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(Dr. Jesu Arockiaraj, highly acclaimed Eminent Scientist with Stanford University World Ranking in Top 2%. Has also received many Awards including Young Scientist Award by Government of India and Government of Tamilnadu)

Greetings!

As we embark on another intriguing journey into aquaculture's thriving and dynamic world, I am pleased to welcome you to the current edition of Auqafocus. I am honored to serve as the Editor-in-Chief of this prestigious magazine, and I am excited to bring you the latest advances, insights, and trends in the ever-changing aquaculture business.

Auqafocus evolves in tandem with the aquaculture landscape. To satisfy the demands of this dynamic area, we are constantly enhancing and increasing our coverage. We aim to cultivate a community of knowledgeable professionals committed to our aquatic health and sustainable production. We intend everyone to engage with us, offer your perspectives, and participate in this exciting adventure.

Aquaculture is a major industry critical in delivering sustainable fish to the world's rising population. The difficulties and potential in this industry are limitless, and Auqafocus is dedicated to serving as your trusted source for in-depth research and up-to-date information.

In this edition, we are thrilled to bring you a diverse array of articles that dive deep into the transformative world of aquaculture. From groundbreaking technology to sustainable alternatives, we explore the trends and innovations shaping the industry's future. The role of IoT in enhancing water quality and nutrition, the rise of giant freshwater prawns, and solutions for shrimp health are just a few of the topics we cover. We also highlight the need for cutting-edge technology in marine biology and the promise of biofloc technology in sustainable aquaculture.

We're here to keep you informed and inspired as we cultivate a sustainable future together. Happy reading!

Regards,
Editors

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REVOLUTIONIZING AQUACULTURE : IOTs role in enhancing water - quality parameters and nutrition in fisheries

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Abstract

Internet of Things (IoT) technology revolutionizes aquaculture by enabling real-time monitoring of water quality, fish health, and feeding processes. This integration of IoT and aquaculture offers improved productivity, sustainability, and reduced losses. Monitoring nodes continuously collect data on critical parameters, transmitting it to cloud platforms for analysis and alerts to farmers. Key IoT applications in aquaculture include water quality monitoring, fish tracking, and automated feeding systems. However, challenges such as data volume and energy-efficient data transmission remain. The future holds promise for further advancements in IoT and AI integration to enhance decision-making and sustainable aquaculture production.

1. Introduction

In addition to conventional farming, aquaculture the food industry with the greatest growth rate plays a vital role as a complementary activity, offering farmers chances for income diversification and social and economic benefits. Several criteria, including environmental conditions, production factors like water quality, and biotic factors, must be continuously checked to avoid yield losses and boost efficiency. Precision aquaculture employs cutting-edge technologies, including artificial intelligence (AI) and the Internet of Things (IoT), to ensure profitability, sustainability, and environmental protection. IoT solutions used in aquaculture often use a local server and save the enormous amount of generated data in an Excel file or a database. It's conceivable to argue that aquaculture has reached the digitalization phase due to the Internet of Things (IoT) ability to provide real-time monitoring solutions remotely and with little or no human intervention (Fig. 1).

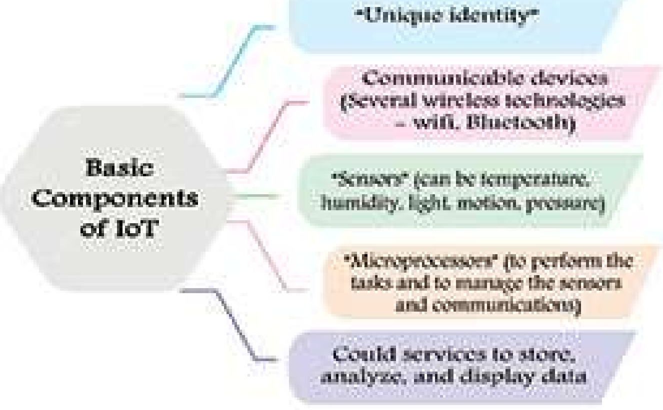

Fig. 1. Basic components of IoT

The network of physical objects is known as the Internet of Things (IoT). Its rapid growth set it apart from the present Internet, which is mostly a network of computers, including mobile devices like phones and tablets. People, animals, plants, household appliances, machinery, products, buildings, and cars can all be considered "things" in the IoT. All physical devices are connected through the Internet of Things (IoT) and can exchange data without the need for human interaction. Remote access and control are both possible for them. This has completely transformed our lives, a truly revolutionary concept. Linking several devices is not a novel idea. The first gadget connected to the Internet was a Coke machine at Carnegie Mellon University in 1982. British businessman Kevin Ashton coined the phrase "Internet of Things" in a 1999 presentation to Procter & Gamble. Radio-frequency identification, or RFID, formed the foundation for the Internet of Things. Due to the combination of numerous supporting technologies, such as embedded systems, wireless communications, microcontrollers, sensors, and micro-electromechanical systems (MEMS), subsequently evolved and gained popularity. The Internet of Things is currently viewed as the future and the next big thing. IoT will spread exponentially in the same way that the Internet did about 20 years ago.

IoT is employed in the aquaculture industry for various purposes, including environmental monitoring, animal tracking, industrial management, precision agriculture, and other areas. A significant amount of data has been gathered using automated controllable systems and networked sensors. This optimizes productivity. Additionally, the productivity of fisheries is increased by reliable data from the Internet of Things, particularly in complex and risky routine activities. Many relevant new technologies are also used to support several sensors and cover a broad region, such as low-power, long-distance wireless communication. These data-driven strategies improve aquaculture operations' effectiveness, sustainability, and productivity.

Farmers gauge the overall quality of the water using time-consuming, outdated procedures. Farmers can't act quickly enough to stop the fish from worsening since they don't get enough alerts. To achieve this goal, efforts have been undertaken to design the architecture of an IoT framework that will assist aquaculture farms in determining the water quality and inform the farmers to take the necessary steps to prevent losses due to fish mortality.

However, there are still a lot of difficulties in aquaculture that need to be resolved. One of the key issues that precision aquaculture could solve is maximizing the yield through effective resource use. This article mainly highlights the importance of IOT in aquaculture, its basic components, workflows, major applications and the various constraints while developing it in fisheries sectors.

2. Importance

Commercial aquaculture is facing several difficulties due to sudden changes in climatic conditions that affect the criteria governing water quality. Aqua farmers currently employ manual check procedures to determine the water's parameters. This will take longer and be inaccurate because water quality parameters may change over time. Aquaculture should innovate to increase potency and reduce losses by monitoring water quality parameters to prevent this drawback. Secondly, the major importance and achievement of IOT in aquaculture is Automated Feeder. Correct feeding is vital at a scheduled period, and aqua-farmers still do this by hand. Therefore, the invention of automated feeding equipment has proven to be a gift for farmers because it eliminates the need for human labour and lessens the problems associated with overfeeding and other issues, for which overfeeding is the primary reason.

IoT-based solutions enable monitoring and adjusting water parameters in real-time, boosting accuracy, prompt notifications, and optimizing aquaculture production. IoT in aquaculture enables accurate feeding methods and enhances feed efficiency. Precision feeding schedules and portion control can be achieved by integrating IoT technologies with feed automation systems, decreasing feed waste and optimizing growth rates. Fish movements, behaviour, and growth can be tracked and analyzed using cutting-edge tracking technology like RFID or acoustic tags, providing a greater understanding of fish health, population dynamics, and stocking density optimization.

