

Project: All India Network Project on AMR in fisheries and livestock

Funding: Indian Council of Agricultural Research, India

Database of Endogenous Viral Elements (EVEs) in penaeid shrimp genomes for enhancing viral PCR-based detection methods

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Endogenous viral elements (EVEs) are viral fragments integrated into host genomes through evolutionary interactions between viruses and hosts. In shrimp, EVEs are implicated in adaptive antiviral immunity via a mechanism known as viral accommodation. However, EVEs may also complicate disease surveillance by causing false-positive results in PCR-based diagnostics, particularly when uninfected shrimp carry EVE sequences that overlap with target regions used in such protocols. To address this challenge and facilitate accurate EVE identification, a bioinformatics pipeline was applied to available genomic and proteomic datasets of *Penaeus monodon* and *P. vannamei*, focusing on five key shrimp viruses: DNA viruses, White Spot Syndrome Virus (WSSV), Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV), and Decapod Iridescent Virus 1 (DIV1); and RNA viruses, Yellow Head Virus (YHV) and Taura Syndrome Virus (TSV). Here, we will discuss identified EVEs across 10 shrimp genomes based on their sequence features and viral origins. Subsequently, a newly developed EVE database housing the identified EVEs will be described for its features to assist in improving PCR-based detection protocols by distinguishing true infections from EVE-derived false positives. The database will serve as a useful resource for EVE research in shrimp aquaculture.

Keywords: Endogenous Viral Elements (EVEs), Shrimp, Viral Detection, Database, International Trade

Project: CAS-NSTDA Joint Research Program

Funding: National Science and Technology Development Agency (NSTDA) and Chinese Academy of Sciences (CAS)

Molecular prevalence of protozoan pathogens in farmed and wild bivalves from the coastal waters of India

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Protozoan pathogens pose a significant threat to farmed and wild bivalves, with several species listed by the World Organisation for Animal Health due to their transboundary potential and global trade impacts. This study presents the most updated molecular prevalence profile of key protozoan pathogens, viz. *Bonamia ostreae*, *Marteilia refringens*, *Perkinsus marinus*, and *Perkinsus beihaiensis* in 15 major molluscan species collected from five coastal states, viz. Goa, Karnataka, Kerala, Maharashtra, and Tamil Nadu. These included *Arca* sp., *Atactodea striata*, *Crassostrea madrasensis*, *Donax* sp., *Gafrarium pectinatum, Geloina bengalensis, Marcia opima, Meretrix casta, Meretrix meretrix, Mytella strigata, Paphia malabarica, Perna indica, Perna viridis, Saccostrea cucullata*, and *Villorita cyprinoides*. A total of 7,253 samples including 4,750 farmed and 2,503 wild bivalves were screened between January 2022 and June 2025 using pathogen-specific PCR assays. Only *Perkinsus beihaiensis* (PB) and *Perkinsus olseni* (PO) were detected. The overall prevalence of PB was 31.51%, while PO was 18.59%. Co-infection was observed in 7.85% of the samples. Infection rates were markedly higher in farmed stocks (PB: 37.41%, PO: 24.46%, co-infection: 10.06%) compared to wild (PB: 19.16%, PO: 7.47%, co-infection: 3.67%). *P. viridis* exhibited the highest infection levels (PB: 35.25%, PO: 22.11%, co-infection: 9.17%), followed by *P. malabarica* (PB: 31.83%, PO: 12.86%, co-infection: 11.25%) and *M. strigata* (PB: 23.88%, PO: 6.39%, co-infection: 1.91%). Notably, *G. bengalensis* (75%) and *P. indica* (33.33%) showed high PB prevalence despite limited sample sizes. *V. cyprinoides* showed lower infection levels (PB: 14.04%, PO: 1.65%, co-infection: 0.82%), while *Arca* sp., *C. madrasensis*, *Meretrix* spp., and *S. cucullata* showed 10% prevalence. All samples of *A. striata* and *Donax* sp. tested negative. These patterns underscore species-specific susceptibility to *Perkinsus* spp., with *P. viridis* and *P. malabarica* as the major reservoirs. The study highlights the widespread distribution of these pathogens and calls for enhanced molecular surveillance and biosecurity strategies.

Keywords: Bivalves, *Perkinsus*, Wild, Farm, Protozoa

Project: Health management and monitoring strategies for marine fish and shellfish

Funding: ICAR-Central Marine Fisheries Research Institute, India

Epidemiology of *Anisakis* larvae in farmed fish within aquaculture zones of Peninsular Malaysia

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The increasing demand for farmed fish as a sustainable seafood source has raised concerns about the safety of aquaculture products, particularly regarding parasitic infestations such as *Anisakis* larvae, which pose health risks to consumers. Fish farmed in cages near areas with marine mammals (final hosts of *Anisakis*) are at the increased risk of *Anisakis* contamination, as these mammals shed larvae into the environment, which then infect intermediate hosts such as farmed fish. This study investigated the occurrence of *Anisakis* larvae in Asian seabass (*Lates calcarifer*), Snapper (*Lutjanus* spp.), and Grouper (*Epinephelus* spp.) cultured along Peninsular Malaysia's West Coast, where marine mammals have been observed. A total of 140 farmed fish (weight ranged from 0.1 to 1.3kg), were examined for *Anisakis* spp. larvae and identified using both morphological and molecular techniques. Results showed no detection of *Anisakis* larvae in grouper (0/60), while a low prevalence was observed in Asian seabass at 7.5% (3/40) and 2.5% in Snapper (1/40). The mean intensity was low, with only a single larva detected in the infected fish. The larvae were identified as *Anisakis* based on the presence of a dorsal lip at the anterior extremity; however, subsequent molecular analysis confirmed their identity as *Hysterothylacium amoyense*. Although less prevalent than in wild counterparts, the detection of *Anisakis* larvae in farmed fish raises concerns about parasite transmission in aquaculture. These findings highlight the need for the ongoing surveillance for better understand *Anisakis* epidemiology in aquaculture environments.

Keywords: Parasite, Aquaculture, Marine Fish, *Anisakis* Larvae, Prevalence

Project: Fish Health Management in Aquaculture

Funding: Development fund under the Department of Fisheries Malaysia, Government of Malaysia

Tilapine viruses: Molecular surveillance and environmental correlates in the wild and Tilapia culture farms in Tamil Nadu, India

