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Review Article

Polyculture of Genetically Improved Farmed Tilapia (GIF Tilapia) and *Penaeus vannamei* using Biofloc Technology—A Review

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ABSTRACT

According to Food and Agriculture Organization of the United Nations (FAO), aquaculture has grown faster and its expansion aimed at meeting the increase of world fish demand, and preserving natural fish stocks. Currently, to produce fish in quantity and quality requires reduction of the environmental impact from aquaculture, through the improvement of culture systems. Disease is the major factor affecting the development and expansion in aquaculture. Losses due to disease in shrimp farming are high. Various approaches to minimize the impact of disease on production are possible. Another approach to keep the pathogen pressure low is polyculture of shrimp and finfish. This practice makes shrimp farming more sustainable by reducing the environmental impact and the incidence of shrimp disease. Antimicrobial peptides in the fish skin kill shrimp pathogens, keeping pathogen pressure of bacteria and viruses low. In polyculture, shrimps can eat tilapia faeces and unused fish feed, while tilapia filter phytoplankton, reducing the risk of low dissolved oxygen levels at night. In addition, shrimp bioturbation at the pond bottom returns nutrients to the water column, enhancing phytoplankton production and consequently the natural feed available for the tilapia. Biofloc technology (BFT) is one of the most applicable and promising systems for sustainable aquaculture development. This technology is essentially based on the recycling of nutrients via microorganisms, primarily (i) heterotrophic bacteria, which convert nitrogen compounds into microbial biomass, in addition to serves as a source of food for aquatic organisms, and (ii) chemoautotrophic bacteria, which convert ammonia to nitrite and nitrate.

KEYWORDS: Aquaculture, biofloc, fisheries, polyculture, sustainability

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1. INTRODUCTION

A quaculture is one of the fastest growing sectors of animal protein production in the world, contributing to the global food security and nutrition in the twenty-first century. Fisheries and aquaculture not only provide livelihood, but also offer dietary essentials for human consumption (Ngasotter et al., 2020). India ranks second in the world for aquaculture production due to its abundance of fisheries resources, making it the third-largest fish-producing nation globally with 7.96% of total production.

Aquaculture animals usually retain 20-25% of protein in the feed, while the rest discharged as ammonia and organic nitrogen in feed residues and excrement (Crab et al., 2007; Piedrahita, 2003), resulting in water deterioration disease outbreaks and heavy financial loss (Samocha et al., 2004; Avnimelech, 2006; Azim and Little, 2008). Another major constraint in the aquaculture industry is to find alternative primary sources of fish meal and fish oil as important and expensive valuable protein and oil sources in the aquaculture-formulated diet (Zhu et al., 2010). Therefore, alternative strategies should be investigated to make the aquaculture industry more sustainable and profitable. To overcome the environmental damage and increase sustainable aquaculture production (Avnimelech, 2009), one of the promising technologies that developed was an ecofriendly culture technology known as biofloc technology (Martínez-Córdova et al., 2017).

The interactions between aquatic farmed organisms in polyculture are mostly determined by the species' biological traits and the stocking densities employed. Improvements in food availability and environmental conditions are major synergistic interactions (Milstein, 1992) in polyculture. Conversely, agonistic behavior and competition for food, space, oxygen, transmission of pathogens and other resources inside the growout unit are the main antagonistic interactions. Therefore, the secret to a successful polyculture is to use ecologically distinct species with complimentary requirements and to arrange a proper stocking density for each.

Among the various shrimp species, *Penaeus vannamei* was the top species produced in 2020. Shrimp are basically benthic and omnivore feeding on detritus, algal films, and bacteria (D'Abramo and New, 2000). *Penaeus vannamei* has a consolidated and expanding producing chain, due to its high feed conversion ratio, disease resistance and can thrive in varying water conditions. This makes it an economically viable option for farmers as it maximizes production and reduces costs. Polyculture involving *Penaeus vannamei* helps to optimize resource utilization, as different species have different feeding habits and occupy different niches within the ecosystem. This can result in increased productivity,

improved water quality, and enhanced overall sustainability of the aquaculture system.

Tilapia is the widely grown fish on earth after carps, and the most prolific species grown in aquaculture. GIF Tilapia is basically pelagic (Wang and Lu, 2015) and omnivorous, filtering phytoplankton (Perschbacher and Lorio, 1993) and feeding on periphyton (Azim et al., 2004). GIF Tilapia was chosen because of its many desirable attributes including short generation interval, importance in developing countries, hardiness, feeding habits, resistance to diseases and general suitability of farming systems. Tilapia has proven to be ideal species for polyculture (Yakupitiyage et al., 1991).

Shrimp and GIF tilapia match the fundamentals described above, mainly because of diverse feeding habits and spatial distribution; they may be raised in the same pond due to separate environmental niches. In polyculture, shrimp can consume leftover fish food and waste, and fish can filter phytoplankton (Perschbacher and Lorio, 1993), which lowers the danger of low dissolved oxygen levels at night times (Santos and Valenti, 2002). Moreover, shrimp bioturbation at the pond's bottom also recycles nutrients into the water column (Kimpara et al., 2011), which increase the phytoplankton production, a natural food for fish.