3. Worlflow

The size of the water bodies (ponds, reservoirs, tanks, etc.) will determine the number of monitoring nodes to be deployed. These sensor nodes continuously detect and record water parameters such as pH, temperature, and dissolved oxygen, which impact the fish's life and are key parameters as they directly impact aquatic animals' health, growth, and carrying capacities. These multiple wireless sensor nodes are deployed in a fishing pond. These sensor nodes gather information on important factors influencing fish health and pond conditions. These sensor nodes wirelessly transfer their data to an edge node, a regional hub for communication and processing. The data is subsequently transmitted from the edge node to the cloud platforms through the Internet for storage and analysis (**Fig. 2**).

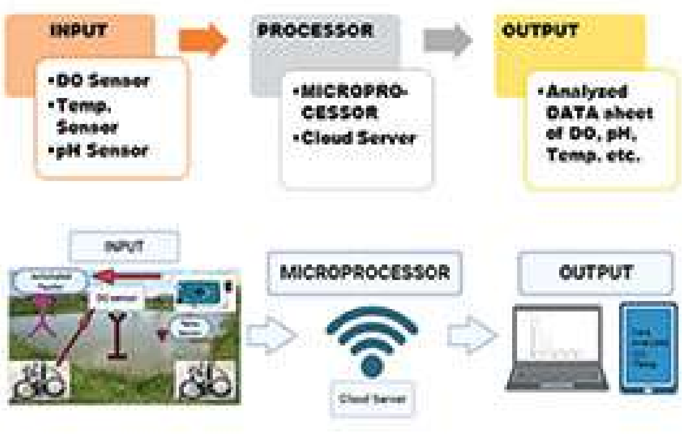

Fig. 2. Workflows of IOT in Fisheries Ponds

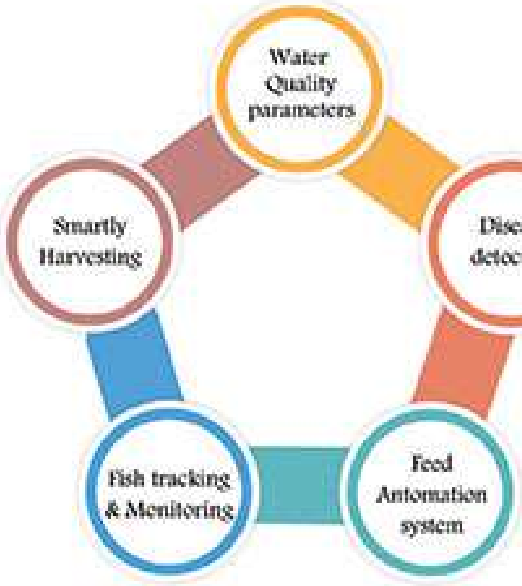

Fig. 3. Applications of IOT in Aquaculture

This cloud platform service enables them to easily aggregate, visualize, and analyze live data streams. An SMS alert is sent to the farm manager if any parameters cross the threshold value. The main three basic pathways are- Input, Microcontrollers and Output. Sensor nodes deployed in the water bodies will act as Input components. The various cloud platforms used are the microprocessor, and as an Output, water parameters data will be displayed on the connected device's screen via SMS or emails. This architecture enables real-time monitoring and analysis of the fishing pond conditions, facilitating informed decision-making and predictive analysis using an AI-trained model. Based on the alert, steps will be taken to boost productivity and lessen the effects of fish loss. The user is automatically notified through email and text messages when a parameter surpasses a specific critical value for the farm. This system enables speedy response by initiating a problem-solving action.

4. Applications of IoT in aquaculture

The Internet of Things (IoT) is essential to aquaculture because it makes it possible to monitor water quality, fish health, and environmental factors in fish farms in real time (**Fig. 3**). IoT technologies optimize aquaculture operations by utilizing sensors, connectivity, and data analytics, boosting productivity and reducing disease risks. IoT enables the sector to adopt sustainable and effective practices, assuring the welfare of aquatic animals and the long-term survival of aquaculture systems. These capabilities include automation, remote control, and intelligent decision-making. Fish farming has seen improved operational effectiveness, optimized resource utilization, and better yields thanks to IoT integration, which has turned the sector into a more sustainable and lucrative business.

Aqua farmers use manual measurements to evaluate the state of the water's various parameters. Manual measurements take a long time and produce inaccurate findings since the factors that gauge water quality constantly change. Therefore, if automatic monitoring is possible, it will be better. Aquaculture uses cutting-edge technology like the Internet of Things (IoT), Computer Vision, and Machine Learning to overcome the challenge of measuring water parameters.

Better regulation of the many chemical, physical, and biological aspects of the water in fish ponds is unquestionably necessary for achieving maximum fish output. Therefore, for effective fish pond management, it is important to understand the water quality, which is controlled by several factors, including temperature, turbidity, water colour, pH, carbon dioxide, alkalinity, electrical conductivity, total dissolved solids (TDS), unionized ammonia, nitrate, and nitrite. Users can use an Android app from anywhere worldwide to monitor the water quality utilizing Wi-Fi and the Internet.

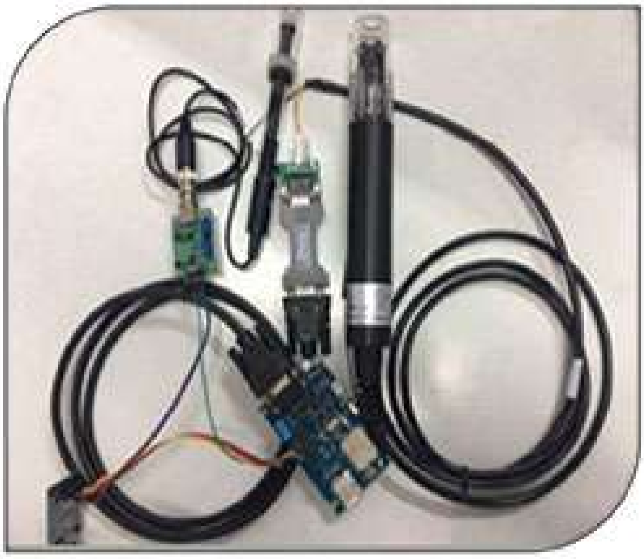

Fig. 4. Sensor measuring DO, Temp., pH (Source- Huan et al., 2020)

Due to a lack of provenance and quality monitoring data, it is challenging to assess fish quality in practice accurately. Real-time and objective quality tracking with the newest Internet of Things (IoT) and Artificial Intelligence (AI) technologies may be possible. Recent advancements in embedded devices and sensor technology can offer a variety of views with previously unheard-of levels of information in the temporal, spatial, temperature, smell, and other environmental domains.

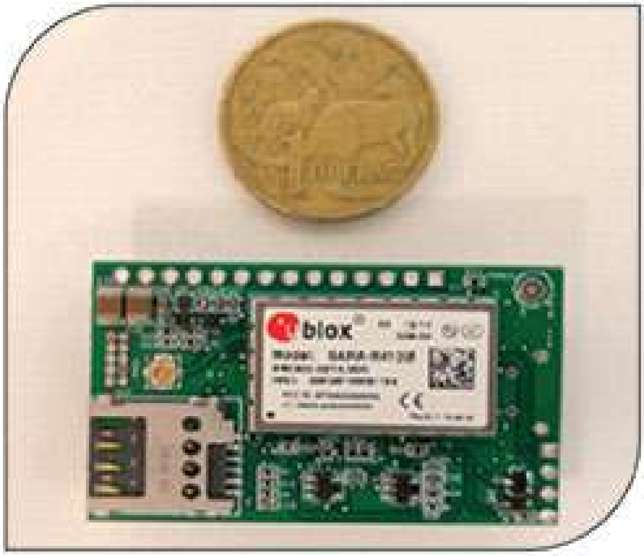

Fig. 5. IoT device under a coin (Source, Wang et al., 2021)

IoT and AI technology made automated quality assessment and tracking possible. Small, water-resistant, and having a long battery life, IoT devices are. They are fastened to the containers fishermen use to transport and store fish across the state. Every IoT device has a Global Positioning System (GPS) sensor to track the box. Additionally, it is connected to an external temperature sensor, allowing Internet of Things devices to sense the temperature of the fish rather than the onboard temperature (Fig. 4-6).