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Tilapia farming is one of the fastest-growing aquaculture sectors globally due to tilapia's adaptability, rapid growth, high protein content and relatively low-maintenance needs. However, the emergence of viral pathogens associated with fatal disease outbreaks in tilapia, has led to significant losses in tilapia production causing substantial economic losses in India. Tilapia Lake Virus (TiLV) and the recently reported novel virus, Tilapia ParvoVirus (TiPV) are the major viral pathogens that cause disease in both wild and farmed tilapia and mass mortality events. This study investigated the prevalence of TiLV and TiPV (2020 - 2024), along with their association with co-infections and the environmental parameters viz., pH, temperature, ammonia and nitrite in both cultured and wild tilapia across Tamil Nadu, India. Tilapia samples (n = 261) collected from the farms and wild (66 sampling sites) showed a prevalence of 51.5% for TiLV (34/66) and 10.6% for TiPV (7/66) and 6.06% of dual infection of TiLV with TiPV (4/66). Histopathological examination of TiLV infected fish revealed the prominence of syncytial giant cells in the liver. TiLV detection with the published PCR primers targeting the genomic segments (1-10) amplified nine segments (except segment 4) for which the sequences were generated and submitted in the GenBank. Molecular diagnostic assays (PCR and LAMP) were developed based on the sequences generated from the TiLV and TiPV strains detected in this study. Diagnostic PCR primers targeting TiLV segments (1, 2, 3, 7, 8 & 9) and the TiLV LAMP assay showed sensitivity up to 1 ng and 1 pg respectively; nested PCR assay developed for TiPV had a detection sensitivity of 1 ng demonstrating their suitability for disease screening. Principal Component Analysis (PCA) indicated that lower temperatures (20-21°C) during the North-East monsoon as a key driver of TiLV outbreaks, with coinfections of bacterial pathogens such as *Aeromonas veronii*, *Streptococcus agalactiae*, *Pseudomonas* sp., and *Enterococcus* sp., leading to disease severity. Transcriptome analysis of TiLV-infected liver revealed 2,379 DEGs, with 1,726 upregulated genes. KEGG analysis identified 680 pathways, with altered metabolic and immune-related functions, suggesting that TiLV may manipulate host metabolism to facilitate infection. These findings emphasize the adoption of effective disease management strategies to mitigate the impact of TiLV and TiPV in tilapia farming in India.

Keywords: Tilapia Lake Virus, Tilapia Parvo Virus, Surveillance, Diagnosis, India

Project: Integrated disease surveillance monitoring system and seed quality assessment to augment aquaculture production in Tamil Nadu

Funding: Tamil Nadu State Government and Government of India under National Agriculture Development Program (NADP)

Epidemiological profile of Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) in farmed *Penaeus monodon* in West Bengal, India

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The Tiger shrimp, *Penaeus monodon* is extensively farmed in West Bengal. However, virus diseases are one of the major threat of shrimps. Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) is an important viral pathogen impacting the global shrimp aquaculture industry, particularly in *Penaeus monodon*. This study aimed to determine the epidemiological profile of IHHNV across key shrimp-farming regions in West Bengal. Study was carried out under the National Surveillance Programme for Aquatic Animal Diseases (NSPAAD) for active surveillance. A total of 1,147 shrimp samples were collected from traditional farms in West Bengal and the sampling sites were districts such as East (Purba) Medinipur, North 24 Parganas and South 24 Parganas. *P. monodon* samples were collected from the sampling sites, hepatopancreas and gill tissues extracted, fixed in 70 % ethanol for DNA isolation, and brought to the laboratory. Genomic DNA was extracted from pleopod and gills and tissue using commercial kits. Further the conventional Polymerase Chain Reaction (PCR) was performed using specific primers of IHHNV recommended by WOAH. For IHHNV amplicon was detected with an amplicon size 309 bp. Results revealed that, out of 1,147 samples, 29 samples were infected with IHHNV(2.52%) of the 261 samples from North 24 Parganas. However, 841 shrimp samples collected from East (Purba) Medinipur and 45 samples from South 24 Parganas had no detection of IHHNV. The concentration of positive detections in North 24 Parganas district, indicates a potential localized endemic zone within the state. These findings emphasize the importance of continuous molecular monitoring to prevent the silent spread of IHHNV and to maintain the health and productivity of India's shrimp farming industry. The study provides essential baseline data for disease risk mapping and supports informed decision-making in national aquatic animal health management programs.

Keywords: Infectious Hypodermal and Hematopoietic Necrosis Virus, Epidemiological Profile, Polymerase Chain Reaction

Project: National Surveillance Programme for Aquatic Animal Diseases

Funding: Department of Fisheries, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India

Design and implementation of IoT with block chain-based traceability system for shrimp supply chain management

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Aquaculture, or the farming of aquatic species like fish, crustaceans, mollusks, and aquatic plants, has grown rapidly over the past few decades and is currently one of the most important sources of high-quality animal protein in the world. Due to its significant global trade potential, high economic returns, and ability to create jobs, brackishwater shrimp farming has drawn a lot of attention within this industry. The complex shrimp supply chain includes broodstock, hatcheries, nurseries, input suppliers, grow-out ponds, harvesting, processing, distribution, and retail sales which makes it very challenging to preserve traceability, control disease outbreaks, and conform with firm international regulatory standards. Traditional supply chain management systems used in shrimp production lack integrated, real-time data recording methods, making it difficult to track shrimp batches from origin to consumption. The disparity raises concerns about the use of illegal chemicals, environmental violations, and product legitimacy. In order to address these issues, a robust, transparent, and technologically sophisticated comprehensive digital traceability system is needed, one that can monitor and record critical data points throughout the production and distribution process. Hence a prototype is developed on shrimp traceability which constitutes internet of things (IoT) sensors for real-time water quality monitoring, blockchain ledger for data transparency, QR codes, radio frequency identification (RFID) tags, and RFID reader for batch identification. The proposed method progresses disease control, antibiotic free status, guarantees regulatory compliance, boosts operational efficiency, and builds consumer confidence in the safety and sustainability of farmed shrimp with improved supply chain transparency, precise food safety records, and prompt disease outbreak notifications. This prototype would encourage the global movement towards safer, traceable, and ecologically aware aquaculture operations by providing the farmers, exporters, and consumers with verified product histories.

Keywords: Shrimp Aquaculture, Traceability, Supply Chain Management, Internet of Things (Iot), Aquaculture Regulations

Project: AINP_FH

Funding: Indian Council of Agricultural Research, India

Statistical methods for evaluating the economic impact of health care intervention in aquaculture

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Health care intervention is designed to improve health outcomes, prevent disease, or treat existing health issues in a population. In aquaculture, healthcare interventions include improved management practices, biosecurity, timely diagnosis and treatments and lately vaccination to prevent or control diseases in aquatic animals. Adoption of these healthcare interventions require budgetary allocations and hence their effectiveness need to be evaluated in monitory terms. Several models have been developed to identify healthcare interventions and evaluate their effectiveness of and associated risk factors. Statistical techniques such as propensity score matching, regression discontinuity designs, interrupted time series analysis, and instrumental variable approaches help establish causal relationships when randomized trials are not feasible. AI-driven methods, including machine learning algorithms like decision trees, random forests and neural networks further improve prediction accuracy and uncover complex patterns in datasets. Combined with traditional approaches such as generalized linear models, survival analysis, and Markov models, these advanced tools enable more precise and data-driven assessments of intervention effectiveness, ultimately helps in better decision-making. Use of healthcare products through biosecurity, vaccination, and alternative treatments is quantified, thereby lowering environmental and health risks. Improved fish health and welfare, leading to higher yields, better product quality, and enhanced food security and nutrition for communities. Economic and ecosystem balance can be achieved by integrating economically responsible practices ensuring reduced negative environmental impacts.