2. FISHERIES AND AQUACULTURE SCENARIO

he fisheries and aquaculture sectors have been ▲ increasingly recognized for their essential contribution to global food security and nutrition in the twenty-first century (Verdegem et al., 2023). With 811 million people suffering from hunger and 3 billion people not able to afford healthy diets, the world is far from its proclaimed goal of ending hunger and malnutrition in all its forms by 2030 (Kakaei et al., 2022). Aquaculture not only supplies dietary essentials for human consumption (Jayasankar, 2018), but provide livelihoods to around 820 million people worldwide (Ngasotter et al., 2020). India is bestowed with an abundance of fisheries resources with 3.15 million ha of reservoirs, 2.36 million ha of ponds and tanks as well as 0.19 million ha of rivers and canals (Handbook, 2019; Datta, 2011). India is the third largest fish producing country, contributing 8% to the global fish production and ranks second in aquaculture production. The fisheries and aquaculture production contributes around 1% to India's Gross Domestic Product (GDP) and over 5% to the agricultural GDP. Global aquaculture production retained its growth trend in 2020 amid the worldwide spread of the COVID-19 pandemic. The total aquaculture production comprises 87.5 mt of aquatic animals, 35.1 mt of algae, 700 tonnes of shells and pearls, reaching a total of 122.6 mt in live weight in 2020. This represents an increase of 6.7 mt

from 115.9 mt in 2018. World aquaculture production of animal species grew by 2.7% in 2020 compared with 2019, an all-time low rate of annual growth in over 40 years. In 2020, farmed finfish reached 57.5 mt, including 49.1 mt from inland aquaculture and 8.3 mt from Mariculture in the sea and coastal aquaculture on the shore. Production of other farmed aquatic animal species reached 17.7 mt of molluscs mostly bivalves, 11.2 mt of crustaceans. Global apparent consumption of aquatic foods increased at an average annual rate of 3.0% from 1961 to 2019, a rate almost twice that of annual world population growth (1.6%) for the same period. Per capita consumption of aquatic animal foods grew by about 1.4% year-1, from 9.0 kg in 1961 to 20.2 kg in 2020.

3. PROBLEMS OF AQUACULTURE

quaculture is the culture of aquatic organisms. People **T**have been involved in different forms of aquaculture for thousands of years. Today, the practice of aquaculture spans the globe. Many of the basic goals have not changed significantly in aquaculture: maximizing growth rate and minimizing production cost. A rapid growth rate minimizes the time to achieve a marketable size and decreases risk. The reduction of production costs makes an operation profitable. To accomplish this, there are number of strategies such as maximizing food conversion and reducing water and land use. Farming requires high protein diets to maintain productivity (Attasat et al., 2013). The downside is the production of a large quantity of nitrogenous and phosphorous waste (Yi et al., 2002). Most nutrients are derived from feed of which only 24-37% and 13-28% of nitrogen and phosphorous, respectively are converted into biomass, while the rest was being lost as organic nitrogen and ammonia in faeces and feed residue (Samocha et al., 2004; Lawrence et al., 2001). This leads to water deterioration, disease outbreaks and heavy financial loss (Azim and Little, 2008; Avnimelech, 2006). A high water exchange frequency is needed to maintain a good pond water quality, displacing the waste into receiving ecosystems (Attasat et al., 2013). The environmental impact of untreated effluents has raised concerns about the sustainability of farming. Another important constraint is the increasing pressure to find alternative primary sources of fish meal and fish oil as important and expensive valuable protein and oil sources in an aquaculture formulated diet (Zhu et al., 2010).

4. SOLUTIONS TO MITIGATE THE PROBLEMS OF AQUACULTURE SECTOR

Major difficulties challenged by aquaculture industry are effluent discharge, feed cost and water. To mitigate the above mentioned problems, advanced aquaculture technologies can be adopted. Hence to sustain the production and safeguard the environment, new

technologies like biofloc (Avnimelech, 1999), polyculture (Jhingran, 1975), raceway technology (Felix, 2006), and aerated lined pond (Felix, 2012) can be applied.

4.1. Biofloc technology

Biofloc technology (BFT) is an innovative and sustainable method of fish production technique that has been recently received a lot of attention at global level. It entails the aquaculture system's growth by its dense microbial communities, known as biofloc (Yu et al., 2023). Biofloc is known to prevent accumulation of toxic nitrogen metabolites (NH₃ and NO₂, etc.) by stimulating and manipulating the carbon/nitrogen ratio (C/N) and converting the metabolites into microbial flocs, even in a zero-water exchange system (Avnimelech et al., 1994; Mc Intosh, 2000). The microbial flocs are formed by heterotrophic bacteria, phytoplankton, zooplankton and protozoa (Avnimelech, 2007). Biofloc technology is an economical alternative for use in decreasing the commercial diets of fish, while simultaneously reducing potential environmental problems (Bauer et al., 2012). The use of biofloc technology has several advantages over other conventional fish farming technologies such as better water quality (Megahed, 2010), disease prevention, lower feed costs, and minimized environmental effect (Naylor et al., 2000).

4.2. Polyculture

Polyculture is a traditional fish farming practice; where compatible species with different feeding habits were stocked in a single-pond for grow out practice to effectively increase production (Zimmermann & New, 2000; Jhingran, 1975). The idea of polyculture is based on the principle that each species stocked has its own ecological and feeding niche that doesn't completely overlap with feeding niches of other species. In monoculture practices, the excess nutrients from uneaten food increase the phytoplankton and ammonia concentrations and change the dissolved oxygen dynamics (Midlen and Redding, 1998). Polyculture adds a subordinate species and improves the performance of the cultured species by enhancing water quality (Wang et al., 1998; Tian et al., 2001). Therefore, polyculture fits the principle of sustainable aquaculture; reduces the impact on environment, increase profitability, provides benefits associated with advanced ecological stability and function by optimizing use of available resources (Wohlfarth et al., 1885; McKinnon et al., 2002).

4.3. Raceway culture system

A raceway is a channel with a continuous flow of water constructed for high density aquatic organism production. The aquaculture term "raceway" is a highly generic name and implies little more than a water impoundment with water flowing through it (Stuart et al., 2009). The Romans

and perhaps even earlier cultures-used complex water management systems to effectively create and manage both aquaculture raceway and static pond system (Wyban et al., 1991). They are usually oval tanks (or polygonal tanks with rounded corners as per Taiwanese shrimp industry of the 1980's) of medium depth and whose diameter is a function of the velocity of the water flow (Shigueno, 1975). The water flow velocity is then a compromise between the flow tolerances of the aquaculture species used and the hydrodynamic characteristics of their waste (Costa Pierce and Barry, 2005). Raceway removes the solids and dissolved waste from the culture unit on a continuous basis, which reduces the discharge of effluent into the environment (Rakocy et al., 1997; Mc Millan et al., 2003). Raceway could potentially address the major challenges associated with the aquaculture effluent production.

