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AQUAMIMICRY AN EMERGING AND INNOVATIVE TOOL FOR AQUACULTURE: A REVIEW

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ABSTRACT:

In the present world, aquaculture is an expanding industry which mainly concerned with feeding and maintaining culture under controlled conditions. As in different culture areas due to water quality deterioration, pathogen spread and breakouts owing to farm runoff have caused immense financial losses to the farmers. To overcome these problems associated with aquaculture, aquamimicry technique usually employed that leads to improved production and follows the goals of sustainable aquaculture. This technique is considered as environment friendly, and also cost-benefit ratio to support economic and social sustainability as it leads to increased production without a significant increase in the use of natural resources (water and land). Additional feeding is maintaining at low cost by using fermented rice bran with suitable probiotic that supports to produce bloom of zooplankton, which further helps to maintain water quality and plankton diversity. Among zooplanktons, copepods act as dominant species replace other zooplankton species indicating the maturity of the system. Microbial population present in the shrimps' gut and surrounding parts act as an essential constituent of aquamimicry system that also creates an ecological model system for prevention of disease outbreaks during culture. Naturally, fed culture species in this system may have high immunocompetence and resistance against several diseases. Shrimp produced in this system are organic and do not contain any harmful chemicals or antibiotics.

KEYWORDS: zooplankton, aquamimicry, probiotics, antibiotics, disease

INTRODUCTION:

In the aquaculture industry, the production of aquatic species with environmental concerns, and various organic technologies must be established (Kumar et al. 2018). In the last few years, new technological inventions have been implemented to minimize harmful environmental effects, increase biosecurity and consequently reduce the production costs of shrimp. Moreover, there are several approaches to managing wastewater and its treatment such as bioaugmentation technology, high level of algal pond system, use of nanomaterials (like nanofiber membranes, nanoadsorbents, polymeric nanoadsorbents), aerobic fermentation for

retrieval of nutrients from waste and different intensive water technologies like biofloc technology (Iber and Kasan, 2021).

The biofloc and aquamimicry methods are considered as effective for the production of shrimp and fish in aquaculture industry (Khanjani and Sharifinia, 2020; Santhanam et al. 2020). Among these, aquamimicry is an innovative tool to efficiently provide a sustainable challenged to the shrimp farming industry. Aquaculture mimicry, also called as aquamimicry is a revolutionary idea that includes mimicking natural estuarine conditions by creating zooplankton diversity (mainly copepods) as an additional nutrition to the cultured shrimp and also promote the beneficial bacteria in culture water (Puja *et al.*, 2018). In this method/technology, a fermented carbon source such as rice bran is used with some probiotics that create phytoplankton and zooplankton population and mimic natural pond conditions. This technology mimics the same conditions as present in pond culture and synergistically works to produce food organisms as present under natural conditions. This technology is predominantly meant to encourage the growth of copepods which are considered as predominant zooplankton species and act as food for culture species. These planktons are considered as supplemental nutrition and improve water quality in fish and shrimp cultures.

Water present in culture tanks primarily mimics the appearance and composition of water that includes microalgae and zooplankton. Ideally, the various zooplankton and microalgae present in cultural water show resemblance with natural estuarine as plankton diversity create or act as a source of food and important nutrient recyclers (Christeson, 2016). Due to this balancing, various physico-chemical fluctuations like pH and dissolved oxygen are minimized so, there is no need for chemicals and antibiotics because rice bran act as a prebiotic source that provides nutrition for the zooplankton and bacteria to create “synbiotics,” which are essentially dietary supplements or constituents that synergistically combine pre-and probiotics. For high stocking intensive culture, the idea of aquamimicry can also be adopted as in this case, a central drainage system is developed from a grow-out pond to a sedimentation pond.