Keywords: Healthcare Intervention, Data Driven Assessment, Ecosystem Balance

Project: All India Network Project on Fish Health

Funding: Indian Council of Agricultural Research, India

Establishment of field disease diagnostic laboratory for servicing and capacity building of marginalized society in brackishwater aquaculture

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India aims to raise the shrimp production to 1.6 MMT by 2030, followed by 3.0 MMT by 2047 using three major shrimp species such as *Penaeus vannamei, P. monodon* and *P. indicus*. To achieve this, different types of farming systems like super-intensive precision shrimp farming, are recommended. The problems are unique to each system warranting specific technical expertise for disease diagnosis and management. As per 2011 census, there are 144.3 million landless laborers in India and they are the significant part of the rural workforce rely on daily wages for their livelihood. To connect these two points, a livelihood capacity building model is developed to select literate unemployed youths from those landless laborers and train them for disease diagnostics and management in brackishwater aquaculture. The target area selected for the implementation of this project is Nagapattinam, Tamil Nadu with a large Coast line of 141 km and brackishwater aquaculture significance. The district has population density of 629 persons per sq.km, higher than the state with the sex ratio of 1026 with the highest scheduled caste population of 31.54% against the state percentage of 20.01%. The district has recorded the literacy rate of 83.6%, higher than the state literacy rate (80.1%). Having good literacy but lack of land resources, this marginalized society has been serving as the laborers in different fields. Whatsoever technological aquaculture-based interventions are implemented with these landless laborers, they are left in the state of poverty once the inputs are withdrawn. Hence, it is another approach to select these educated and unemployed youths from this marginalized society, and teach and train them with basic aquaculture diagnostic and management skills. This will be a kick-start opportunity for those youths, to become a technical support to brackishwater aquaculture over the years of their involvement and experience.

Keywords: Shrimp Aquaculture, Capacity Building, Diagnostic Skills, Shrimp Diseases, Livelihood Option

Host range susceptibility to *Ecytonucleospora hepatopenaei* (EHP) infection in brackishwater aquaculture

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Microsporidian parasite, *Ecytonucleospora hepatopenaei* (EHP) causing hepatopancreatic microsporidiosis (HPM) clinically manifested by slow growth, size variation, white feces syndrome (WFS) and mortality is presently considered to be important pathogen in shrimp aquaculture. To understand the host variation in susceptibility to this pathogen, two separate sets of 42 days multiple-dose challenge and susceptibility study experiments were conducted in triplicates using shrimp and crab species. One set of experiment was conducted with the healthy juveniles of three economically important shrimp species such as *Penaeus indicus, P. monodon* and *P. vannamei*, while another set of experiment with the healthy juveniles of green crab, *Scylla serrata, red crab, S. olivacea*, and crablet of *S. olivacea*. Shrimp hepatopancreas (HP) with gut having EHP load of 22.31×10^6 copies g^{-1} was fed to each group for five days. Samples were collected for haematology, bacteriology, histopathology, PCR and qPCR at weekly intervals. The water quality parameters and total *Vibrio* count were recorded throughout the experiment. qPCR analysis revealed that there was significantly highest EHP load in HP of *P. vannamei* (64.35×10^6 copies g^{-1} of HP tissue) on 42^{nd} day of experiment followed by 1.61×10^6 copies g^{-1} of HP in *P. monodon* and the least in *P. indicus* (0.02×10^6 copies g^{-1} of HP tissue), which is indicative of shrimp species variation in susceptibility to EHP. qPCR analysis of crab HP shown that there was very low level of EHP load with consistent decrease in load and reaching zero after 42^{nd} day post challenge, is suggestive of no establishment and/or no proliferation of EHP spores in HP tissues in crab species.

Keywords: Microsporidiosis, qPCR, Shrimp Diseases, White Feces Syndrome, Retarded Growth

Surveillance of aquatic animal diseases in the tropical archipelago of India: Insights from Andaman and Nicobar Islands

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The present study underscores the surveillance of aquatic animal diseases in the freshwater and marine ecosystems of the Andaman and Nicobar Islands (ANI), a tropical Indian archipelago. Through passive and active surveillance, the research identified a range of infectious and non-infectious diseases, including viral (2%), bacterial (23%), parasitic (34%), fungal (2%), and environmental issues (39%) from the aquatic animals of ANI. Binary logistic regression analysis revealed that the odds of disease occurrence in South Andaman (odds ratio: 0.22) are higher than in the other two districts of ANI. As a part of passive surveillance, baseline data with geo-referenced details were collected from over 1,700 fish ponds, and the stakeholders were provided with guidance on better management practices. All these findings were submitted to the National Database on Aquatic Animal Diseases on periodic interval for effective management. To enhance stakeholder awareness, a total of 53 capacity-building programs were conducted, benefiting 1,327 participants comprising of farmers, officials from state departments and other stakeholders. Mass media initiatives, including television programs, radio talks, and other awareness activities, further disseminated knowledge on aquatic animal health management. The "ReportFishDisease" mobile app was popularised among the stakeholders to enable real-time disease reporting in ANI. The study also provided technical support to the stakeholders on better management practices, with notable success in shrimp farming at South Andaman and recognized as a success story by ICAR, New Delhi. Policy recommendations were offered to the local authorities to strengthen aquatic animal health management. These efforts fulfilled the international obligations for the Quarterly Aquatic Animal Disease (QAAD) report, reflecting the current status of aquatic animal diseases in ANI at regular intervals. The findings are crucial for developing effective health management strategies, with the ultimate goal of reducing disease-related losses and enhancing fish production in the region.

Keywords: Surveillance, Infectious Diseases, Non-Infectious Diseases, Pathogens, Andaman And Nicobar Islands

Project: National Surveillance Programme for Aquatic Animal Diseases (NSPAAD) in Andaman and Nicobar Islands

Funding: Department of Fisheries, Government of India, New Delhi

Molecular identification and genetic diversity of *Anisakis typica* in *Decapterus* sp. from Endau-Rompin, Malaysia

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Parasitic nematodes of the genus *Anisakis* are known to infect a range of marine fish species, raising concerns for both aquatic animal health and seafood safety. While previous studies have reported *Anisakis* spp. in Malaysian waters, data remain scarce for small pelagic fish such as *Decapterus* spp. In this study, 30 fish specimens were collected from Endau-Rompin, Johor, and examined for nematode infection. Eight *Anisakis* larvae were isolated from the visceral organs and subjected to molecular analysis using the 18S rDNA marker with TK1 and NC2 primers. Phylogenetic analysis confirmed all specimens as *Anisakis typica*. Sequence comparison revealed the presence of four distinct haplotypes among the eight sequences, with a haplotype diversity of 0.6429. This indicates a moderate level of genetic variation within the local A. typica population. These findings contribute to the understanding of parasite diversity in wild fish populations and highlight the importance of molecular surveillance in monitoring emerging parasitic threats. The application of genetic tools in parasite diagnostics supports improved aquatic animal health management and enhances food safety awareness in tropical fisheries.

Keywords: *Anisakis typica, Decapterus* sp., Molecular Identification, Parasitic Nematodes, Aquatic Animal Health

Project: Detection of zoonotic parasite *Anisakis* spp. through molecular tools: An emerging public health concern

Funding: ASEAN-India Science & Technology Development Fund (AISTDF)

White faecal syndrome in *Penaeus vannamei*: Correlation studies with respect to salinity, culture status and disease prevalence

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In the year 2008, species *Penaeus vannamei* was introduced into Indian waters was marked as the significant phase in shrimp aquaculture because of its fast growth rate. Diseases is one of the major impediment for the growth of shrimp aquaculture. In the recent years disease syndromes evolved due to multiple etiologies have caused huge havoc to the sector. One such syndrome is White Faecal Syndrome (WFS) which is a gastrointestinal disorder, characterized by reduced feed consumption, growth retardation, presence of loose shell, white/golden brown intestine, white faecal strings floating on water surface of affected pond and sometimes with mortality. This study analyzes the data of 175 shrimp pond samples collected from different farms during 2022 to 2025 in Thiruvallur district of Tamil Nadu, out of which 22 samples were found to have WFS. Correlation of WFS affected farm was made with the following parameters like water (salinity), culture status (days of culture, DOC), and other shrimp disease pathogens. As far as water parameters of WFS affected pond ($n = 22$) are considered, majority of ponds ($n = 17$) had salinity <10 ppt, three ponds within 20-30 ppt, two ponds with 40 ppt was observed. Culture status of WFS affected ponds ($n = 17$) were observed between 40-80 DOC, three nos. below 40 DOC and two above 140 DOC duration. Correlation with other disease status revealed that 16 were *Ecytonucleospora hepatopenaei* (EHP) positive, six were Wenzhou shrimp virus 8 (WzSV8) positive and two were IMNV positive out of 22 WFS ponds. Histopathology of WFS shrimp showed remarkable lesions in hepatopancreas like sloughing of tubular epithelium and lack of epithelial differentiation. High prevalence of WFS was noticed in salinity below 10 ppt, during the culture period of 40-80 days and were majorly positive for EHP and followed by WzSV8 pathogen.