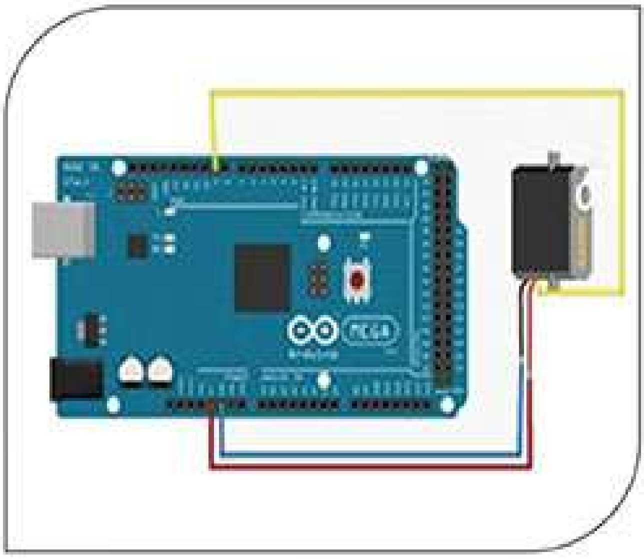

Fig. 6. The automation circuits of fish feeding systems (Source- Riansyah et al., 2020)

Even though fish must be fed on time, there are instances when this presents a problem while the owner is away from the fish pond. Furthermore, excessive feeding will make the feed deteriorate. The feed dose needs to be precise. The fish-feeding system in a mini aquarium is controlled online via a website, and the automatic fish-feeding system is through the Android application (Table 1).

Table 1. IOTs in various Aquaculture Systems

S.No.	Aquaculture System	Parameters checked	Microprocessors	References
1.	Aquaponics System	pH, Temp., Turbidity, NH_3, NO_3^{3-}	Arduino	Udanor et al (2022)
2.	Hydroponics with Aquaculture sytem	Temp., pH & Humidity	Arduino	Tamana et al (2021)
3.	Recirculating Aquaculture System	Temp. & Water-level	Raspberry Pi	Al-Hussaini et.al. (2015)
4.	Biofloc Technology	Temp., pH, DO & TDS	Arduino UNO	Rashid et al (2022)

5. Major constraints

Requires more variables than we can currently monitor to obtain the most complete picture of what is happening in farms. Creating new probes to measure microbes, micropollutants, or other physicochemical properties makes this a technological challenge.

The capacity to transport a significant volume of data from the farm using the least amount of energy is another significant barrier to the IoT revolution in this industry. By examining their behaviour, activity, and potential diseases directly underwater, developing real-time monitoring using high-quality HD video feeds to enable deep image processing would create new prospects for livestock surveys. Additionally, it will be possible to scan the microenvironment, such as the weather or local activity, to stop poaching.

6. Conclusions and future perspectives

We now can create cutting-edge solutions that make life easier thanks to the Internet of Things (IoT), which has evolved into a highly adaptable solution for several use cases, from smart cities to the smart aqua farming business. IoT and cloud technology development has opened up new avenues for developing innovative farming techniques. To improve sustainability, this study focuses on disease control and water quality monitoring to examine the possible effects of IoT in aquaculture. It covers a variety of IoT applications in aquaculture, such as fish tracking and monitoring systems, feed automation systems, environmental control systems, and water quality monitoring.

Integrating AI and machine learning, developing sensors, and expanding IoT infrastructure are the future directions for wise decision-making, predictive analytics, and sustainable aquaculture production.

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CRESCO Objectives

CRESCO aims to cultivate and produce huge quantitative of Algae for Aquaculture Industry for use as Feed and Nutrition agent It aims for Mass production of Spirulina and value added products with an initial Target of 1Ton per month. Gradually it aims to develop large scale Algae ponds and be one of the largest producer of Spirulina – both for domestic consumption and also for International exports.

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Cresco aims to produce large quantities of Algae and Microalgae. This would encompass production of economically important algal species like *Spirulina sp, Chlorella sp, Dunaliella sp, Hematococcus sp, Nanochloropsis sp, Diatoms, etc.* These are highly useful in the Aquaculture industry as Fish Nutrition.

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CULTIVATING A SUSTAINABLE FUTURE :
Giant freshwater prawn's rise amidst Southeast Asia's shrimp crisis

FAITT

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Abstract

Southeast Asia's shrimp farming industry faces challenges due to environmental factors and a recent downturn. The Giant Freshwater Prawn emerges as a sustainable alternative, fostering economic growth and environmental conservation. Transitioning to innovative practices like mono-sex farming empowers small-scale farmers, ensuring economic viability and sustaining seafood demand. GK Aqua leads in all-male prawn seed production, exporting disease-free seeds globally. Founder Giva Kuppusamy champions mono-sex prawn farming for its economic and environmental benefits, promoting responsible aquaculture

Redefining Southeast Asia's shrimp farming With a surging global seafood demand, Southeast Asia has risen as a key player in shrimp farming, driving economic growth, however impeded by environmental factors. The region's shrimp industry recently faced a notable downturn, prompting a re-evaluation of methods. Amidst this challenge, the Giant Freshwater Prawn (Macrobrachium rosenbergii) has emerged as a sustainable, high-density farming alternative, holding great promise for seafood production.

Giant freshwater prawns transforming Southeast Asia Giant Freshwater Prawn is indigenous to Southeast Asia, offering diverse benefits through environmental impact and ensuring economic feasibility. Farmers can adept innovative techniques like integrated multi-trophic aquaculture (IMTA), where species integration reduces water consumption pollution risks and boosts economic returns by cultivating them. Transitioning to this form of farming creates a dual advantage, conserving the environment and reviving economies. The crisis within shrimp production addresses the need for sustainable aquaculture practices, and freshwater prawns provide a practical solution. This transformation empowers small-scale farmers to enhance their earnings and escape unsustainable practices, one of the most crucial steps in meeting the ever-increasing seafood demand sustainably.

Moreover, one of the growing sustainable practices in freshwater prawns is monosex farming. This solution increases production yield and shorter harvest cycles, giving farmers higher economic value. Monosex farming reduces reliance on wild stocks and transfer of vertical disease transmission that affects prawn productivity.

Fig. 1. Adult giant freshwater prawn

GK Aqua's global expansion in all-male prawn seed production Based in Malaysia, GK Aqua is one of the most promising mono-sex prawn producers, where they have successfully engineered the biotechnology approach to produce all-male seeds. This biotechnology company has successfully expanded their international presence by exporting all-male seeds to several regions, such as the Middle East, Southeast Asia, and Latin America.

GK Aqua offers farmers high-quality all-male seeds through specific-pathogen-free (SPF) broodstock production. With state-of-the-art in-house molecular laboratory facilities (Fig. 1-2), GK ensures that all the broodstock is maintained disease-free throughout the whole process of pre- and post-mating to produce highly resilient post-larvae.

Fig. 2. GK Aqua Molecular Laboratory

Championing sustainability. Monosex freshwater prawn farming revolution Founder of GK Aqua (Fig. 6-9), Giva Kuppusamy, mentioned that it is essential to provide a solution for farmers who have worked hard to contribute to the production of food globally and contribute to continuing efforts in food security challenges nowadays. Monosex freshwater prawn farming offers significant advantages for sustainable aquaculture, such as controlled disease management and simplified feed management strategies, leading to higher survival rates and improved product quality. This approach boosts the economic viability of prawn farming and aligns with responsible and environmentally conscious aquaculture practices (Fig. 9).