HISTORY:

Sutee Prasertmark and Veerasan Prayotamornkul were two shrimp farmers in Thailand who established this aquamimicry technique in 2013. It was based on the hypothesis that these farming practices are more workable by following the same conditions as present in the natural aquatic environment. The main idea behind this practice occurred in Thailand, during the disease outbreaks in the 1990s. At that time, it was found out that in some extensive shrimp ponds, they were growing healthy and disease-free. As the farmers, had limited resources available, so using these practices includes no extra formulation of feed. As a substitute for this, only rice bran was used because of its impending reason for the enhanced performance in the pond ecosystem. Over

time, this protocol gradually developed after extensive trial and error. Using this concept, many farmers outside Thailand, try this concept in their worst-performing ponds.

Pond preparation

A filter bag of size around 200-300 μm is usually filled up to a depth of around 80-100 cm, a suitable probiotic like *Bacillus* sp. was added and the pond is chain-dragged for seven days. Lined ponds with heavy ropes are used to minimize tearing the liner. To avoid the development of biofilms that mainly cause toxicity to shrimp culture, a gentle dragging is necessary to promote the mixing of probiotics with the soil. Farmers usually add tea seed cake (20 ppm) along with rice bran or wheat bran in the fermented form at 50-100 ppm. Excessive addition of these supplements results in more copepod blooms, which should happen within two weeks. Whereas, for proper mixing of tea seed cake, probiotics and nutrient full aeration must be maintained in the pond.

Grow-out, Sedimentation and bio filter ponds

In the grow-out pond water circulation is usually maintained through the eight long-arm paddlewheels (3-hp at 85 rpm) which are arranged as such to promote better circulation around the pond for solids to concentrate in the center. Different fishes like catfish and milkfish are cultured in these ponds, which mix the detritus and subsequently promote the oligochaete worms which are rich in essential amino acids like methionine and lysine that can be eaten by fishes. (Vijayan, 2019).

To maintain water quality in grow-out ponds, additional probiotics should be added every month. Sediments present in this pond encourage the production of worms and other benthic invertebrates that the fish can consume. Meanwhile, if ropes or lines are present, these are frequently and strongly colonized by horse mussels. Further for the accumulation of sediments in the sedimentation pond, the depth of the pond should be 4-m in the center and 2-m on the edges. In this pond. Fishes should be stocked at low density, mainly bottom-dwelling fishes (catfish, milkfish) are reared depending upon the salinity of the water. Their feeding on and stirring up the debris help clean the pond ecosystem, ultimately fish can provide food for farm workers. From the sedimentation pond, water overflows to another pond i.e. bio-filter pond, to increase the retention time. In this pond, fishes like tilapia can be added at low density. From bio-filter pond water overflows back to grow-out pond with little or no nitrogenous waste material. After every three years, sedimentation pond should be properly cleaned.

Currently, the ratio of these ponds is 1:1 (treatment to grow-out ponds), which obviously requires relatively large areas of land in relation to production. However, trials are currently in proceeding to substantially diminish this ratio by regulating water flows, carbon inputs, and different combinations of live organisms in the treatment ponds.