Keywords: WFS, White Faecal Syndrome, Shrimp Disease

Project: National Surveillance Programme for Aquatic Animal Diseases (NSPAAD)

Funding: Pradhan Mantri Matsya Sampada Yojana, Government of India

Risk factors for infectious Salmon Anemia Virus infection and clinical infectious salmon anemia in Atlantic Salmon and Rainbow Trout: A systematic review

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Infectious salmon anemia is one of the most important viral diseases for the farmed Atlantic salmon industry. This article presents a systematic review aimed at identifying risk factors associated with clinical ISA, ISAV infection or related mortality, as well as those influencing the time since stocking to ISA, ISAV occurrence or related mortality. This systematic review was reported according to the PRISMA guidelines. The existing scientific literature in four databases (MEDLINE (via PubMed), CAB Abstracts via (CAB EBSCO), Scopus, Earth, Atmospheric & Aquatic Science Collection via ProQuest) was searched using predefined search terms for each database to find scientific literature published until 28th June 2024. Two authors independently screened articles and extracted data. A total of 514 studies were identified through the search in four databases and Google Scholar. Seventeen studies were left for full-text screening following title and abstract screening. Finally, after full-text screening, 10 studies were included in the final systematic review. Of the included studies, four were carried out in Norway, two in Canada, two in Chile, one in the USA, and one included both USA and Canadian marine sites. All studies were observational in nature, including cohort, case-control, and case-cohort designs. Proximity to a neighboring marine site with ISA was associated with an increased risk of both clinical ISA and time since stocking to ISAV infection. Additionally, higher smolt weight at the time of stocking was identified as a risk factor for several outcomes: clinical ISA, ISAV infection, primary ISA, and time since stocking to ISAV infection. Risk of bias assessment indicated that all included studies were at high or very high risk of bias. These findings highlight the need for more robust, well-designed studies that account for key confounders and minimize bias to better understand the risk factors associated with ISAV infection and clinical ISA.

Keywords: Atlantic Salmon, Disease Control, Fish Diseases, Network Analysis, Risk-Based Surveillance

Mortality in farmed striped catfish *Pangasianodon hypophthalmus* attributed to *Edwardsiella ictaluri* infection in Uttar Pradesh, India

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Edwardsiella ictaluri is a highly pathogenic intracellular bacterium, known to cause significant losses to the aquaculture sector. The pathogen has been reported to affect several economically important fish species including striped catfish *Pangasianodon hypophthalmus* in many countries. In the present study, we provide evidence for association of this pathogen with large-scale mortalities in farmed striped catfish from Uttar Pradesh, India. The diseased fish exhibited emaciation, erratic swimming, haemorrhagic vent, pinpoint ulcerations on skin. Wet mount examination of gills and skin scrapings showed no parasitic infestation. Post-mortem examination revealed white foci in the kidneys. Histopathological examination of the diseased fish showed focal necrotic areas in kidney, spleen and liver tissues. Notably, bacterial pathogens isolated from kidney of the diseased fish were presumptively identified as *E. ictaluri* on basis of biochemical tests. The isolates were confirmed as *E. ictaluri* on basis of PCR amplification and sequencing of 16S rRNA, gyrB and *E. ictaluri*-specific fimA genes. Importantly, *E. ictaluri* was also detected in infected tissues from diseased fish by species-specific PCR. Further, following experimental infection with *E. ictaluri* isolate (PKV1) by intraperitoneal injection, 100% mortality was observed in striped catfish within 5 days, indicating that the isolate is highly virulent. Additionally, in the experimentally-infected fish, the clinical signs and histopathological lesions were similar to those in naturally-infected fish, and *E. ictaluri* could be reisolated, thereby fulfilling Koch's postulates. Based on the findings, the cause of mortality was attributed to infection with *E. ictaluri*. This forms the first report of *E. ictaluri* infection from India, and highlights the need for implementation of strict biosecurity measures for management and preventing the spread of this pathogen.

Keywords: *Pangasianodon hypophthalmus*, Striped Catfish, *Edwardsiella ictaluri*, Aquaculture, gyrB gene

Funding: Pradhan Mantri Matsya Sampada Yojana Government of India

First report of *Lactococcus petauri* infection in marine fish species in Japan and its pathogenicity in Striped Jack and Thread-sail Filefish

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Lactococcus garvieae serotype-I has been a causative agent of lactococcosis in Japan since the 1970s, followed by the emergence of *L. formosensis* serotype-II around 2012 and *L. garvieae* serotype-III around 2021. We isolated two isolates from diseased fish that did not correspond to any previously identified serotypes. In this study, we characterized these isolates by examining its genetic and biochemical properties and confirmed their pathogenicity through infection experiments. We also investigated whether similar strains existed in archived collections. Two isolates were subjected to phylogenetic analysis based on a partial gyrB sequence, serotyping using each anti-sera, and biochemical characterization using the API Rapid ID 32 STREP system. Pathogenicity was also assessed by intraperitoneal injection of the two isolates into striped jack *Pseudocaranx dentex* and thread sail filefish *Stephanolepis cirrhifer*, and monitoring mortality over a 21-day period. Finally, multiplex PCR using three primer pairs specific for serotype I-III was performed on 735 clinical isolates previously diagnosed with lactococcosis. All PCR-negative isolates were subsequently identified by phylogenetic analysis using partial gyrB sequences. The two isolates were placed within the *Lactococcus petauri* clade, showed no agglutination with antisera for serotypes I-III, and assimilated sucrose. Each isolate caused 40-90% mortality at 10^5 CFU/fish in striped jack and thread sail filefish. Striped jack exhibited clinical signs consistent with typical lactococcosis, including eye inflammation, systemic hemorrhaging, and caudal peduncle ulcers. In contrast, thread sail filefish showed only eye inflammation and internal hemorrhaging. Multiplex PCR showed that 14 out of 735 clinical isolates were PCR-negative for serotypes I-III, and these 14 isolates were identified as *L. petauri*. We report the first isolation of *L. petauri* from diseased marine fish in Japan. *L. petauri* was pathogenic to both striped jack and thread sail filefish. A survey of clinical isolates revealed that *L. petauri* was most frequently isolated from Thread sail filefish. *L. petauri* represents a novel serotype distinct from those previously reported in Japan, suggesting that commercial FKC vaccines may not provide effective protection.