4.4. Lined pond culture system

Earthen ponds are conventionally used for fish farming with some boundaries like continuous water seepage; complications with soil-water chemistry haven't been understood with difficulties in determining the causes of physio chemical parameters related problems and organic load accretion (Weber et al., 2009). Hence, lining the ponds with High Density Polyethylene (HDPE) sheets has many advantages like reducing seepage, reducing pumping cost, complications due to soil-water interactions can be prevented and for undertaking advanced farming practices are more appropriate (Felix, 2006; Felix and Venkataramani, 2007). The initial investment will be largely compensated by the huge advantages in terms of net profit. Lining materials should be long lasting and cost effective (Felix, 2011).

5. OVERVIEW OF BIOFLOC TECHNOLOGY

Biofloc technology (BFT) is an innovative and sustainable method of fish production technique that has been recently received a lot of attention at global level. It entails the aquaculture system's growth by its dense communities, known as biofloc (Yu et al., 2023). The biofloc system was developed under the same principle that regular waste water treatment plants, in which the microbes grow from faeces of the cultured organisms being transforming it into less complex organic products that can be consumed by other organisms and return to the food chain (Avnimelech and Kochba, 2009).

In aquaculture, the "biofloc" system acts like retention trap for the nutrients in the pond, and reduces maintenance costs because it can be used as food supplement for the commercial organisms being cultured, which provides an added value by improving the food consumption rate (Azim and Little, 2008). To establish the BFT, it is necessary that the system has a cover that prevents the accumulation of

solid organic matter at the bottom, adding carbon source that stimulates the growth of heterotrophic bacteria, and to keep constant aeration in the water column, which will help the combination of physical, chemical and biological factors, required for the floc formation (Emerenciano et al., 2011).

To develop the biofloc, biological polymers substances are required to keep the components together, creating a matrix that encapsulate the cells. This matrix protects the microorganisms from their predators, provides direct access to nutrients and works as substrate (De Schryver et al., 2008). Species biodiversity that inhabit the flocs depends on the microbiota found in the water body; some of them may function as biological control agents against pathogens through competitive exclusion or due to probiotic capabilities (Ray et al., 2010). However, to achieve the establishment of heterotrophic bacteria in the biofloc, it is necessary to adjust the carbon/nitrogen (C:N) relation in the water body, and is required around 15 units of carbon to assimilate one nitrogen unit, this is obtained by adding a food of low protein and one carbohydrate such as soya hull pellet powder in sufficient amount (Avnimelech, 1999; Emerenciano et al., 2012). When this rate is adequate, bacteria that grow inside of the microsystem starts to use compounds that can be toxic to the culture such as organic carbon, ammonia nitrogen, nitrates, nitrites, and phosphates as energy sources, oxidizing them so algae, fungi, and other bacteria and filtering organisms can use them (Avnimelech, 1999; Avnimelech, 2007).

The non-consumed nitrogen by the organisms in the culture can be used to produce microbe protein, instead of generating toxic compounds which also helps controlling toxic inorganic nitrogen, residual food, and the rest of the phytoplankton production will also be broken down into simpler compounds (Avnimelech, 1999). It is very important to note that this process reduces the total amount of dissolved oxygen available for the organisms, so the existence of an adequate concentration of this element in the water becomes very important (Abarzúa et al., 1995; Avnimelech, 1999; Mc Graw, 2003). Proliferation of bacterial colonies and microorganisms generates an increase in the biofloc biomass, this increase must have a density between 10 and 15 ml so the system can keep functioning properly (Avnimelech, 1999; De Schryver et al., 2008; Emerenciano et al., 2011).

Nevertheless, uses of biofloc technology for sustainable development in aquaculture are critical because of the essential role of microorganisms in the establishment and control of ecosystem facilities, especially nutrient cycling, water quality control, and disease regulation in the culture system (Timmis et al., 2017). Although, several microbial biotechnologies have been applied or are still

in the developmental pipeline to increase the productivity in aquaculture by creating the ecofriendly environment to support the growth of other aquatic organisms. These organisms are key agents of pollutant removal and recycling (Bossier and Ekasari, 2017; Liu et al., 2019). BFT is one of such novel microbial biotechnologies that have been developed with an excellent ecofriendly technology not only for higher productivity but also for sustainable development (Emerenciano et al., 2017; Abakari et al., 2021) (Table I).

6. OVERVIEW OF POLYCULTURE

Polyculture is also referred as co-culture or integrated aquaculture (Bunting, 2008). Polyculture utilizes the concept that a mixed stock of selected species, with complementary or minimal competing feeding habits and different ecological requirements, can exploit the resources of the different ecological niches in system effectively, thereby resulting a maximum production for given input quantities (Pitt and Nguyen, 2004; Douglass et al., 2008).

Table 1: Recent use of biofloc technology (BFT) in fish and shrimp culture					
Fish/ shrimp species cultured	Technology used	Effect on fish / Shrimp	Reference		
Nile tilapia (Oreochromis niloticus)	Biochar – based BFT	No remarkable negative effects of biochar on growth and physiological performance.			
Nile tilapia (Oreochromis niloticus)	Jaggery – based BFT	Improved growth and survival; higher immunity to Aeromonas hydrophila greater antioxidant capacity.			
Genetically Improved Farmed Tilapia (Oreochromis niloticus)		Enhanced growth and survival; improved immunological parameters	Menaga et al., 2020		
Amur minnow (Rhynchocypris lagowski)	BFT with differential protein	Enhanced growth; boosted immune Yu et al., 202 response and digestive enzyme activity; higher expression of antioxidant-related genes.			
Shrimp (Litopenaeus vannamei)	Wheat flour-based zero water exchange BFT	Affected growth performance	Kim et al., 2021		
Shrimp (Litopenaeus vannamei)	Biofloc – based super intensive tank system	Better growth performance in outdoor conditions than in indoors	Xu et al., 2021		

In addition, the density of organisms within the system may be a limiting factor because oxygen consumption increases as a function of biomass (Martinez-Cordova et al., 1997).