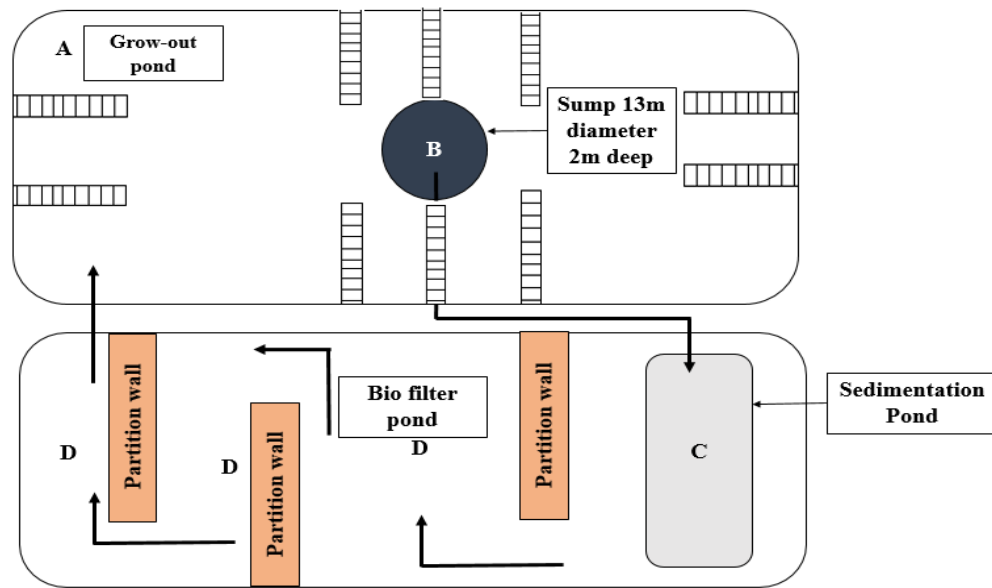


Figure 1: Basic layout of different pond systems in farm pond where aquamimicry system was accepted for intensively culturing species

Carbon source preparation and use

In aquamimicry, a carbon source that is used mainly is fermented rice bran (FRB) with some probiotics that create phytoplankton and zooplankton blooms and simulate natural pond conditions. These planktons play an important role in improving water quality and act as supplemental nutrition in fish and shrimp cultures (Vijayan, 2019). The composite carbon source, such as rice or wheat bran (without husk), is mixed with water (1:5-10 ratio) and probiotics under aeration for 24 hours.

When the bran is in finely powdered form, the entire mixture is added slowly to the pond. If crumbled, the supernatant milk/juice is added to the pond and the bran solids are fed to fish in the biofilter pond. According to Romano (Stottrup, 2006), FRB is made by adding water, some hydrolyzing enzymes and probiotics to rice bran powder and is then allowed to soak overnight. The pH of the incubation water should be between 6-7 and adjusted if necessary. The prepared mixture is allowed to ferment for 24 h and then added to cultured ponds at the rate of 500–1,000 kg ha⁻¹ (Romano, 2017). After shrimp stocking, which can be at densities of 30-100 animals/square meter, the amount of fermented rice bran to be added depends on turbidity level and system. In general, for extensive farming 1ppm is recommended, while for the intensive system, 2-4 ppm is used. By using a Secchi disk, a turbidity level measure, which should be around 30-40 cm that is ideal for shrimp culture. If higher than this, less bran should be added and vice versa. During the grow-out period, additional probiotics should be added each month to help maintain water quality and to promote the formation of biocolloids (flocs composed of detritus, zooplankton, bacteria, etc.).

PROCESS:

This process can be initiated by the addition of rice bran with probiotics and kept for fermentation for around 24 h. Fermented rice bran at the rate of 500-1000 Kg/ha will be supplemented to the pond for the proliferation of dominant zooplankton species i.e. copepods and after one-week post-larvae will be stocked of around 10-20 individuals/m². Zooplankton diversity can be maintained as a feed by the addition of fermented rice bran at the rate of 1ppm during early stocking and also for creating minor biofloc (< 25 mL/L as measured by an Imhoff cone). Later for feeding, fermented soybean meal will be used which completely reduced the feed cost. Additional development of biofloc will be very much helpful to fulfill the nutritional requirement and thus reduce the feed cost (Chakravarty *et al.*, 2018; Romano, 2017).

As a natural food for shrimp

Due to abundance of natural planktonic communities in this system, which mimics the natural estuary conditions that helps in encouraging and balancing the growth of culture species. Commonly, shrimps were fed on natural feed present in water resources, such as zooplanktons, phytoplanktons, and artemia (Romano, 2017). In this system, prebiotic sources derived from fermented carbon (e.g., rice bran) and *Bacillus* sp. act as probiotic sources, both these act synergistically and maintain natural conditions by creating plankton's diversity, especially copepods. These live foods can be used as balancing foods for shrimp culture. Furthermore, beneficial bacterial species present in this system can provide suitable culture conditions for the growth and welfare of shrimp. Raising species in this pond usually do not feed directly on phytoplankton, in fact they consume tiny organisms and bacteria that eat these phytoplanktons.

This culture of planktons and biofloc components help in balancing the water quality and environment ideally resembles a natural estuary conditions. As a result, consumption of feed and water exchange can be minimized. This technology is evidenced best for waste utilization, as with the constant use of carbon sources and probiotics, planktonic growth can be sustained.