Keywords: *Lactococcus petauri, Lactococcosis, Pseudocaranx dentex, Stephanolepis cirrhifer*

Project: Pathogen characterization of a novel lactococcosis agent for risk management and disease control
Funding: Japan Fisheries Research and Education Agency, Japan

Infestation dynamics, site-specific distribution and histopathological changes associated with the Sea louse *Caligus rotundigenitalis* in Pearlspot *Etroplus suratensis* (Bloch)

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The pearlspot (*Etroplus suratensis*) is a commercially important brackishwater species valued for its culinary appeal and adaptability to varying salinities. However, parasitic infestations, particularly by the sea louse *Caligus rotundigenitalis*, pose a significant threat to its aquaculture. This copepod parasite infests gill and skin tissues, causing tissue damage, inflammation, impaired respiratory function, and increased mortality in cultured fish. Despite its impact, limited information is available regarding the infestation dynamics and site-specific distribution of *C. rotundigenitalis* in pond-reared pearlspot. In the present study, fifty moribund pearlspot showing visible signs of sea lice infestation such as erratic swimming, bottom settling, and feed refusal were collected from a brackishwater pond and analyzed for parasite distribution and histological changes at attachment sites. Parasites were categorized as egg-bearing (females with egg sacs) and non-egg-bearing (immature stages and adults without egg sacs), and counted across five body regions: head, dorsal body, ventral body, caudal fin, and sub-opercular region. The results indicated a significantly higher abundance of egg-bearing lice on the caudal fin (mean count: 22.65±4.9), followed by the head (10.25±3.5) and sub-opercular region (6.85±2.4). Non-egg-bearing forms were most prevalent on the head (23.9±5.5), dorsal body (14.25±3.9), and caudal fin (11.15±2.3). Notably, no non-egg-bearing individuals were observed in the sub-opercular region. Histopathological analysis of infected gill and skin tissues revealed structural alterations at parasite attachment sites, including epithelial damage and inflammatory responses. This study establishes a pathological association between *C. rotundigenitalis* and *E. suratensis*, highlighting site-specific distribution of the parasite and its tissue-level impact. The observed behavioural signs may serve as early indicators of infestation, facilitating timely therapeutic intervention. These findings highlight the importance of improved parasite surveillance and targeted management strategies to mitigate sea lice-associated mortality in brackishwater pearlspot aquaculture.

Keywords: Brackishwater Aquaculture, *Etroplus suratensis*, *Caligus rotundigenitalis*, Ectoparasite, Histopathology

Project: Genome editing approaches for improving growth and reproduction of brackishwater teleost and Indian white shrimp (*Penaeus indicus*)

Funding: ICAR-Central Institute of Brackishwater Aquaculture, India

Piscinoodinium sp. a silent killer associated with epizootics and mortality of freshwater fishes in the Andaman and Nicobar Islands

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Piscinoodinium is a well-known parasitic dinoflagellate genus that infects freshwater fish, and the infection on the host is known by many names, including 'freshwater velvet', 'rust disease', 'gold dust disease', 'freshwater oodinium', and 'pillularis disease'. In tropical and sub-tropical countries, *Piscinoodinium* sp. causes severe morbidity, large-scale mortality, and epizootics in aquarium and food fish. *Piscinoodinium* sp. has garnered little attention because most reports of the parasite have been in fish held in aquaria. Very recently, *Piscinoodinium* sp. has been documented from the Andaman Islands, and are known to cause mortality in both exotic and endemic freshwater fishes. The *Piscinoodinium* sp. infection was recorded in 5 d old *Betta splendens* larvae, sub-adults, and adults,*Poecilia reticulata* with 9.6-12.6-100% prevalence and mortality rate of 59.6-97.2% & 38.4-72.4%, respectively. The infection was also observed in endemic fish species *Aplocheilus andamanicus* with a prevalence and mortality rate of 100%. The mortality occurred within 3-4 days in fries and subadults. In adult fish mortality was slow, which occurred over a period of 10-15 days. Infectivity study with a brackishwater goby, *Mugilogobius chulae* also demonstrated typical symptoms and signs observed in other freshwater fish hosts, and caused mortality in a period of one week. The molecular characterization of the SSU rDNA gene of *Piscinoodinium* sp. infecting *B. splendens* from the Andaman Islands demonstrated that the species is genetically distinct, and future work may be attempted to study its morphology for better understanding about the species-level identity. Further, surveillance may be undertaken for this overlooked parasite.

Keywords: Parasite, Dinoflagellate, Endemic, Biosecurity, One health

Project: National Surveillance Programme on Aquatic Animal Diseases phase 2

Funding: ICAR-National Bureau of Fish Genetic Resources, India

Microbiological investigation of disease-causing bacteria in Wild freshwater fishes from Mula and Mutha Rivers, India

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This study presents the first comprehensive microbiological assessment of three wild freshwater fish species *Gymnostomus fulungee, Garra mullya*, and *Puntius sophore* collected from local fish markets and the Mula and Mutha rivers in Pune, Maharashtra, India. A total of 29 specimens were analyzed (*G. fulungee*, n=10; *G. mullya*, n=10; *P. sophore*, n=9). The total length and body weight of each species were recorded. *G.fulungee* exhibited mean total length of 18.82±4.80 cm and a mean body weight of 50.26 ± 24.17g. For *G. mullya*, the mean total length was 10.63 ± 1.00cm and body weight 11.89 ± 3.22 g respectively. *P. sophore* showed mean total length of 9.77 ± 2.73cm and a mean body weight of 17.62 ± 17.13g. Standard culture and subculture methods were used to screen for bacteria on tryptic soy agar (TSA) and biochemical identification was the next step. Numerous potentially harmful and opportunistic bacteria were isolated from organ-specific locations. *Citrobacter murliniae* and *Plesiomonas shigelloides* were isolated from liver in *G. fulungee*, whereas *Enterobacter wuhouensis* was found in the gills. *G. mullya* produced *Bacillus amyloliquefaciens* from the intestine, *Pseudomonas alloputida* from the gills, and *Citrobacter murliniae, Acinetobacter baumannii*, and *Staphylococcus pasteuri* from the anterior kidney. *Aeromonas veronii* and *Citrobacter trautae* were found in the liver and gill tissues of *P. sophore* respectively. The tissue-level effects of the isolated bacteria were verified by histopathological analysis of infected organs, which showed cellular degeneration. This study underscores the need for continued microbial monitoring in wild freshwater systems. The presence of known and potentially emerging pathogens such as *Pseudomonas alloputida* and *Enterobacter wuhouensis* in asymptomatic wild fish suggests their ecological relevance and pathogenic potential. These findings contribute valuable baseline data for understanding fish-associated microbiota and support future strategies in fish health management, biosecurity, and public health protection.