Many benefits have been achieved in shrimp polyculture systems when using fish as subordinate species, despite the fact that polyculture is not common and rarely researched. The benefits of polyculture include the diminution of ecological impacts and improvement in yield and water quality (Muangkeow et al., 2007; Troell et al., 2009). Polyculture can contribute to minimizing the environmental impact of farm effluents, particularly those related to nitrogenous wastes, which are further converted into toxic metabolites; the main reason for this is that some subordinate species can fed on and assimilate most of the waste generated from shrimp aquaculture. A higher efficiency of nitrogen utilization has been observed in polyculture systems compared with monoculture systems (Zhen-xiong et al., 2001), with a consequent decrease in nitrogen excess, improvement in water quality and diminution of the environmental impact resulting from

effluent discharges. Yokoyama et al. (2002) asserted that in polyculture systems, the wastes from aquaculture are assimilated through the food web within pond microcosm formed by the co-cultured organisms and natural pond biota. There is also evidence that the diversity of species within a specific environment influences a variety of ecosystem processes including productivity, decomposition and nutrient cycling (Hooper et al., 2005; Balvanera et al., 2006; Douglass et al., 2008). Belton and Little (2008) affirmed that shrimp culture has severely affected the ecosystem and concluded that integrated aquaculture practices, such as polyculture, are good alternatives for reducing contamination (Table 2).

7. SPECIES INTERACTIONS

The interactions between farmed aquatic organisms in polyculture or co-culture depend mainly on the biological characteristics of the species and the stocking densities used. Major synergistic interactions are improvements in food availability and environmental

Table 2: Effect of polyculture on the environment and biology				
Fish/shrimp species cultured	Environment	Biology effect	Reference	
Macrobrachium rosenbergii Oreochromis niloticus	Reduced phytoplankton densities and pH levels	Higher average weight, and more efficient feed conversion of shrimps (confined tilapia)		
Macrobrachium rosenbergii Oreochromis niloticus	Reduced phytoplankton biomass, improved the water quality	Controlled luminous bacteria	Tendencia et al., 2004; Tendencia, 2003; Tendencia et al., 2006	
Macrobrachium rosenbergii Oreochromis niloticus	Improved system sustainability		Santos and Valenti, 2002	
Ophicephalus striatus Chanos chanos		Did not affect the growth of milkfish	Cruz and Laudencia, 1980; Xu, 2013	
Mugil liza Litopenaeus vannamei	Modified bacterial nitrification, reduced total suspended solids	Enhanced growth of mullet, but impaired shrimp's growth	Holanda et al., 2020	
Indian major carps, rohu (<i>Labeo rohita</i>), catla (<i>Catla catla</i>), and mrigal (<i>Cirrihinus mrigala</i>)	Maintenance of NH ₄ -N, NO ₂ -N and NO ₃ -N in the acceptable range of water quality	Satisfactory growth performance (higher rate of specific growth)	Deb et al., 2020	

conditions (Milstein, 1992). On the other hand, the major antagonistic interactions are competition for food, space, oxygen, and other resources within the growout unit and agonistic behavior. Thus, the use of ecologically different species that have complementary requirements and planning a suitable density for each are the keys for successful polyculture.

Shrimp and tilapia match the fundamentals described above mainly because of differing feeding habits and spatial distribution. Shrimp are benthic and omnivorous in production ponds, eating commercial feed, detritus, waste, and feces (D'Abramo and New, 2000). Tilapias are pelagic and omnivorous, filtering phytoplankton (Wang and Lu, 2015), eating periphyton (Getachew, 1993; Tadesse, 1999; Sakr et al., 2015), and consuming commercial feeds. In polyculture, shrimp can eat tilapia feces and unused fish feed (Santos and Valenti, 2002), while tilapia filter phytoplankton (Perschbacher and Lorio, 1993), reducing the risk of low dissolved oxygen levels at night. In addition, shrimp bioturbation at the pond bottom returns nutrients to the water column (Kimpara et al., 2011), enhancing phytoplankton production and consequently the natural feed available for the tilapia. Therefore, shrimp and tilapia exploit different niches in the pond environment, show positive synergism and low antagonistic interactions; thus, they can be cultivated together with success.

Generally, polyculture influences water quality in a positive manner and it may decrease the need to exchange water in ponds. The benefits of introducing tilapia into shrimp ponds on the physical and chemical aspects of water quality were noted by Rouse et al. (1987) and Alston (1989) in polyculture, and by Danaher et al. (2007) and Tidwell et al. (2000) in co-culture (stocking tilapia in cages within ponds). The main benefits cited are the stabilization of dissolved oxygen and pH. These are because the tilapia consumes excess algae, lowering nocturnal respiration and the excess of photosynthesis during the day, and the shrimp consume detritus, reducing respiration at the pond bottom.

Among the various shrimp species, *Penaeus vannamei* has a consolidated and expanding production chain, due to its adaptability, rapid growth, and adaptability to polyculture with fish (Hossain and Islam, 2006). Similarly, in the farmed fish species, Genetically Improved Farmed Tilapia (GIF tilapia) is emerging as an important cultivable fish after carps. GIF tilapia strain has better growth, meat quality and good market value than normal tilapia strain (Sgnaulin et al., 2000). Besides that, *P. vannamei* and GIF tilapia has differing feeding habits and spatial distribution which is highly suitable for this hybrid system.