Biofloc vs aquamimicry

This technology is more or less similar to biofloc technology except few differences like amount of carbon source and the dominated communities are zooplanktons like copepod that will serve as additional nutrition to the cultured shrimp or fish and less feed input compare to bio floc technology. In biofloc system, for maintaining the floc volume, C:N ratio 15:1 should be fixed with proper aeration (Poh, 2014). Supplementary feeding should be done via addition of carbonaceous substrate which helps in proliferation of heterotrophic bacteria after maturation of water. On the other hand, in case of aquamimicry after stocking of culture species, additional carbon source should be added dependent on the level of intensification (extensive or intensive) and turbidity of water. Furthermore, this technology can be beneficial for both extensive and intensive system with the limited supply of aeration, whereas in biofloc technology, proper

aeration is required to maintain suspended particles in the water and used in intensive culture system. Feed accounts for more than half the production cost in most aquaculture practices and in aquamimicry (Deepak *et al.*, 2020). Despite this, biofloc technology has its own limitations that includes usually high aeration in order to maintain suspended particles in culture water, high infrastructure and installation expenditures, great energy consumption, high carbon footprint, and the complicated management of total suspended solids in the system (Gaona *et al.*, 2016). Compared to the BFT, aquamimicry is a new shrimp farming method that can be used to overcome these limitations by modulating the conventional methods.

Nutritional value of culture species

Ideally, the water quality and its diversity in this system mimics the appearance and composition same as present in natural estuarine water that includes microalgae and zooplankton. This concept mainly applies in aquaculture as an alternative source of protein, therefore it helps in decreasing the anti-nutritional factors (ANF) and increasing the nutritional value of food and cereals in addition to feeding formulation (Dawood and Koshio, 2016). Thus zooplanktons presence, especially copepods that are highly rich in minerals, long chain polyunsaturated fatty acids, trace elements, group of free amino acids (e.g. taurine) and pigments that makes shrimp's immunocompetence and also improve their feeding efficiency (Conceicao *et al.*, 2010; Biesebeke, 2018; Butto and Haller, 2016). Usually, the shrimp produces by this technology are highly rich in astaxanthin, that is responsible for red colouring in shrimps, which also increase the market value of shrimp called as organic shrimp. Shrimp culture via this method is appearing red in colour when cooked, likely from the consumption of natural foods which include the marketing value as organic shrimp (Romano and Kumar, 2017). In this context, the effect of probiotic i.e *Bacillus subtilis* with fermented rice bran on water quality, antioxidant activity performance and immunity of *L. vannamei* in different salinities conditions with zero water exchange system was also found to be improved as present under natural conditions (Abdel-Tawwab *et al.*, 2022). Another study also revealed that, the biochemical composition of rice bran after fermentation was also enhanced i.e 16.79%, 14.92%, 17.36% and 50.94% for protein, fat, ash and total carbohydrates, respectively (Albuquerque, 2019). Initially, rice bran was used in the culture method but later it was replaced by standardized fermented rice having higher effectiveness. Because using rice bran as a fermented medium increases the availability of nutrients and also considered as low cost and low-risk process. According to different studies, it was found out that rice bran is highly rich in nutrient but at the same time it contains higher fiber contents (Law, 1984). Therefore, by using the fermentation method, lipid, ash, fiber, and phytic acid were successfully removed from rice bran, and the technique was standardized (Flores-Miranda, 2014).