Keywords: *Gymnostomus fulungee, Garra mullya, Puntius sophore*, Bacterial Pathogens, Fish Disease

Project: Disease diagnostic and quality testing laboratory for aquatic animals

Funding: Pradhan Mantri Matsya Sampada Yojana, Ministry of Fisheries, Government of India; DST-FIST, Government of India; DBT-BUILDER scheme, Government of India; and Department of Fisheries, Government of Maharashtra

Parasite watch: A two-year active surveillance study in the freshwater fish farms of Odisha, India (2023 - 2025)

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Aquaculture, a rapidly growing sector vital to livelihoods and global food supply, faces major challenges from disease outbreaks caused by various pathogens. Parasitic diseases, reported as the most common fish ailments in India, pose a significant threat to aquaculture sustainability by causing reduced growth rates, poor feed conversion, and increased mortality in farmed fish. Active surveillance plays a crucial role in the early detection of parasitic threats and the implementation of timely control measures. The present study, conducted from April 2023 to June 2025, aimed to assess the prevalence and distribution of parasitic infections in freshwater fish farms across 14 districts of Odisha, India. A total of 561 farms were surveyed, involving fish species such as *Labeo rohita, Cirrhinus mrigala, Catla catla, Pangasianodon hypophthalmus, Piaractus brachypomus*, and *Oreochromis niloticus*. Skin mucus and gill scrapings were examined microscopically to detect parasitic presence. Of the farms surveyed, 312 (55.6%) were found positive for parasitic infections, while 249 (44.4%) were negative. The monogenean parasite *Dactylogyrus* sp. was the most prevalent, observed in 66.3% of infected farms. This was followed by mixed infections (24.4%), crustacean parasites *Argulus* spp. (4.8%), *Ergasilus* sp. (0.3%), and protozoan *Trichodina* sp. (4.2%). Among mixed infections, *Dactylogyrus* sp. & *Trichodina* sp. co-infections were most common (13.5%), followed by *Argulus* sp. & *Dactylogyrus* sp. (5.4%), and other combinations in smaller percentages. The study found a correlation between poor water quality, particularly low pH and alkalinity, associated with higher parasite incidence. Seasonally, infections peaked during the monsoon (60.28%), followed by summer (55.56%) and winter (50%), indicating favorable conditions for parasites during the rainy season. This active surveillance underscores the importance of regular monitoring and the adoption of improved farming practices. The findings provide crucial baseline data for disease risk assessment and policymaking, supporting the sustainable development of aquaculture.

Keywords: Aquaculture, Active Surveillance, Parasite, Prevalence

Project: National Surveillance Programme for Aquatic Animal Diseases

Funding: National Fisheries Development Board, India; ICAR-NBFGR, India; and Pradhan Mantri Matsya Sampada Yojana, Department of Fisheries, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India

Prevalence patterns of *Enterocytozoon hepatopenaei* (EHP) in shrimp ponds across Punjab, India: A probabilistic sampling-based study

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The present study investigated the prevalence of OIE-listed diseases in the shrimp cultured in the inland saline waters of Punjab, India, using a randomized sampling strategy involving 61 farms from four districts of Punjab. The study followed the two OIE-recommended methods; Method I, which involves the farmer-reported mortality events, yielded no samples due to the absence of such mortality events. Method II employed by targeting 10% of farms and one-third of ponds per selected farm per district was selected for shrimp sampling. It revealed that no viral or bacterial pathogens (WSSV, TSV, IHHNV, and AHPND) were detected through molecular method. However, the microsporidian parasite *Enterocytozoon hepatopenaei* (EHP) was detected on multiple shrimp farms through nested PCR and sequencing. Out of 61 farms, 17 shrimp farms tested positive for EHP, resulting in an overall farm-level prevalence of 28%, with district-wise variation ranging from 0% in Bathinda to 78% in Mansa. Whereas at the pond level, 33 out of 142 ponds (23%) tested positive for EHP infection. A total of 511 pooled biological samples were examined, including 460 five-shrimp pools and 51 ten-shrimp pools. Of these, 108 (23.5%) and 14 (27.5%) pools tested positive, respectively, resulting in an overall pooled sample prevalence of 23.9%. Further, in the study, epidemiological tools such as Epitool were applied to understand how pool size influences prevalence estimates. It is found that prevalence was estimated at 5.21% (95% CI: 4.29-6.26) for the five-shrimp pools study and 3.16% (95% CI: 1.72-5.26) for the ten-shrimp pools study. These findings highlight the importance of continuous EHP monitoring, enhanced farm management cum biosecurity practices, and further investigation into environmental and operational risk factors driving disease outbreaks in the shrimp aquaculture farms of Punjab.

Keywords: Shrimp Aquaculture, Disease Surveillance, Farm-Level Epidemiology, Microsporidian Infection, Molecular Diagnosis

Project: National Surveillance Programme for Aquatic Animal Diseases Phase-II

Funding: Pradhan Mantri Matsya Sampada Yojana, Department of Fisheries, Ministry

of Fisheries, Animal Husbandry and Dairying, Government of India; National Fisheries Development Board, India; and ICAR–National Bureau of Fish Genetic Resources, India

Role of passive disease surveillance in identifying bacterial pathogens in Indian aquaculture systems with special reference to Punjab freshwater aquaculture systems

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Bacterial infections in aquaculture continue to be a serious concern. It is noteworthy that the majority of bacterial infections, such as *Aeromonas* species, are opportunistic in nature and can function as primary or secondary invaders based on the host's health status. Thus, for aquaculture systems to effectively control and manage disease, prompt identification and treatment are crucial. In this regard, fish samples were received/collected from various districts of Punjab using a passive surveillance method as part of the National Surveillance Programme for Aquatic Animal Diseases. The species submitted for disease investigation included *Ctenopharyngodon idella, Pangasianodon hypophthalmus, Catla catla, Labeo rohita, Cyprinus carpio* var. *Koi, Heteropneustes fossilis*, and *Cirrhinus mrigala*. All these suspected fish samples were processed for disease diagnosis. Clinical examination (Level I) revealed signs such as hemorrhagic lesions, scale loss, and ulcerative wounds, etc. Samples from gill, kidney, and skin tissues were subjected to bacteriological analysis. Colonies displaying typical morphological characteristics on the Rimler-Shotts (RS) agar, like pale yellow to yellow, opaque, and round, were isolated for further identification. Species-level identification of the bacterial pathogens was done using Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometry. The results of this study showed that *Aeromonas veronii* was detected in suspected diseased samples of *Ctenopharyngodon idella, Pangasianodon hypophthalmus, Catla catla, Labeo rohita*, and *Cyprinus carpio var. Koi* that were collected from the various regions of Punjab (Ludhiana, Patiala, and Mansa). Further, the suspected fish samples (*Heteropneustes fossilis, Catla catla, Labeo rohita*, and *Cirrhinus mrigala*) from the districts of Ferozepur (30°39'47.9"N, 74°27'20.4"E) and Ludhiana (31°10'31.4"N, 75°31'58.4"E) were found to be infected with *Aeromonas hydrophila*. And *Acinetobacter junii* was detected in fish samples of *Cirrhinus mrigala* and *Labeo rohita* that were collected from Ludhiana (30°38'46.6"N, 75°44'43.4"E). This study emphasizes how crucial disease surveillance is to reduce financial losses in the aquaculture sector.