In shrimp farming regions, tilapia is often grown in cage or hapa inside shrimp ponds, or is produced in supply channels or head ponds. In Latin American countries such as Brazil and Mexico, red tilapia hybrids are now cultured in brackish ponds traditionally used only for shrimp farming (Alceste et al., 2001). In the Philippines, more than 60% of the shrimp farms employ tilapia—shrimp polyculture (Cruz et al., 2008). Farming tilapia and shrimp together, improves shrimp health and increases profits (Yuan et al., 2010; Hern'andez-Barraza et al., 2012). Shrimp production was generally higher in polycultures than in monocultures (Li

and Dong, 2002). Tilapia/shrimp polyculture is important in the control of the luminous bacterial disease caused by *Vibrio harveyi* (Cruz et al., 2008). Studies have shown that the presence of genetically improved farmed tilapia (GIFT) reduced the luminous bacteria population and increased shrimp survival (Tendencia et al., 2006). The ability of tilapia to control luminous bacteria has been extensively studied (Tendencia et al., 2004; Tendencia and Choresca, 2006). Tilapia polyculture maintained a stable plankton environment, and increased shrimp survival (Cruz et al., 2008). Stocking performance, feeding strategies and productivity in shrimp/tilapia systems have been studied (Wang et al., 1998; Hernandez-Barraza et al., 2012).

8. HISTORY AND STATUS OF PENAEUS VANNAMEI

Pinaeus vannamei is native to the tropical East Pacific from the Gulf of California, Mexico to northern Peru (Holthuis, 1989). It is now the most widely cultured shrimp in the world (Liao and Chien, 2011). It is currently raised in at least 27 countries, with major production operations occurring in the US, Mexico, Central America, tropical South America, China, India, and southeast Asia. Shrimp is one of the significant high exported commodity in world fish trade. The white leg shrimp (*Penaeus vannamei*) production has increased steadily over the last decade, and in 2020, a total of 5.8 mt were reached, making it the most-produced animal species in aquaculture. In fact, the culture of P. vannamei generates profits of over 33 billion USD year⁻¹ worldwide. Major producers are Asian and South American countries, and in 2019, imports of *Penaeus* shrimp to Europe reached 284.270 tonnes with a total value of 1.98 billion EUR. Among the various shrimp species, *Penaeus vannamei* has a consolidated and expanding production chain, due to its adaptability, rapid growth, and adaptability to polyculture with fish (Hossain and Islam, 2006).

9. HISTORY AND STATUS OF GENETICALLY IMPROVED FARMED TILAPIA (GIF TILAPIA)

The GIFT strain was developed by World Fish Centre (WFC; formerly known as International Centre for Living Aquatic Resources Management, ICLARM) through several generations of selection from the base population involving 8 different strains of Nile tilapia Oreochromis niloticus. GIFT program succeeded 12–17% average genetic gain per generation over five generations and cumulative increase in growth rate of 85% in O. niloticus (Eknath and Acosta, 1998). The Indian government recognizes GIF tilapia farming a key sector in aquaculture, particularly considering the success of other tilapia industries in tropical and subtropical regions around the world

(Menaga and Fitzsimmons, 2017). Nile tilapia (*Oreochromis niloticus*) had 3.69% of share world production quantity of all species whereas 3.05% of share of world production value of all species. Capture production of tilapia and other cichlids are 7, 12,740 tonnes in 2012 and 8, 50,770 tonnes in 2018 respectively. International exports of fishery commodities by FAO ISSCAAP of Tilapia and other cichlids in world exports are 40,273 tonnes in 1998 and in 2018 was 8,11,960 tonnes with a share in total exports of 0.11% in 1998 and 1.21% in 2018. The desirable characteristics of this genetically improved strain are of high yielding, better growth, efficient converter of organic and agricultural wastes into high quality protein, resistant to diseases, very hardy, tolerant to overcrowding conditions and adaptability to polyculture with shrimp (Sgnaulin et al., 2000).

10. BIOFLOC ON WATER QUALITY

D eing an aquatic animal, fish growth and health conditions Dare directly anchored by water quality. Elevation of ammonia nitrogen in water body of fish pond results in fish toxicity. The traditional solutions is to exchange water of the pond frequently or applicant sophisticated labor expensive technique as rotating biological contactors, trickling filters, bead filters and fluidized sand biofilters which are usually used in intensive aquaculture systems to remove toxic nitrogen from water in production units. Microbial process is the key step in waste water treatment, as the waste degradation and elimination could be managed. Activation of the ammonia assimilating bacteria by adding carbohydrates enhances the water quality in the pond (Avnimelech, 1999; Luo et al., 2014; Wang et al., 2016 and Khanjani et al., 2016). The balance between carbon and nitrogen in biofloc system lead to enhance the water quality by minimizing the level of NH₄+, NO₂ in ponds (Wang et al., 2016). Similar positive effect on water quality parameter (ammonia, nitrite and nitrate) was noticed in biofloc system in culture of grey mullet (Haridas et al. 2021), giant river shrimp (Hosain et al., 2021), Nile tilapia (Elayaraja et al., 2020; Azim and Little, 2008), Amur carp (Ezhilarasi et al., 2019), Pacific white shrimp (Lin and Chen, 2003; Samocha et al., 2004; Kuhn et al., 2010) and Zero water exchange system (Burford et al., 2004; Wasielesky et al., 2006; Ray et al., 2010; Vinatea et al., 2010).

The pH should be ranges from 6.5 to 9 depending on the culture species. The pH fluctuated between day and night due the action of photosynthesis and respiration. Growth and survival of the culture stock as well as the efficiency of the biofloc components are affected when pH is reduced or increased from their recommended values (<6 and >8.5) respectively. Emerenciano et al. (2017) reported that pH became acidic level could affect the nitrification process in biofloc system.