Zooplankton diversity

In the aquaculture industry, for successful farm management, it is essential to sustain natural feed and water quality into a culturable pond. Microscopic creature present in pond ecosystem forms the basis of food chains and food webs in all aquatic system. Zooplankton species which mainly feed on phytoplanktons and are directly correlated with the growth of aquatic species, especially prawns and shrimp. These forms of zooplanktons are motile and distributed themselves in upper and lower strata of culture water. Zooplankton mainly serve as food supplement for carnivorous and omnivorous fishes (Alam *et al.*, 1987). Larval stages of some fishes (carp) and shrimps feed mostly on zooplankton because they provide necessary amount of protein required for growth, survival and the development of organs especially gonads of fish (Bardach *et al.*, 1972). The zooplankton contributes about 47% of food items for *Catla catla* while 23% for the shrimp. Their population shows or affects the culture system by maintaining water quality via making a balance between O₂ and CO₂, proper oxygen levels, and augmentation of organic substances accumulated in ponds. Due to this balancing, various parameters like pH regulation, preventing the development of microalgae and pests, and stabilizing water temperature (Das and Bhuyan, 1974). The association amongst the physico-chemical parameters and plankton development of pond water and their relation with monthly fluctuations of zooplankton are of great importance and basically very much essential in case of fish culture and fisheries management. In aquamimicry, the production system contains the beneficial zooplanktons and microalgae that are produced in the water, and the system mimics natural estuarine waters. The system does not necessitate the use of any chemical or antibiotics, since rice bran itself provides nutrition to the zooplanktons, and the bacteria present are responsible for the development of pre-and probiotics creating a healthy environment. The abundance and diversity of plankton also affect the survival and growth rate of cultured fish.

Application of probiotics in aquamimicry system

The growth of aquaculture as an industry has accelerated over the past decades, this has resulted in environmental damages and low productivity of various crops. The need for increased disease resistance, growth of aquatic organisms and feed efficiency has brought about the use of probiotics in aquaculture practices. In this system, suitable carbon ingredients (energy source) along with probiotics, which mainly consume unutilized nitrogen, generated through feed and excreta and finally converted to floc and help to maintain the population of copepod as a source of proteinaceous feed (Deepak *et al.*, 2020).

Prebiotics constitute a carbon source on which probiotics can grow. Within a week of the application of this mixture, the dominance of copepods bloom occurs. The first use of probiotics occurred in 1986, to evaluate their capacity to increase the growth of hydrobionts (organisms solely live in water bodies). After that, probiotics were used against bacterial

infections and to improve water quality in pond system. Now a day, there is predictable evidences that these probiotics can enhance the reproduction rate, nutrient digestibility and increase tolerance to stress conditions. Presently, there are commercially available probiotics products prepared from several bacterial species such as *Carnobacterium* sp., *Lactococcus* sp., *Bacillus* sp., *Enterococcus* sp., and yeast *Saccharomyces cerevisiae* among others, and their use is regulated by careful management recommendations (Martinez cruz *et al.*, 2012).

The use of probiotics in modern aquaculture is increasingly rising, which in addition to helping to preserve the guts' microbiota (Romero *et al.*, 2014) helps to promote the nutritional value of plant feedstuffs through fermentation (Van Nguyen *et al.*, 2018). Their use in aquaculture systems such as the BFT and aquamimicry also improves the zootechnical process of the system (De Melo *et al.*, 2015). Probiotics are living microorganisms that are added to the aquaculture environment through food or water to exert beneficial effects on the host health and its growth performance by modulating the guts' microflora (Balcazar *et al.*, 2006; Merrifield *et al.*, 2010; Dawood *et al.*, 2018; Vidal *et al.*, 2018). The use of probiotics in aquaculture is a suitable alternative to therapeutics for controlling occurrences of infectious diseases and they can contribute to improve water quality (Liu *et al.*, 2010; Jahangiri and Esteban, 2018; Biswas *et al.*, 2019; Nathanailides *et al.*, 2021). Probiotics produce enzymes that limit the activity of pathogenic bacteria by reducing the gut's pH, making it difficult for these bacteria to live at low pH (Martinez Cruz *et al.*, 2014). In addition, these probiotics by quorum quenching mechanism can interfere the quorum sensing among opportunistic bacteria

In addition, probiotics through quorum quenching mechanism can disturb quorum sensing among opportunistic bacteria that regulate virulence factors, and the formation of biofilms, which are the critical parameters in the occurrence of infectious diseases in farmed aquatic animals (Subramani and Jayaprakashvel, 2019). Limiting the pathogenic bacteria in the culture system can provide a better environment for the proliferation of beneficial bacterial species that helps to improve digestion and absorption of nutrients (Lara-Flores, 2011). Some spore-forming *Bacillus* species belonging to gram-negative bacteria act as suitable probiotics for use in aquaculture and can easily be stored at room temperature without affecting their normal biological function. Specific probiotics, including *B. subtilis*, produce essential vitamins such as vitamin B1 and vitamin B12 (Ghosh *et al.*, 2007). Therefore, these probiotics are increasingly used to enhance growth, and resistance to pathogenic microbes in farmed shrimp.