Keywords: Aquaculture, Disease, Early Detection, Disease Surveillance, Molecular Diagnosis
Project: National Surveillance Programme for Aquatic Animal Diseases Phase-II
Funding: Pradhan Mantri Matsya Sampada Yojana, Department of Fisheries, Ministry

of Fisheries, Animal Husbandry and Dairying, Government of India; National Fisheries Development Board, India; and ICAR–National Bureau of Fish Genetic Resources, India

A machine learning approach for shrimp diseases prediction using culture and climatic parameters

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Shrimp aquaculture, a dominant economic activity of coastal India, is highly vulnerable to disease outbreaks, especially White Spot Syndrome Virus (WSSV) and *Enterocytozoon hepatopenaei* (EHP), causing substantial production losses. Accurate disease prediction using environmental and climatic indicators helps sustainability of shrimp farming. This study assesses the impact of climatic and culture parameters on disease incidence, aiming to optimize machine learning models and determine the minimum data requirements for accurate forecasting. Data were collected from 290 shrimp farms covering Tamil Nadu, Andhra Pradesh, and Odisha states between April 2015 and February 2017 under National Innovations in Climate Resilient Agriculture (NICRA) project. Pond culture parameters like stocking density, days of culture, water source and salinity, and climatic variables like rainfall, maximum and minimum temperatures obtained from IMD were included for building the models. Climate data at monthly, fortnightly, weekly and daily intervals were tested for four months. Seven machine learning models viz., Logistic Regression, Random Forest, XGBoost, Neural Network, Naive Bayes, K-Nearest Neighbour (KNN), and Support Vector Machine (SVM) were evaluated for predicting WSSV and EHP incidence. The dataset was split into 80% for training (n = 232) and 20% for testing (n = 58). Weekly average climate data provided better accuracies over daily and monthly datasets in predicting both diseases. Among all models, Random Forest achieved the highest accuracy in majority cases. Notably, average weekly data for one month prior to the incidence of disease was found to be sufficient to predict the disease incidence. Combining culture parameters with climate data can improve model accuracies. The study on building disease prediction models with the climatic variables, prior to incidence of disease offers a valuable decision-support tool for proactive disease management in shrimp aquaculture.

Keywords: WSSV, EHP, Temperature, Rainfall, Random Forest

Project: National Initiative on Climate Resilient Agriculture

Funding: Indian Council of Agricultural Research, India

Surveillance and diagnostic findings of Tilapia Lake Virus (TiLV) in Malaysia from 2017-2025

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TiLV is an emerging viral pathogen that significantly impacts global tilapia aquaculture. First identified in 2014, it has been reported in multiple countries, primarily affecting tilapia species. In Malaysia, the first confirmed TiLV outbreak occurred in 2017. Since then, numerous cases have been diagnosed following the availability of specific diagnostic procedures. This paper summarizes the diagnostic findings and studies on TiLV in Malaysia from 2017 to 2025, with a focus on host susceptibility, detection in wild populations, and implications for disease control. Although primarily affecting tilapia, studies suggest the virus may have a broader host range. From 2017 to 2025, the National Fish Health Research Centre (NaFisH) conducted TiLV surveillance using molecular diagnostic techniques, primarily reverse transcription polymerase chain reaction (RT-PCR). Samples were collected from both cultured and wild freshwater fish species, including during disease outbreaks and routine epidemiological surveys. A total of confirmed TiLV cases were identified, predominantly involving red and black tilapia species. Interestingly, the virus was also detected in other fish species during natural outbreaks, including *Anabas testudineus* and species of *Barbonymus schwanenfeldii* and *B.gonionotus*. Moreover, asymptomatic wild fish, both tilapia and *Barbonymus* spp. were tested positive for TiLV during surveillance study, suggesting the possibility of subclinical or carrier states in these species. Experimental infection trials further revealed that tilapia are more susceptible to TiLV than *B. shwanenfeldii* based on higher observed morbidity and mortality rates. These findings indicate that TiLV has become endemic in parts of Malaysia and poses a continuing threat to the aquaculture industry. The detection of TiLV in asymptomatic wild fish raises concerns about the role of carrier species in viral persistence and transmission, complicating biosecurity and control strategies. The potential for virus dissemination through public fish release programs highlights the need for enhanced risk management measures.

Keywords: Tilapia Lake Virus, Diagnostic Cases, Surveillance Study

Project: Research & Development in fish health program in aquaculture

Funding: Department of Fisheries, Malaysia

Prevalence and distribution of White Spot Syndrome Virus in farmed shrimps from Gujarat, India

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White spot disease is caused by the white spot syndrome virus (WSSV) and is probably the most extensively studied crustacean virus to date and the same was classified in Genus *Whispovirus* in the family *Nimaviridae*. This study was aimed to detect the WSSV in major shrimp species such as *Penaeus vannamei* and *Penaeus monodon* in the state of Gujarat in India under National Surveillance Programme for Aquatic Animal Disease (NSPAAD). Shrimp samples (*P. monodon* and *P. vannamei*) were collected from the sampling sites, hepatopancreas and gill tissues extracted, fixed in 70 % ethanol for DNA isolation, and brought to the laboratory. Molecular detection was performed using two step PCR protocol, the primary PCR amplified a 1,447 bp fragment, while the nested PCR targeted a 941 bp region of the WSSV VP28 gene. A total of 2,145 shrimp samples were collected from prawn farms across eight districts of Gujarat includes Anand, Bharuch, Bhavnagar, Junagadh, Navsari, Porbandar, Surat and Valsad. Among the tested samples, 227 were confirmed WSSV positive (10.58%). Comparative species analysis revealed a higher infection rate in *Penaeus monodon*, indicating species- specific susceptibility. The highest WSSV prevalence was recorded in Navsari in which a total 1176 samples were tested, and 139 samples were found to be positive (11.8%) and from Bharuch district, 324 shrimp samples were tested, and 29 samples found to be positive (8.9%). However, WSSV was not detected in any of shrimp samples collected from Anand, Bhavnagar, Junagadh and Porbandar. The relatively higher infection rate in *Penaeus monodon* highlights the need for targeted biosecurity interventions for that species. The data suggest localized WSSV hotspots and possible farm level factors influencing virus occurrence. The study emphasizes the need for continuous surveillance, use of specific pathogen free broodstock and region-specific disease management strategies to reduce the risk of WSSV outbreaks in Gujarat.

Keywords: White Spot Disease, *Penaeus vannamei, Penaeus monodon*, Gujarat, Two Step PCR

Project: National Surveillance Programme for Aquatic Animal Diseases (NSPAAD)

Funding: The Department of Fisheries, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India

Isolation and identification of a putative novel pathogen, *Kosakonia sacchari* from Diseased Cyprinus carpio in Hisar, Haryana

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Bacterial infections pose a significant threat to aquaculture, especially in intensive and semi-intensive systems, where fish are often subjected to suboptimal water quality and stress, increasing their susceptibility to pathogenic invasions. The study was conducted to assess the bacterial diversity in different aquaculture system across Hisar district, Haryana including village ponds, Recirculating Aquaculture Systems (RAS) and Biofloc systems. Fish exhibiting clinical symptoms such as external lesions, hemorrhages, and behavioral abnormalities were collected and subjected to microbiological and molecular characterization. Bacterial isolates were isolated from the intestine of moribund *Cyprinus carpio*. Initially, identification was performed through morphological and biochemical tests, followed by molecular characterization of selected isolates through universal 16s rRNA gene. BLASTn analysis result revealed isolates' similarity up to 99 percent with *Kosakonia sacchari* which was later re-ensured by phylogenetic analysis. The detection of *K. sacchari* from intestinal tract of a diseased fish is a novel finding and suggests towards the opportunistic pathogenic behaviour of isolate. The presence may be linked to poor water quality or immunosuppression in fish due to environmental stressors, common in intensive aquaculture systems. To the best of our knowledge, this is the first report of K. sacchari from a freshwater fish in India. The emergence of such atypical bacteria in aquaculture systems underscores the need for continuous monitoring and microbial profiling to detect early signs of disease emergence. While *K. sacchari* has not been widely documented as a fish pathogen, its isolation from a diseased host necessitates further investigation into its virulence, transmission dynamics and potential zoonotic implications as the genus is well reported as human pathogen.