Exploring EHP infections in shrimp aquaculture and the potential of neem as a natural solution

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Abstract

Enterocytozoon hepatopenaei (EHP) infection in shrimp aquaculture, highlighting its impact on the industry and potential mitigation strategies. It covers EHP's life stages, transmission pathways, and clinical manifestations, emphasizing growth retardation, lethargy, reduced feed intake, and soft shells. The study also explores diagnostic methods such as PCR and qPCR, and examines the use of natural products like neem for treatment. EHP's implications for shrimp health and the challenges of control are discussed, underscoring the need for effective diagnostic tools and management approaches to prevent economic losses.

Introduction

Aquaculture plays a vital role in global food security by providing a significant portion of the world's seafood consumption. It encompasses a wide range of activities, from raising fish in freshwater ponds to cultivating oysters in coastal waters, and it can take place in natural or artificial environments. Proper management and sustainable practices are crucial to ensure the health of the aquatic ecosystem and the production of safe and high-quality seafood products. India holds the second position globally in aquaculture production and is a significant shrimp supplier to the United States and the European Union. With a 17.5% market share in farm-raised shrimp aquaculture, India shifted from Black tiger shrimp (*Penaeus monodon*) to Pacific white leg shrimp (*Litopenaeus vannamei*) due to white spot disease. *L. vannamei* exhibits superior

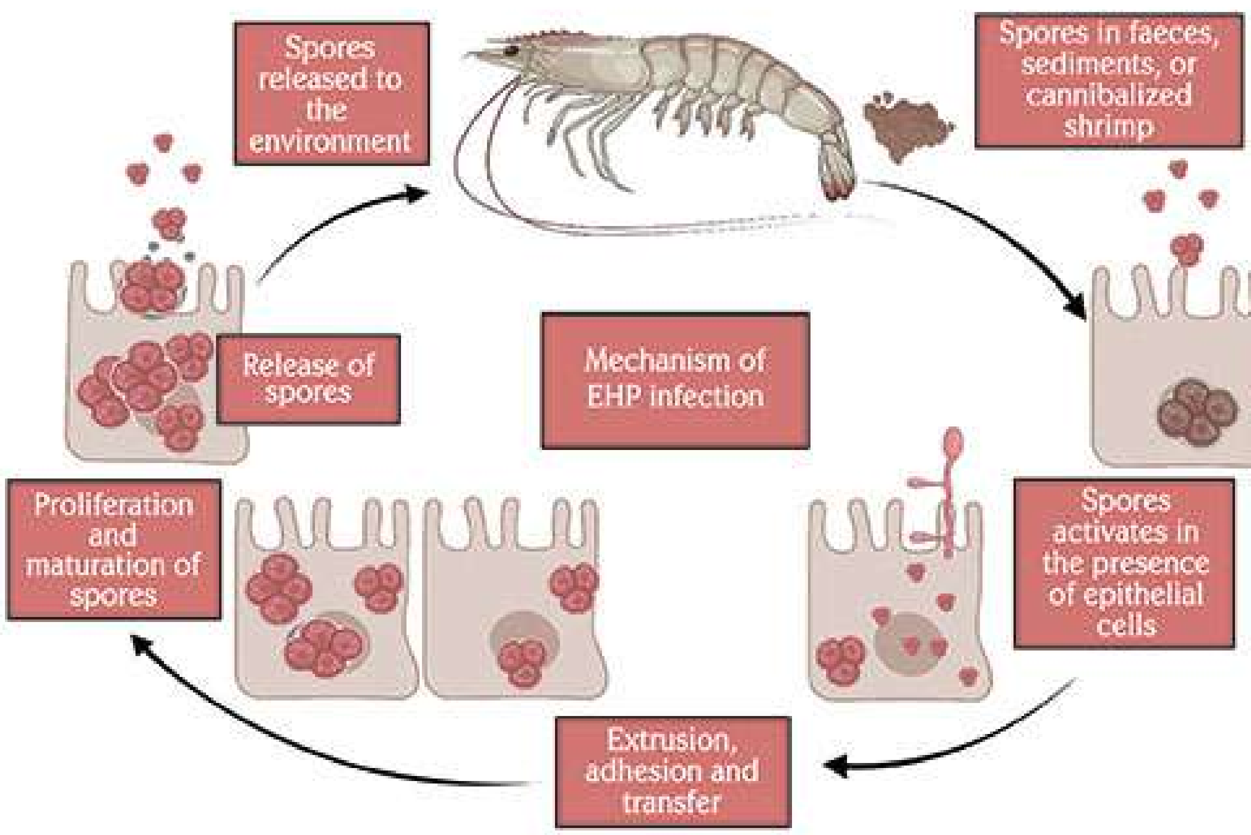

Fig. 1. General Life cycle and transmission of EHP

Table 1. Clinical manifestations of EHP in shrimp

Clinical Manifestations	Description
Growth Retardation	EHP-infected shrimp often exhibit reduced growth rates, leading to size variability.
Lethargy	Infected shrimp may show decreased activity levels and overall sluggishness.
Reduced Feed Intake	Shrimp with EHP infections tend to consume less feed than their healthy counterparts.
Empty Midgut	Infected shrimp may have an empty midgut, indicating reduced feeding and digestion.
Chronic Mortalities	Long-term infections can lead to recurring mortalities within the shrimp population.
Soft Shells	Infected shrimp often exhibit soft shell conditions, which can impact overall health.
White Feces Syndrome (WFS)	EHP has been linked to WFS, characterized by white GI tract discolouration and white faecal threads.

aquaculture traits to P. monodon and Fenneropenaeus indicus, including better survival, rapid development, adaptability, and disease resistance. The shift resulted in 5.4 million tonnes of seafood in 2019.

Shrimp aquaculture

Shrimp aquaculture plays a pivotal role in driving economic growth and generating significant employment opportunities, particularly in the developing countries of Asia. With a substantial market share of 24.9% in the global trade of farmed shrimp, India holds the position of the second-largest aquaculture product producer. In 2018, India stood as the primary prawn supplier to the United States and the second-largest to the European Union. In response to the threat of white spot disease, Litopenaeus vannamei emerged as a replacement for the Black tiger prawn (Penaeus monodon) in India. The Pacific whiteleg shrimp (L. vannamei) has emerged as the preferred crustacean species for human consumption through aquaculture. This notable crustacean, known for its prominence in aquaculture, yielded a farm production of 5.4 million tonnes and contributed significantly to the economic value of 2.6 million rupees in 2019.

Current affairs

Challenges of unknown origin have impacted shrimp farmers' expectations in L. vannamei production, hindering the industry's ability to meet global demand. Infectious ailments induced by microorganisms, such as bacteria, viruses, and microsporidia, threaten the shrimp farming sector across Asia. A microsporidian named EHP has afflicted Penaeus monodon in Thailand since 2003. Initially identified as EHP in 2009, it infects shrimp's hepatopancreas cells.

In nations where EHP occurrences have been recorded, including China, Indonesia, Malaysia, Vietnam, and Venezuela, primary shrimp cultivation occurs across diverse environmental settings, encompassing coastal regions, brackish zones, and rural locales. Initially, there were reports of early-stage shrimp mortalities attributed to early mortality syndrome (EMS) in several parts of India. Nevertheless, a comprehensive investigation conducted by the Central Institute of Brackishwater Aquaculture (CIBA) in Chennai, India, effectively debunked the presence of EMS in the country. Consistent shrimp deaths within culture ponds have also been linked to suspected cases of running mortality syndrome, yet the precise causative agent for this phenomenon remains unidentified.