Temperature is an important parameter because it affects the oxygen consumption, pH, ammonia and metabolic rate of fish. Temperature ranges from 28 and 30°C is ideal for tropical fish culture but it varies depending on the cultured species. Wilen and Balmer (1999) reported that temperature should be ranges from 18 to 25°C is favor for biofloc development and which influences the floc characteristics and also microbial metabolism.

Dissolved oxygen is a crucial parameter in biofloc systems, the oxygen demand will be higher, because of the interaction between the bacteria, algae and fish size. The recommended dissolved oxygen to be maintained is 6–8 mg l⁻¹ to ensure proper functioning of the system. Luo et al. (2014) stated that increasing the C: N ratio and maintaining a high DO (6 mg l⁻¹) in the culture water enabled the assimilatory activity of heterotrophic bacteria to convert ammonium into bacterial biomass in biofloc system.

Alkalinity is the total concentration of bases in the water which includes carbonate, bicarbonate and hydroxide ions. Total alkalinity ranges from 70 to 150 mg l⁻¹ which provide a well buffered environment and suitable for growth of the fish and pond primary productivity (Boyd et al., 2002). Azim and Little, (2008) reported that, total alkalinity was stable in normal culture system and oscillates in AMF system due to buffering action. Hardness is a measure of alkaline ions of Calcium, Magnesium in water with other ions like Aluminium, Zinc and Hydrogen. Acceptable range of hardness in aquaculture is 50–150 ppm.

Emerson et al. (1975) state that toxicity on aquatic organisms has been attributed by ammonia (NH₃). Emerenciano et al. (2017) reported that less than 0.1 ppm of NH₃ suitable for fish culture and toxicity level mainly depends on pH of water in biofloc system. Hargreaves (2013) documented that ammonia or nitrite higher during initial stage can be reduced by adding carbohydrate. At primary stage, carbohydrate was added to lower ammonia concentration and to stimulate the heterotrophic bacterial population. The concentration of nitrites was lower and more efficient than nitrification in biofloc system (Perez-Fuentes et al., 2016).

11. FLOC PARAMETERS

Numerous studies have demonstrated that different microorganisms have amassed in biofloc systems, which play an important role in transforming the nitrogen compounds into functional feed for other organisms (Roberto et al., 2017). The created floc feed, contains various active compounds and probiotic strains, and helps in supplying energy, nutrition, and disease resistance to the fish (Ahmad et al., 2017). According to Crab et al. (2012), the applied carbon source, to promote the floc, encourages the proliferation of particular bacteria, protozoa, and algae,

which affects both microbial diversity and nutritional quality of floc. Floc characteristics are influenced by dissolved oxygen, temperature, mixing intensity and carbon source (Crab et al., 2010). The metabolic activity of cells within biofloc depends on dissolved oxygen. In higher aeration, flocs tend to be larger and denser (Wilen and Balmer, 1999). Temperature is the main source of microbial metabolism. It may influence floc characteristics. More deflocculation of activated sludge flocs occurs in low temperature leading to decline in microbial activity within flocs (Wilen et al., 2000). Krishna and Loosdrecht (1999) studied that higher water temperature leads to higher production of sludge. Crab (2010) reported that at optimal water temperature about 20–25°C, flocs are stable.

Avnimelech (2007) studied that floc volume continuously increased due to the addition of carbon source with intensive aeration and subsequently fed by culture species. The desirable range of floc volume, a quantitative characteristic of biofloc, for finfish culture was 25 to 50 ml 1⁻¹ (Hargreaves, 2013). Krishna and Loosdrecht (1999) mentioned that the temperature between 20-25°C is suitable to obtain stable flocs with an intermediate floc volume index of about 200 ml g⁻¹. Crab et al. (2010) observed that biofloc with a higher floc volume index are produced at lower DO-levels in the biofloc ponds. Better FVI provide, the aquatic organisms enough opportunity to filter the flocs from suspension before they sediment to the bottom of the ponds. The optimum level of floc physical and chemical characteristics and suspended solids (SS) and Floc volume index (FVI) should be 0.2 to 1.0 g 1⁻¹ and >200 ml g⁻¹ respectively (De Schryver et al., 2008). Mueller et al. (1967) observed that the sludge floc density measured after removal of the water was 1.09 g cm⁻³. Smith and Coakley (1984) reported that density of solid material in the flocs, with a typical constant value of 1.40 g ml⁻¹. Li et al. (1986) reported that the activated sludge floc cell density was around 1.01 g cm⁻³. The porosity was increased in smaller flocs (<200 µm) than that of the larger flocs. Li et al. (1986) stated that the porosity of the activated sludge

Hargreaves (2013) documented that BFT system should operate with less than 500 mg l⁻¹ of suspended solids. The range between 200 to 500 mg l⁻¹ is appropriate for good system and this level can control ammonia without excessive water respiration. TSS between 100 to 300 mg l⁻¹ ideal for the best feed consumption in shrimp raceway BFT systems. In BFT system, TSS level was gradually increased throughout the experiment based on the addition of suitable carbon source (Long et al., 2015). Ray et al. (2010) studied

ranged from 82.5 to 95%. Chu and Lee (2004) reported

that flocs are usually irregular in shape, size and have broad

distribution with highly porous condition and observed

around 99% of porosity has been observed.

that over accumulation of TSS concentration in the biofloc system causes gill clogging. Van Wyk (2006) observed that limited water exchange, higher organic matter input, and improved growth rates of heterotrophic bacteria contribute to an increase of TSS level in BFT systems.