Keywords: *Kosakonia sacchari, Cyprinus carpio*, 16S rRNA, Aquaculture Pathogen, Hisar

First report of *Amyloodinium* sp. (Brown, 1931) (*Dinoflagellida*) infestation in Indian pompano (*Trachinotus mookalee* Cuvier, 1832)

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A study was carried out to identify the cause of mass mortalities in fry stage of Indian pompano (*Trachinotus mookalee* Cuvier, 1832) reared in marine hatchery of ICAR-CMFRI at Visakhapatnam regional centre. Affected fish exhibited clinical signs including surfacing, hyperventilation, anorexia, slime overproduction and postmortem gaping. Microscopic examination and histopathological analysis of the gills, skin and fins confirmed the presence of trophonts belonging to the causative dinoflagellate *Amyloodinium* sp. (Brown, 1931). Trophonts were nearly spherical in shape, ranging in length from 21.34 to 80.64 μm (66.39 $\pm$ 10.99 μm) were located in the studied fish tissues. Over 90% of the sampled fish were infested, with the highest parasite loads observed on the skin, followed by the gills and fins. This is the first report of *Amyloodinium* sp. infestation in Indian pompano.

Keywords: *Amyloodinium* sp., India Pompano, Marine Hatchery

Project: Development of seed quality indices for marine finfishes

Funding: ICAR-Central Marine Fisheries Research Institute, India

Winter associated pathogenic fungus *Protoachlya paradoxa (Saprolegniaceae)* infection in Bengal yellowfin seabream (*Acanthopagrus datnia*)

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Bengal yellowfin seabream (*Acanthopagrus datnia*), locally known as Datun, is widely distributed across the coastal areas of the Bay of Bengal, is a local delicacy due to its white and tender meat. Hence, there is a high demand for the species in local markets of West Bengal and Odisha. The cultivation of *A. datnia* has been practiced for a long period within the traditional aquaculture systems including bheries of West Bengal, (India) and Bangladesh. During winter, fungal infection in fishes is a common threat for aquaculture in Kakdwip and adjoining areas of West Bengal, India. Since there is a gap in the understanding on fungal infection in breams, a potential species for aquaculture, the present study was undertaken. *Protoachlya paradoxa* is a pathogenic fungus in the *Saprolegniaceae* Family under the Oomycota Division, found in the aquatic and terrestrial environment. Heavy mortality of *A. datnia* was observed in winter during December-January 2025 at Kakdwip area of West Bengal, India. During this period, the average water temperature varied between 16 to 22 °C. The infected fishes appeared weak, with presence of hemorrhagic lesions on the skin. White to gray cotton-like growth observed on skin and fins of infected fish. Fish samples were collected, the pathogen was isolated on Sabouraud Dextrose Agar (SBD) agar and identified through conventional as well as through nucleotide sequencing of the internal transcribed spacer (ITS) region. The isolated fungus was identified as *P. paradoxa*. Histopathological studies revealed that the skin epidermal layer was occupied by numerous fungal hyphae. Muscle tissues appeared with necrotic lesions. The results of the study suggested that *P. paradoxa* is a potential pathogen infecting *A. datnia*. The study provided base line information on pathology of *P. paradoxa* infection in *A. datnia*, which strengthen our understanding on fungal infection in brackishwater fish species.

Keywords: *Protoachlya paradoxa, Acanthopagrus datnia*, Bengal Yellowfin Seabream, Fungi, Pathology

Project: Captive breeding and seed production of candidate brackishwater species of Eastern region of India

Funding: ICAR-Central Institute of Brackishwater Aquaculture, India

Mapping of shrimp farms and shared environmental risks in disease spread: A case study in Chengalpattu District, Tamil Nadu

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Aquaculture is highly vulnerable to a range of diseases that can lead to considerable economic losses, posing a significant threat to shrimp farmers. The shared use of resources, unregulated planning and resource utilization, and insufficient management strategies may contribute to the proliferation of diseases in shrimp aquaculture farms. Though mapping of disease outbreaks provides insights into the presence of various pathogens and the incidence of diseases across different regions and farms, there are challenges associated with tracking disease occurrences and ensuring traceability with the primary influencing factors. A systematic analysis of spatial data concerning disease occurrence, which is associated with the prior selection of farms exhibiting spatial dispersion and the characteristics of related water bodies, will facilitate the development of management strategies aimed at disease prevention. Geographical Information Systems (GIS) platform will help integration of the environmental characteristics, spacing of farms, the resource utilization patterns by various stakeholders, the drainage patterns of creeks, and the management strategies, which will offer valuable insights into the factors causing diseases. The recurring occurrence of White Spot syndrome virus disease outbreak was mapped in the shrimp farms at Pudupattinam, Chengalpattu District, Tamil Nadu. Initially, we created a map that illustrates the disease affected farms. Subsequently, we performed spatial analysis to obtain the geographical characteristics of the impacted areas. The disease farm locations were linked with the source water canals and creeks, the spatial pattern indicated that the linkage of source water canals and its tributaries to farms. The research demonstrated that GIS can serve as a crucial tool for decision-making by offering insights into the spatial distribution of diseases and assisting in the strategic designing and placement of farms for aquaculture expansion.

Keywords: Disease Spread, Environmental Risk, Spatial Analysis, Shrimp Farms, India

On-site diagnostic platform for disease surveillance in aquaculture

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Diseases cost the global shrimp aquaculture industry an estimated USD 6 billion annually. While India leads the global shrimp export market, farmers lose almost USD 1 billion (≈ ₹4,000 crore) to White Spot Syndrome Virus (WSSV) and *Enterocytozoon hepatopenaei* (EHP) annually. Although laboratory-based polymerase chain reaction (PCR) diagnostics is the gold-standard for disease detection, it remains impractical for routine pond-side surveillance as they require specialised facilities and staff to operate. Farmers also face a long wait time for results, which do not arrive in a timely manner. Forte Biotech has developed RAPID, a portable, on-site, Loop-Mediated Isothermal Amplification (LAMP) platform that can deliver qualitative and quantitative results for pathogens such as EHP and WSSV in under an hour. This study aimed to develop and validate RAPID, for field-use in rural aquaculture. Field validation was conducted on shrimp tissue samples collected from commercial sources across Vietnam and Singapore. Results obtained with RAPID were benchmarked against commercial quantitative PCR (qPCR). The assay demonstrated 91.6 % sensitivity and 90.1 % specificity for EHP, with a limit of detection of 10 genomic copies per reaction. Similar performance was observed for WSSV and AHPND. In operational settings, RAPID detected incipient WSSV outbreaks up to eight days earlier than scheduled PCR surveillance, enabling timely intervention. Usability trials showed that farm technicians with minimal training could process samples, perform DNA extraction using proprietary systems, and obtain results easily. Crucially, the same RAPID is species agnostic with wider diagnostic applications. Assays for Tilapia Lake Virus and *Streptococcus iniae* are in development, positioning RAPID as a universal point-of-care platform for India's ₹57,000 crore aquaculture sector. RAPID delivers laboratory-grade accuracy in a rugged, affordable format tailored to India's farming systems, empowering farmers to implement proactive biosecurity, slash economic losses and pave the way for broader aquatic animal health management.

Keywords: Diagnostics, Point-of-care Testing, Biosecurity, EHP, WSSV

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