During 2016, instances emerged where L. vannamei, cultivated in ponds, exhibited the presence of EHP across multiple regions in India. Consequently, the shrimp farming sector encountered considerable financial losses. Notably, specific eastern Indian states, such as Andhra Pradesh, feature ponds comprising a borehole and estuary water blend, resulting in varying salinities ranging from 0 to 30 ppt, with an average of approximately 10 ppt. Conversely, western regions like Gujarat experience pond water salinities ranging from 30 to 44 ppt. It is worth highlighting that EHP occurrences have been documented across both high and low-salinity conditions. Reports indicate a staggering production loss of 0.77 million tonnes in Indian prawn farms due to EHP infection, translating to a revenue decline of $567.62 million.

EHP

Microsporidia are parasitic microorganisms that can infect various hosts, including mammals, invertebrates, certain protists, fishes, and crustaceans, by forming spores. The distribution of microsporidians has been extensively documented in freshwater, brackish water, and marine environments. Among these microorganisms, EHP has gained prominence as a pathogen. Taxonomically, EHP belongs to the Enterocytozoonidae family, suborder Apansporoblastina, phylum Microsporidia, and kingdom Fungi. Various shrimp species like Penaeus japonicas, Penaeus monodon, and Penaeus vannamei, commonly raised in shrimp farming, can harbour EHP in their hepatopancreas.

Life cycle

EHP follows a life cycle with two distinct stages. The intracellular spore-forming stages colonize the shrimp hepatopancreas's digestive epithelial cells, causing harm to the hepatopancreatic (HP) tubule epithelial cells. The extracellular stage involves an active spore phase within the digestive tubule, with multiplication occurring in the hepatopancreatic cell cytoplasm. When infected with EHP, host cells display irregular or regular basophilic inclusion bodies in the cytoplasm, resembling the Plasmodium sporogony stage, with or without spores. Host cells are frequently infected as the spore is released from its polar tube, breaching the plasma membrane and releasing its contents (sporoplasm) into the cytoplasm. The sporoplasm subsequently develops adjacent to the host cell's cytoplasm, resulting in a spreading plasmodium. Upon separation from sporoblasts, this plasmodium generates complete spores while remaining proximate to the host cell cytoplasm. Sporogony involves synthesizing spore extrusion precursors, including the polar filament and anchoring disc.

EHP spores are found within the gastrointestinal tract and the lumen of HP tubules. Horizontal transmission in shrimp farming occurs as spores are ingested through contaminated shrimp excrement or via cannibalism of infected shrimp, where ruptured HP epithelial cells produce mature spores. EHP has been documented across a spectrum of salinity conditions **(Fig. 1)**.

The hepatopancreas is pivotal in digestion, absorption, lipid, glycogen, and mineral storage. An EHP-infected hepatopancreas can significantly impact shrimp's health and physiology, potentially disrupting nutritional processes, metabolic pathways, and enzyme production. While EHP infections do not cause substantial mortality, they are linked to size discrepancies and growth retardation. Correlations have been observed between the detection of elevated EHP gene levels and increasing growth retardation in shrimp.

Clinical manifestations

Clinical manifestations of EHP include growth retardation, which contributes to size variability, lethargy, reduced feeding, and empty midguts. Infected shrimp often exhibit soft shells. EHP is also associated with White Feces Syndrome (WFS), characterized by white gastrointestinal tract discolouration and whitish faecal threads floating on the water's surface.

Despite the availability of EHP-free post-larvae for stocking shrimp farms, feeding them locally captured marine organisms positive for EHP can result in their infection. Additionally, the faeces of infected shrimp carry numerous spores that facilitate horizontal transmission to healthy shrimp. (EHP) exhibits complex life cycles involving various spore types generated according to the host species, often spanning multiple phyla. While some spores may appear physically similar under a light microscope, their functionality can differ, with one type infecting one host while another targets an alternative host. These intricate cycles may involve transitions between monokaryotic (haploid) and diplokaryotic nuclei, where the latter consists of two tightly enclosed haploid nuclei. In certain species, the diplokaryotic nucleus may merge to form a conventional diploid nucleus, which then undergoes eukaryotic meiosis, yielding four haploid nuclei.

EHP produces oval-shaped, monokaryotic spores measuring around 1.1 ± 0.2 µm x 0.6 ± 0.2 µm. These spores exhibit 5–6 coils of the polar filament on one side and an anchoring disc on the other. Recent PCR results have revealed EHP spores within various shrimp cellular components, including R (reserve), B (blister), and F cells of the hepatopancreas, as well as in midgut epithelial cells and shrimp faecal specimens. Embryonic cells (E-cells) near the hepatopancreas typically remain unaffected **(Table 1)**.

Similar to other microsporidians, EHP spores possess a double-layered spore wall. The outer layer (exospore), approximately 10 nm thick, is electron-dense, while the inner layer (endospore), approximately 2 nm thick, is electron-lucent. This spore wall offers protection against harsh conditions, rendering the spore components environmentally resilient during dissemination. EHP's spore wall proteins are categorized within the SWP12 clade, responsible for host cell attachments. The first EHP spore wall protein (EhSWP1) was identified through whole-genome sequencing and is associated with host cell attachment function. Additionally, these proteins contribute to spore protection. This evolutionary trait likely provides advantages for EHP to infect other shrimp species or expand its host range.

Like other invertebrates, shrimp employ their innate immune system to counter invading pathogens. This defence mechanism encompasses both humoral and cell-mediated immunity. Non-specific enzymes like lysozyme (LZM) and superoxide dismutase (SOD) in bodily fluids collaborate to ward off bacterial infections. Shrimp physiological and immunological status is influenced by metabolic factors such as triglycerides (TG), total protein (TP), cholesterol (CL), glucose (GLU), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), as well as parameters like total hemocyte count (THC), prophenoloxidase (PO), superoxide anion (SOA), total antioxidants capacity (T-AOC), and catalase (CAT).

Table 2 . Different diagnostic methods to detect EHP in shrimp

Diagnostic Method	Description
PCR (Polymerase Chain Reaction)	Amplifies specific DNA segments for EHP detection; highly sensitive and specific.
qPCR (Quantitative PCR)	Measures EHP DNA concentration quantitatively; aids in assessing infection severity.
LAMP (Loop-Mediated Isothermal Amplification)	Rapid amplification method, cost-effective and suited for field use.
In Situ Hybridization Assay	Uses labelled probes to detect EHP-specific RNA in tissue sections; offers spatial information.
Histopathology	Microscopic examination of tissue sections for EHP-induced cellular changes provides visual evidence.
Nested PCR	Two-step PCR that enhances specificity targets specific EHP genes to avoid cross-reactions.

Diagnosis

Numerous diagnostic methodologies have been documented for the detection of EHP to date. These include loop-mediated isothermal amplification (LAMP), in situ hybridization assay, histopathology, polymerase chain reaction (PCR), and quantitative PCR (qPCR), which relies on the small subunit rRNA (SSU rRNA) gene. Nested PCR targeting the spore wall protein gene (*swp*) or b-tubulin gene may also mitigate potential misinterpretations stemming from sequence similarities among SSU rRNA genes among closely related microsporidia.

EHP has been recognized as a significant threat to shrimp farms in various countries. Preventative strategies involve treating prawn ponds with quick lime (CaO) at six tonnes per hectare to eliminate residual spores and potential carriers before introducing EHP-free post larvae (PL). The main focus of EHP infection control efforts is these measures. However, their effectiveness in preventing the spread of the EHP virus to healthy shrimp is limited. Unfortunately, no curative treatments are known for EHP infection, and clear clinical symptoms are absent in infected prawns, posing challenges for surveillance and containment. As a result, developing a rapid and straightforward diagnostic approach for EHP infection is imperative to avert disease outbreaks and economic losses **(Table 2)**.