Role of copepods in aquamimicry technology

In both intensive and extensive fish culture systems, relationships between fishes and crustaceans representing the subphylum Copepoda must be controlled to maximize fish production. Aquamimicry technology is also known as usually based on production of copepods under natural conditions, which is generally eaten by shrimp species present in pond ecosystem.

This method mainly negates the use of rigorous mixing, oxygenation and any external feed source present in the culture system (Romano and Kumar, 2017). Copepods are crustaceans that are commonly used due to their small size and short life cycle, which act as a food material for marine animals, nutrient recycling and energy conversion in food chain (Radhakrishnan *et al.*, 2019; Chakravarty *et al.*, 2018). They usually richer in LC-PUFA including dicosahexanoic, arachidonic and eicosapentaenic acids, which are necessary for growth and development of culture species and have higher nutritional value than rotifers and artemia species (Wilcox *et al.*, 2006; Satoh *et al.*, 2009). In addition, it has been observed that the copepods also contain high amounts of carotenoids, peptides, free amino acids (e.g., taurine), vitamins and minerals like selenium, iodine, copper and manganese) (Karlsen *et al.*, 2015; Taher *et al.*, 2017).

The marine copepods are considered to be “nutritionally superior live feeds” for commercially important cultivable species, as they are a valuable source of protein, lipid, carbohydrates and enzymes (amylase, protease, exonuclease and esterase), which are essential for larval survival, growth, digestion and metamorphosis (Stottrup, 2000; Kleppel *et al.*, 2005). Copepods are known to have greater digestibility (Schippe *et al.*, 1999) and a relatively high weight-specific caloric content (Sun and Fleeger, 1995). In addition to their nutritional and physical superiority as live feed, copepods are also highly suitable for culture because of their eurythermal and euryhaline characteristics, which allow them to tolerate wide environmental fluctuations (Carli *et al.*, 1995).

Copepods play major roles in pond ecosystems, serving as 1) food for small fish, 2) micropredators of fish and other organisms, 3) fish parasites, 4) intermediate hosts of fish parasites and 5) hosts and vectors of human diseases. These copepods are very advantageous in the food chain and act as the perfect source of food and nutrient recyclers in marine ecosystem. The orders harpacticoid, calanoid, and cyclopoid are the three major candidate species of copepods for aquaculture production and usually dwell in fresh, marine and estuarine environments. The advantage of harpacticoids over other rotifers is that they can grow at high densities. Cyclopoid copepods are rarely used in aquaculture, and a density of ~5,000/L is possible to achieve in cultures. Environmental factors such as light, salinity, temperature and available nutrients can affect the abundance and the diversity of phytoplankton and zooplankton species in this system. Abbaszadeh *et al.*, (2022) reported that the inclusion of copepod (*Calanopia elliptica*, 0.2 organisms mL⁻¹) in a BFT nursery system remarkably improved growth, immune parameters and enhanced feed efficiency in *L. vannamei*.

CONCLUSION:

The recent major issue in the world is ever-increasing seafood demand, cultivable land resources, and water scarcity. To overcome all these problems, the preeminent way out is

intensive sustainable aquaculture. In the recent years, Aquamimicry is considered as new concept that creates natural estuarine conditions by increasing copepods that can further act as a supplementary food to a cultured species. It is believed that, using this balanced approach in aquaculture by enhancing both biofloc and microalgae farmers could increase more production of cultivable species. The concept is greatest known for reducing the stress associated with fluctuating water quality, and it minimizes favourable environmental conditions to pathogens. Adopting aquamimicry in shrimp farming offers more sustainability than conventional farming.

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