12. GROWTH PERFORMANCE

Nombining of biofloc with polyculture offers several advantages such as better growth characteristics (Hisano et al., 2019), minimizes the feed and water input, and effluent production (Reinoso et al., 2019; Martins et al., 2020). Barbosa et al. (2022) studied on the polyculture of fresh water shrimp with Nile tilapia using an aquaponic system and biofloc technology and stated that no significant difference were observed with respect to zootechnical performance. Polyculture of tilapia and river shrimp in a system of biofloc expressed a higher growth, which was reflected in a greater productivity, meeting the challenge of producing more in a smaller volume of water (Reinoso et al., 2019). Hisano et al. (2019) stated that polyculture of Nile tilapia and freshwater shrimp in BFT provides better growth performance comparing to RAS. Poli et al. (2019) demonstrated that feasibility of increasing yield by integrating L. vannamei and O. niloticus in a biofloc system. Polyculture of Nile tilapia (O. niloticus) and marine shrimp (L. vannamei) displayed better economical and performance indicator in comparison with monoculture (Bessa Junior et al., 2012).

Polyculture technology in biofloc system improved the growth performance of mullet and P. vannamei with better utilization of residues and microbial aggregates as food source which met the nutritional requirements up to 50% (da Rocha et al., 2012). Similarly, Hoang et al. (2020) studied on polyculture of mullet (M. cephalus), tilapia (O. niloticus) with white shrimp (P. vannamei) and stated that shrimp-fish polyculture with a stocking density of fish at 10% of the initial shrimp biomass resulted in better growth of mullet, tilapia and white shrimp. Integration of shrimp and mullet in biofloc increases yield without compromising fish health and shrimp growth (Legarda et al., 2019). Jatoba et al. (2011) studied on the polyculture of Nile tilapia (O. niloticus) with marine shrimp (L. vannamei) fed with Lactobacillus plantarum resulted in increased final weight and feed efficiency. Co-culture of shrimp and tilapia resulted in better yield and physiological responses (Apun-Molina et al., 2015). Polyculture of Nile tilapia and shrimp at suitable stocking densities and appropriate feeding rates results in positive interactions and better growth performance in recirculating system (Hernández-Barraza et al., 2013). Polyculture is more efficient with the combination of 2 tilapia and 5 shrimp m⁻² (Simão et al., 2013). Hosseini Aghuzbeni et al. (2017) studied on

the polyculture of white shrimp with mullet and stated that polyculture improves growth and production of white shrimp. Integrated system with a low tilapia-shrimp ratio (the ratio of 0.01 and 0.025) were effective to improve the growth and nutrient conversion ratio without lowering shrimp growth (Muangkeow et al., 2007).

13. DIGESTIVE ENZYME ACTIVITY

Biofloc plays an important role in stimulating the activities of digestive enzymes (Moss et al., 2001) by promoting the breakdown of nutrients into simple molecules which further converted into building blocks (Dong et al., 2018). In general, ability of an organism to digest, absorb and utilize the nutrients was directly indicated by the digestive enzyme activity. Knowledge of digestive enzymes in an organism helps to determine its digestive capabilities, which in turn helps the selection of ingredients to be included in a diet.

Xu and Pan (2012) reported the enhancement of protease and amylase activities of the L. vannamei grown in the biofloc based system and suggested that appropriate extracellular enzyme activities are present in biofloc. Few beneficial bacteria such as Bacillus sp. in the ingested biofloc could facilitate the modification of physiological and immunological status of the host, through the colonization in the gastrointestinal tract (Zhao et al., 2012; Xu and Pan, 2013). Xu et al. (2013) suggested that the microbial flocs could exert a positive effect on the digestive enzyme activity of L. vannamei which can facilitate feed digestion and utilization. In biofloc system different microorganisms can produce different kind of enzyme to breakdown the protein, lipid and other particles, when these particles ingested in shrimp it will also improve the digestion of the animal. Anand et al. (2013) reported that the dietary inclusion of biofloc enhance the digestive enzyme activity with high growth rate in Penaeus monodon.

Wang et al. (2015) reported that protease enzyme activity was higher in the biofloc cultured crucian carp compared to the clear water system. Long et al. (2015) reported that BFT treatment had a stimulatory effect on the digestive enzyme activity. Biofloc are rich source of heterogenous microbial cells, bioactive compounds and thus exogenous enzymes of biofloc have enhanced the digestive enzyme activities of *L. rohita* (Ahmad et al., 2019, Ahmad et al., 2016; Mahanand et al., 2013). Najdegerami et al. (2016) stated that common carp fingerlings can adapt well to nutritional conditions and that microbial flocs stimulated the production and/or activity of digestive enzymes, resulting in improved digestion of nutrients in the gut.

14. HEMATOLOGY

The most convenient tool for the fish culturist to assess clinical status is hematology. Hematological parameters

such as hematocrit, hemoglobin, number of erythrocytes and white blood cells are indicators of fish health with respect to nutrition and feeding. Hematological parameters act as physiological indicators to changing external environment as a result of their relationship with energetic (metabolic levels), respiration (Hb levels) and defense mechanisms (leucocytes level) and provide an integrated measure of the health status of an organism which manifest in changes in growth (Ishikawa et al., 2007). Normal ranges for various blood parameters in fish have been established by different investigators in fish physiology and pathology (Rambhaskar and Srinivasa Rao, 1987; Zhou et al., 2009). The analysis of blood indices has proven to be a valuable approach for analyzing the health status of farmed animals as these indices provide reliable information on metabolic disorders, deficiencies and chronic stress status before they are present in a clinical setting (Bahmani et al., 2001).

Bakhshi et al. (2018) studied on common carp reared in biofloc and reported that increase in RBCs count in comparison with control (without carbon source). No significant effect was observed on hematology parameters of tilapia, O. niloticus reared in biofloc (Long et al., 2015; Azim and Little, 2008; Mabroke, 2018; El-Husseiny et al., 2018). Tilapia, O. niloticus reared at low density in biofloc has shown significant difference in WBC in comparison with clear water (Hwihy et al., 2021). Significantly decreased hematological indices (RBC, WBC, HGB and blood platelets) were reported by increasing stocking density of Nile tilapia reared in biofloc treatment (Mehrim, 2009; Kpundeh et al., 2013 and Zaki et al., 2020). Mansour et al. (2017) studied on Nile tilapia, O. niloticus reared in biofloc with dietary plant protein levels and stated that fish reared under BFT showed higher WBC counts than fish maintained in clear water. Mansour and Esteban, (2017), has reported that O. niloticus reared in biofloc culture has increased hemoglobin and hematocrit value.