Treatment

To address EHP infections or enhance the immune response of affected shrimp against EHP, natural products such as herbal compounds like neem have been investigated. Neem, scientifically known as Azadirachta indica, is deeply rooted in Ayurveda, the ancient medicinal system of India. Ayurveda extends beyond mere treatments and procedures; it represents a holistic way of life that aligns with fundamental truths. Traditional Ayurvedic remedies often feature ingredients like aloe vera (Aloe barbadensis), neem, and sandalwood (Santalum album), which possess antibacterial properties and cool the blood, benefiting conditions related to Pitta.

Neem, a versatile plant, has been utilized for over two millennia in India and neighbouring regions. Its diverse biological functions are well-documented through scientific research. Various parts of the neem tree, including the seeds, flowers, kernel, twigs, bark, roots, and leaves, have been employed in traditional and folk medicinal systems for their therapeutic attributes. Neem leaves, for instance, exhibit anti-inflammatory, antibacterial, antiviral, antioxidant, hepatoprotective, antimutagenic, anticarcinogenic, and other properties, making them integral to traditional healing practices. Neem seeds possess antimalarial, antipyretic, and antifungal qualities and properties similar to the leaves. The neem flower contributes medicinal benefits such as phlegm clearance, bile regulation, and intestinal worm treatment, containing sesquiterpenes, hydrocarbons, fatty acids, steroids, and aromatic compounds.

Neem is rich in over a hundred primary and secondary metabolites, making it a valuable source of bioactive compounds. Primary constituents encompass protein molecules and derivatives of fats or carbohydrates, while secondary compounds include flavonoids, steroids, saponins, and alkaloids. Neem's therapeutic components are broadly categorized into isoprenoids and nonisoprenoids. The primary active component is tetranortriterpenoids, limonoids or C-seco meliacins. Azadirachtinoids, isomeric meliacins, are a significant subgroup, with azadirachtin E standing out for its efficacy. Other constituents include phenolic compounds, carotenoids, steroids, ketones, and terpenoids.

Over time, diverse techniques have been developed to extract neem with varying potency for personal and commercial applications. Indigenous methods involve water extraction, cold maceration, steam distillation, and solvent extracts. Aqueous extracts, achieved with minimal equipment, are comparably effective. Crushing neem seeds, kernels, or leaves, soaking them overnight, and filtering yields extract. Alternatively, granular material can be soaked in a cotton bag for hours, producing a refined extract containing water-soluble amino acids, bitters, and carbs.

In conclusion, neem's multifaceted therapeutic attributes, documented through ancient practices and scientific research, make it a promising natural compound for combating EHP infections and enhancing the immune response in affected shrimp.

Conclusion

The introduction of Litopenaeus vannamei revolutionized shrimp aquaculture and led to Enterocytozoon hepatopenaei (EHP) infections. These infections, caused by microsporidian parasites, challenge the industry globally. Diagnostic methods like PCR and LAMP aid in identifying EHP, but preventive measures and effective treatments are lacking. EHP's intricate life cycle affects shrimp health, causing growth retardation and size variability. With its diverse biological properties, neem shows promise as a potential natural remedy to combat EHP infections and bolster shrimp immunity. While advancements in diagnostics provide early detection, the industry faces ongoing challenges in managing EHP and minimizing economic losses. Understanding EHP's impact and exploring remedies like neem offer avenues for further research and innovation. The quest for sustainable shrimp aquaculture necessitates continuous scientific exploration and collaboration.

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Marine biology and biomineralization suffer from a lack of access to cutting-edge Omics technologies

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1. Introduction

Marine biology is a vast field that focuses its research on organisms ranging from bacteria to the biggest animal on earth. To date, various biomolecules have been studied to characterize marine organisms, their diseases, adaptations, natural products, etc. In this current article, we will focus mainly on the research on biomineralization in marine organisms such as mollusks that could be representative of all the other fields in marine biology (Fig. 1).

Biomineralization is a process by which organisms produce inorganic minerals (biominerals) under the control of organic macromolecules. Biomineralized structures offer excellent protection to the organisms from the external environment and predators. Although the biomineralized structures are made of 95% inorganic minerals, the 5% of organic molecules make it 3000 times fracture resistant than naturally occurring inorganic crystals (Currey, 1977). The organic molecules are secreted by specialized cells lining the biomineralized structures. Studying these organic molecules, including proteins, is critical in conservation and biomimetic material.

Proteomics is the study that identifies and quantifies all the proteins in the sample. In mass spectrometry-based proteomics, proteins are identified based on their mass and charge. Proteomics on the mollusk shell became popular in the past few decades due to the composite structures formed by fabricating proteins, polysaccharides, and calcium carbonate crystals (Arivalagan et al., 2017). However, most studies still use primitive biochemical studies and mass spectrometers, resulting in incomplete data. The current article aims to unravel the recent trends in proteomics and how this could improve the in-depth proteomics analysis.

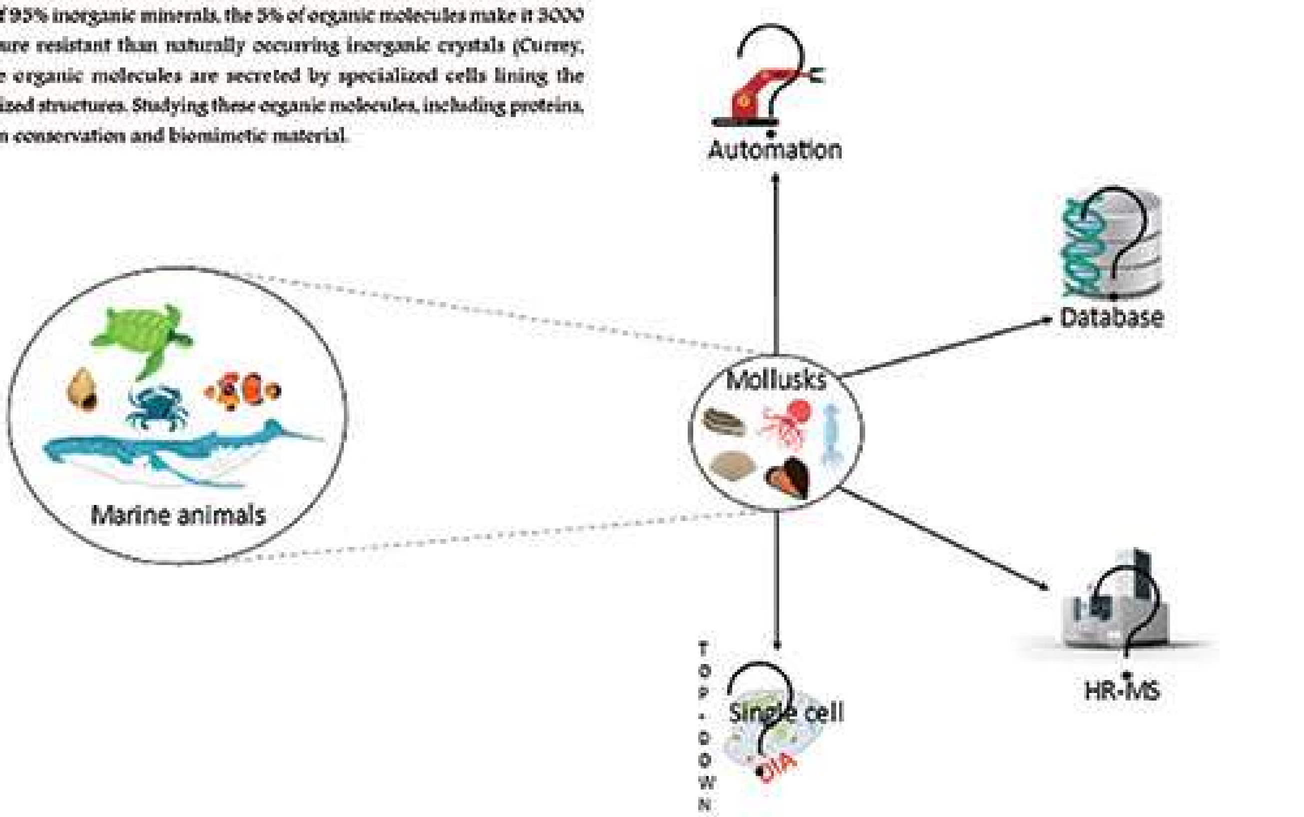

Fig. 1. The cutting-edge technologies and other areas where proteomics is lagging in marine biology and biomineralization

2. Non-model organisms

Most of the proteomics works use the bottom-up approach, where the proteins are digested into peptides and analyzed. The peptides are then matched to proteins using computational tools that rely on proteomic or genomic databases. It is often easier to work with organisms such as yeast, Thale cress, fruit flies, and humans whose genome was completely sequenced (Heck & Neely, 2020). However, non-model organisms were either partially sequenced or not sequenced at all. Post-2015, the new era of genomics started, resulting in an explosion of genomic data, thanks to the continuous drop in the cost of data generation and assembly (Wetterstrand, 2016). Even then, very few marine organisms were sequenced. The genomic database cannot completely solve the issue; annotation plays a significant role in proteomics data search. Although several freely available tools exist, it is a labor and time-consuming task. Only a single work cannot produce a complete genomic database with annotation.

Molluscs is a taxa with only very few species sequenced and annotated. Recent works that aim to identify biomineralization proteins rely on genomic or transcriptome studies on the lining cells that secrete biomineralization proteins (Clark et al., 2020). These studies suffer from incomplete databases and annotations. To understand the complex biomineralization process by marine animals, in-depth proteome profiling is required, which is possible only when the database is complete.

3. High-resolution mass spectrometers and computational tools

It is fascinating that proteomics has advanced greatly from 1D and 2D electrophoresis to advanced tandem high-resolution mass spectrometers (HR-MS) within a decade. In the age of mass spectrometers, it evolved in all aspects, from ionization methods and analyzers to detectors. Current HR-MS is capable of high speed, sensitivity, and accuracy. To give an idea, the recent MS can identify more than 5,000 proteins in a 10-minute gradient.

Most of the research works make use of the HR-MS process clinical samples. They use it for biomarker discovery, drug development, understanding disease mechanisms, etc. Future works on biomineralization should consider using HR-MS, which would result in a complete catalog of proteins and the identification of several isoforms/proteoforms containing post-translational modification.

4. Proteomics workflows

Proteomics always refers to shotgun bottom-up proteomics. However, several complementary workflows exist that could provide more detailed information. To date, proteomics works using Data Dependent Acquisition (DDA) that fragments only the most intense peptides. The low-abundant peptides can be identified only with extensive chromatographic separation, fractionation, or enrichment steps. In data-independent acquisition (DIA), all the peptides were fragmented, resulting in less biased complete analysis (Gillet et al., 2012).

Similarly, the Top-down proteomics workflow complements the bottom-up, where the intact protein is directly analyzed in the mass spectrometer. It has a significant advantage over bottom-up in characterizing isoforms containing PTMs. Little to no DIA or top-down work has been carried out so far. These works might result in identifying new candidate proteins and isoforms in biomineralization, which would aid in understanding the biomineralization process better.

The recent greater leap in proteomics is single-cell proteomics. The HR-MS has advanced to the level of cataloging proteins from a single cell. Few instruments can precisely separate single cells and dispense nanoliters, making single-cell proteomics possible. Applying this workflow to epithelial cells lining the mollusk shells would provide valuable insight into the mechanism of the biomineralization process.

5. Automation and other advanced instrumentations

Sample preparation is the most critical step in any proteomics experiment. Most of the proteomics sample preparation involves enzymatic digestion that usually consumes several hours to days, slowing the overall workflow. Moreover, hundreds of sample preparations can easily introduce human error, leading to analytical inaccuracy (Fu et al., 2023). Recent advancements in instrumentation and automation minimizes sample handling error and fastens the sample preparation efficiently, facilitating the high throughput in the sample analysis. Several instruments, such as AssayMap Bravo, Andrew+, Proteograph, etc., offer sample preparation from enrichment, denaturation, reduction, alkylation, and digestion till sample cleanup. With minimal programming, the automation can be taken to a level where human interference is not needed until result interpretation and manuscript preparation.

In marine biology research, especially in biomineralization, sample preparation is tedious, including demineralization and matrix separation. Also, there are still many questions to answer, including proteins specific to shell species, phenotype, region, and microstructure, which might demand huge sample preparation. Adding automation would enable high throughput sample preparation and analysis.

The important part of advanced mass spectrometers, workflows, and automation is the cost. Huge money is not only needed to build such infrastructure but also to analyze huge sets of samples. The pharmaceutical industry is one of the sectors that can spend several billion dollars each year on research and development. Hence, most of the above-mentioned cutting-edge technologies can be accessed only by the pharma industries. Exploring the ocean also has provided more valuable products such as antibiotics, anti-cancer, and anti-inflammatory substances. In the future, marine biology must be provided with access to cutting-edge proteomics techniques that would enable high throughput sample analysis, leading to the discovery of novel compounds. Biomineralization can lead to the discovery of complex mechanisms operating and biomimetic materials.

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COPEFLOC TECHNOLOGY : A sustainable Aquamimcry Concept for Aquaculture

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Abstract

Copefloc technology is a novel approach in aquaculture that emulates natural ecosystems to generate copepod zooplankton blooms and beneficial microorganisms. Like biofloc systems, this technology offers sustainable shrimp and fish farming benefits by reducing production costs, improving water quality, and providing excellent nutrition. The technology involves adding probiotics to carbon sources like fermented rice bran, resulting in phytoplankton and copepod blooms. Copefloc is adaptable to semi-intensive and intensive aquaculture systems and provides numerous advantages, including reduced feeding frequency, lower production costs, improved water quality, disease resistance, and enhanced product quality. While it has many merits, its effectiveness in indoor culture systems is limited. In summary, cope-floc technology offers a sustainable and natural approach to aquaculture, paving the way for organic and pellet-free practices

Fig. 1. Microscopic image of copepod

Introduction

Copefloc technology is a recent concept in shrimp farming that aims to mimic the environment of a natural estuary by producing copepod zooplankton blooms and beneficial microorganisms that serve as live food and improve water quality in shrimp culture. Although this technology is similar to biofloc technology in some aspects, the amount of carbon absorbed is lower and less dependent on nitrogen input ratios. This article highlights the process and development of copefloc, merits and demerits of the technology.