15. BLOOD BIOCHEMISTRY

Blood biochemistry parameters can be also used to detect the health of fish (De Pedro et al., 2005). Exogenous factors, such as management (Svobodova et al., 2008), diseases (Chen et al., 2005) and stress (Cnaani et al., 2004), always induce major changes in blood composition. For example, significant fluctuations were detected in the concentrations of glucose, cholesterol and other basic components in response to handling and hypoxic stress (Skjervold et al., 2001). Cholesterol is mainly synthesized in liver and is an intermediate product in lipid, carbohydrate, and protein metabolism. The levels of glucose are considered to be specific indicators of sympathetic activation during stress conditions (Lermen et al., 2004). Basic ecological factors, such as feeding regime and stocking density, also

have a direct influence on certain biochemistry parameters (Coz-Rakovac et al., 2005).

Studies on mullet, *M. cephalus* reared in biofloc has increased total protein and globulin levels when integrated with *L. vannamei* (Legarda et al., 2019). Similarly, increased total plasma and globulin were reported in mullet, *M. cephalus* reared in biofloc treatment (Kakoolaki et al., 2016; Akbary et al., 2018). No significant effect was observed on total protein of tilapia, *O. niloticus* reared in biofloc and control group (Long et al., 2015). The biochemical parameters of the blood serum were significantly improved in biofloc treatment in comparison with other treatments in *O. niloticus* (Azim and Little, 2008; Martins et al., 2017; Sayed and Moneeb, 2015).

16. IMMUNE PARAMETERS

Temolymph parameters are mostly used to monitor ▲ Lthe physiological condition, nutritional quality and status of immune systems in crustaceans exposed to various stressors (Matozzo et al., 2011; Porchas Cornejo et al., 2011). The circulating haemocyte count of crustaceans in terms of both increase in quantity and quality and ProPO activity indicated enhanced immune status in crustaceans and hence disease resistance (Rodriguwz and Le Moullac, 2000; Chiu et al., 2007). Increased haemocyte count was reported in L. vannamei (Xu and Pan, 2013; Panigrahi et al., 2017, 2018 and 2019; Abbaszadeh et al., 2019; Tong et al., 2020), P. monodon (Kumar et al., 2017) and P. indicus (Panigrahi et al., 2020) in biofloc based systems. Shrimps evidently consume microbial floc in situ in biofloc systems (Crab et al., 2012), that increases THC and ProPO activity in P. vannamei biofloc systems (Kim et al., 2014). Similarly, higher PO activity was observed in L. vannamei (Ekasari et al., 2014), P. monodon (Kumar et al., 2017) and P. indicus (Panigrahi et al., 2020) when grown in biofloc systems than in clear water system. This improvement might be attributed to the consumption of large quantity of bacteria (Bacillus and Lactobacillus) associated with biofloc by the shrimp that has probably released immune-stimulatory substances in the intestinal tract and could significantly enhanced the immune status in *P. vannamei* juveniles in biofloc system.

17. BACTERIAL COMMUNITY IN BIOFLOC SYSTEM

17.1. Culture water

Avnimelech (1999) studied that addition of carbon source increase the total heterotrophic bacterial count in biofloc system. De Schryver et al. (2008) stated that different carbon sources were used for biofloc production and revealed that carbon source determines the composition of the floc. This could have an effect on the bacterial populations that are closely associated with the flocs.

bacterial populations were significantly higher in the biofloc groups than the control due to the addition of sweet potato as carbon source and earlier study was carried out by using wheat and corn flour as carbon sources for biofloc production in freshwater tilapia culture. Manan et al. (2017) studied that *Aeromonas* and *Pseudomonas* species were identified as heterotrophic bacteria from their experiment and organic compounds were used as the source of energy by heterotrophic bacteria derived from the organic matter. *Vibrio* load was decreased in biofloc system through higher diversity of phytoplankton and algae and also can also compete with dominant number of heterotrophic bacteria (Manan et al., 2017). Emerenciano et al. (2013) discovered that the natural probiotic in the biofloc could fight against

Caipang et al. (2015) reported that the total heterotrophic

Kumar et al. (2017) observed highest mean total bacterial count in *P. monodon* reared in biofloc generated from molasses with *Bacillus* being the most dominant bacterial group followed by *Vibrio* and *Lactobacillus*. Similarly, Panigrahi et al. (2019) also reported that carbohydrate supplementation systems significantly increasing the total heterotrophic bacteria (THB) count in biofloc system when compared with control group in *P. vannamei*. Recently, Khoa et al. (2020) found significantly high THB and *Vibrio* loads in biofloc treatment tanks reared *L. vannamei* when compared with control. Furthermore, Sundaram et al. (2021) recorded significantly high THB loads in substrate integrated biofloc systems than clear water control system in *P. vannamei*.

17.2. Gut flora

the *Vibrio* sp.

Del'Duca (2015) reported that the bacterial community composition of gastrointestinal tract in tilapia was resembled the composition in water than the sediment in the pond. Total bacterial abundance of the intestinal tract of juvenile tilapia was significantly higher than that of water. In gastrointestinal tract of juvenile tilapia and water, the abundances of *Lactobacillus brevis*, *Lactobacillus collinoides* and *Pseudomonas fluorescens* were significantly higher than other bacterial groups and proportional value of Bacillus was significantly higher in fish than in water (Del'Duca, 2015). Chethurajupalli and Tambireddy (2021) found significantly higher THB, *Bacillus*, *Lactobacillus* and *Vibrio* count in biofloc treatment reared *L. vannamei* compared to control.

18. CONCLUSION

Polyculture study suggests this hybrid system as sustainable, profitable and environmentally beneficial technology.

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