Photographs of Copefloc bloom in the field

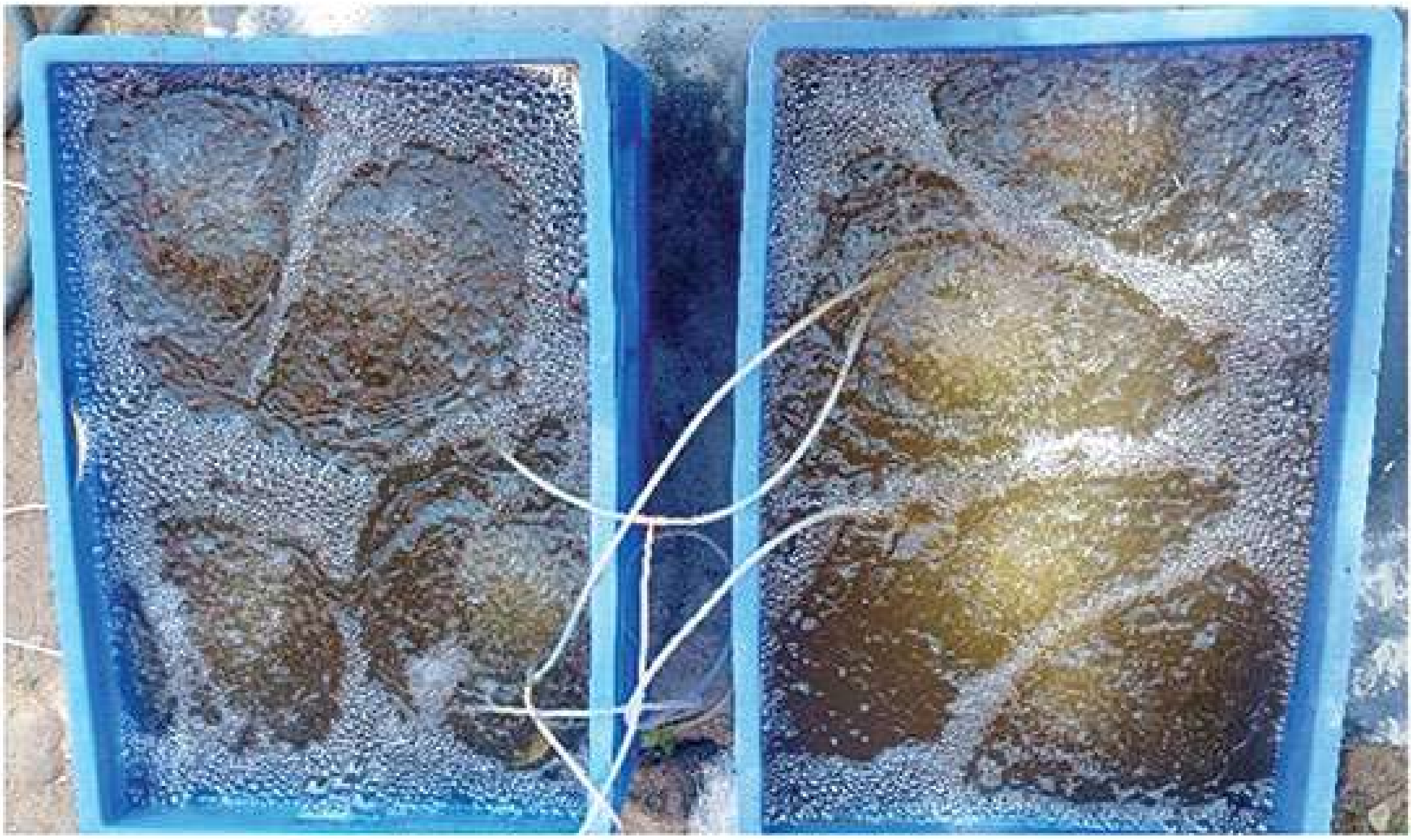

Fig. 2. Microscopic image of copepod

What is Copefloc ?

Copefloc technology is based on the aquamimcry concept, a novel idea first developed in Thailand. It involves *in-situ* waste absorption that resembles a natural ecosystem to generate zooplankton (copepod) blooms (**Fig. 1**) and promote the growth of beneficial bacteria to be made available that serve as an excellent source of nutrition for fish and shrimp in farming practices.

How to develop a copefloc system ?

Copefloc is produced by adding probiotics to a carbon source such as fermented rice bran (FRB) or wheat bran (FWB) to develop phytoplankton and zooplankton blooms. Fermented rice bran (FRB) is produced by adding probiotics to finely powdered rice bran and water at a ratio of 1.5 to 1.10 and allowed to ferment overnight. The fermentation process aids in the breaking down of anti-nutritional factors like fibre and phytic acid from the rice bran, making it favourable for the growth of copefloc. Rice bran, being a prebiotic source, combines with the probiotic added to establish a symbiotic effect. For the production of copefloc bloom in the fish and shrimp culture ponds (**Fig. 2**).

FRB can be applied at a rate of 500-1000 kg/ha. Within a week of application, the colour of the water turns golden brown, which indicates copefloc development in the system. To maintain copepod blooms, FRB can be incorporated every month at a rate of 10 kg/ha, along with probiotics to maintain the water quality.

Types of aquaculture systems suitable for the adoption of copefloc technology

Copefloc technology is suitable for semi-intensive and intensive fish and shrimp farming systems. A stocking density of 10 nos/m2 may be followed in fish farming. In shrimp farming, post larvae (PL 12-15) can be stocked at a density of 30-40 nos/m^2. FRB may be added at a dose of 1 ppm when the water turbidity is 30-40 cm (Secchi disc reading). In an experimental copefloc-based *Penaeus vannamei* culture, with a stocking density of 40 PL/m^2, a yield of 5.53 tons with a survival rate of 94% (CIBA 2021)

Important features of the copefloc system

Copepods are an excellent live feed for fish and shrimp as they are rich sources of protein, lipids, carbohydrates, and enzymes (exonuclease, esterase, protease, and amylase exonuclease, which are necessary for growth, larval survival, digestion, and metamorphosis. Furthermore, it has been noted that the copepods contain significant levels of carotenoids, peptides, free amino acids (such as taurine), vitamins, and minerals, including selenium, iodine, copper, and manganese, which provides a characteristic red colour, increasing the market potentiality. In addition, copepods can withstand a wide range of environmental fluctuations. To retain the zooplankton as a feed and to create minimal biofloc of <25 mL/L (Imhoff cone reading) FRB at a rate of 1ppm/day throughout the culture period (**Fig. 3**). The copefloc development not only meets the nutritional requirements of the cultured fishes but also lowers the feeding interval from thrice to twice a day and proportionate costs involved for the feed which generally constitutes about 50-60% of the production costs in intensive farming practices.

Benefits

Copefloc technology is suitable for adoption in fish and shrimp culture practices due to the following benefits.

- The overall production costs for the fish/shrimp culture are reduced as there is no exogenous feeding.
- Copefloc technology does not require any filtration system, and the water exchange is minimized as excess sediment is removed.
- The water quality issues are reduced due to pollution in the pond bottom, viz., black soil formation and odour related to overfeeding and high protein feed.
- Disease incidence is reduced as live feed forms are considered nutrient capsules rich in vitamins that are known to improve the overall health disease resistance in fish/ shrimp at a higher risk in the intensive culture systems.
- Copefloc also improves the colour and attractability of cultured fish and shrimp as they are rich in amino acids, polyunsaturated fatty acids, and astaxanthin, imparting red to the skin and flesh.

However, implementing this concept in indoor culture systems is less effective.

Conclusion

Owing to the various advantages over traditional farming practices, copefloc technology is more sustainable for developing organic shrimps by incorporating natural inputs eliminating the use of any hazardous chemicals or antibiotics to yield nutritious and safe products to the consumers. Copefloc technology will also pave the way for pellet-free aquaculture practices, increasing its sustainability.

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FAITT PARTICIPATED IN THE LDF INDIA EXPO HELD ON 21-23 OF SEPTEMBER, 2023 AT HITEX EXHIBITION CENTER, HYDERABAD IN COLLABORATION WITH ITS ASSOCIATE COMPANY CRESCO ALGAE FORT, WHICH IS INVOLVED IN SPIRULINA PRODUCTION. THIS EVENT MARKS A MOMENTOUS MILESTONE FOR FAITT, A NON-PROFIT ORGANIZATION COMMITTED TO FOSTERING ADVANCEMENTS IN THE AQUACULTURE SECTOR THROUGH TECHNOLOGICAL INNOVATIONS FROM DOMESTIC TO GLOBAL. IN THE EXHIBITION FAITT & CRESCO SHOWCASED THEIR SPIRULINA PRODUCTS AND SUPPLIED ENERGY JUICE MADE UP OF SPIRULINA AND OTHER TRADITIONAL INGREDIENTS. SPIRULINA JUICE HAVE RECEIVED GOOD APPRECIATIONS FROM THE VISITORS AND OTHER PARTICIPANTS OF THE EVENT.

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