**“Carbon and Nitrogen ratio in Shrimp Aquaculture Systems”**

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**Aquaculture and Shrimp farming**

Looking at the current population explosion and the speed of human-centered land encroachment, scientists have expressed concern about the availability and production of food to meet global demand. The agricultural sector alone would not be able to cope with such a huge global demand of 7.6 billion people, which does not seem to be decreasing in any way. About 20% of global populations consume fish due to which the sector of aquaculture has been originated. On the other hand, it has grown many folds with advancements in science and technologies in fish production and post-harvest which has become a prominent commercial sector. Fisheries sector is broadly classified into two types *viz*, marine and inland fisheries. In marine fisheries, fishermen venture into the sea for fishing with their fishing vessels and gears to harvest the fish, whereas in inland fisheries, fishermen go for fishing in rivers, lakes and reservoirs. But, with the advancements in technology and growing demand of fish as food source, people started growing fish in captivity under controlled and semi-controlled condition in the ponds. The history of fish culture tracks back to 2000 BC which originated in China where, they started growing carps in captivity by providing the required condition for the fish to grow. Providing suitable conditions in captivity means to provide the required physical and chemical environment in the culture system by keeping the physico-chemical parameters in optimum level as required by the species cultured. Physico-chemical parameters of soil and water are the key to successful culture of fish which is why emphasis has been given to the scientific study of physical and chemical parameters of soil and water.

One of the world's fastest growing aquaculture sub-sectors is fin/shellfish farming. India has a vast coastline of 8,118 km in geographical area and an exclusive economic zone (EEZ) of 2.02 million km2. Socio-economically underdeveloped small and small-scale fishermen, whose lives are closely intertwined with the ocean and sea, who dominate sea fishing, In order to improve fishing resources and replenish natural stocks whose populations have decreased due to overexploitation or environmental degradation, marine fin fish culturing has become more and more popular. Shrimp aquaculture has been used for many years in Southeast Asia and is a traditional kind of coastal farming in several nations. A variety of issues have arisen as a result of the recent tendency toward more intense forms of culture. Experiences in the area, however, indicate that, with the adoption of suitable management practices, shrimp farming can be socially, environmentally and economically viable and help produce food and reduce poverty in coastal areas. The creation and application of such management strategies must take into account technical, economic, social and environmental challenges.

According to NFDB (2020), India ranks third in fisheries production and second in aquaculture. Fisheries alone has employed 145 million people and contributed to 1.07% of the GDP and export earnings of Rs 334.41 billion. Presently Andhra Pradesh tops under culture and production of shrimp. Commercial shrimp farming started from the year of 2009–2010 (MPEDA, 2021). Total world fisheries and aquaculture production attained another high record of 178.5 mmt in which, 96.4 million tonnes is capture fisheries while 82.1 million tonnes is from aquaculture production in 2018 (FAO, 2020).

According to FAO (2020), in the period 1961–2017, the average annual growth rate of total food fish consumption increased by 3.1%, exceeding the annual population growth of 1.6%. Food fish consumption per capita increased from 9.0 kilograms (live weight equivalent) in 1961 to 20.3 kilograms in 2017. Aquaculture can control overexploitation, create jobs and provide the world with protein-rich food. The Government of India plans to increase fish production from 137.58 lakh to 220,000 tonnes in 2018-19 under the Pradhan Mantri Matsya Sampada Yojana (PMMSY) of the Ministry of Fisheries, Animal Husbandry and Dairying, Government of India.

**Important shrimp species in aquaculture**

Mainly crustaceans, tiger shrimp (Penaeus monodon), Indian white shrimp (Penaeus indicus), white leg shrimp (Penaeus vannamei), red tail shrimp (Penaeus penicillatus), banana shrimp (Penaeus merguiensis), green tiger shrimp (Penaeus indicus) an important shrimp used in coastal aquaculture The Pacific white shrimp or white-legged shrimp Litopenaeus vannamei (Boone, 1931) is classified in the Phylum Arthropoda, Class Crustacea, Order Decapoda, Family Penaeidae, Genus Litopenaeus, and Species vannamei (Perez and Kensley, 1997). It is native to the tropical western Pacific coast of Latin America, from southern Mexico in the north to Peru in the south, between latitudes 32°N and 23°S. This peneid is very abundant on the coast of Ecuador in Esmeraldas (border province of Colombia), where females are available throughout the year (Huang et al., 2003). Litopenaeus vannamei is extremely euryhalic, capable of inhabiting low salinity waters of 1-2 psu and hypersalinity waters up to 40 psu (Menz and Blake, 1980). Juveniles and juveniles live on a muddy bottom in warm water (25–32 °C) with a salinity of 28–34 psu at a depth of 70 cm and a burrow. Adults prefer higher salinities of 34-35 psu and prefer slightly deeper water (30-50m). Young stage abundance showed an inverse correlation with salinity and a positive correlation in a Mexican coastal lagoon system (Rivera et al., 2008).

Recently, *L.* *vannamei* acquired by coastal aquaculture farmers as itis one of the most intensively cultivated shrimps all over the world and reduced risk of catastrophic diseases (Perez and Kensley, 1997; Boyd, 2002; Zhu *et al*., 2006). The intensification of production systems leads to adverse changes in water quality and has increased the risk of diseases due to higher stocking densities and feeding rates (Nasrin, 2016). Shrimp farming has been practiced in India since 40 years but commercial and large scale shrimp culture started in 1990’s.

Initially, shrimp farming was only done on an experimental scale in India. An important step towards large-scale shrimp aquaculture was taken soon after the Indian Council of Agricultural Research (ICAR) Central Inland Fisheries Research Institute first demonstrated brackish water fish farming in West Bengal in 1973. Later, a research project coordinated by ICAR on brackish water aquaculture was taken up. up across India in 1975 in West Bengal, Andhra Pradesh, Odisha, Tamil Nadu, Goa and Kerala. Meanwhile, the Central Marine Fisheries Research Institute (Vijayan and Kailasam, 2020) demonstrated successful shrimp seed production at Narakkal, Kerala. Commercial shrimp hatcheries were established by the Marine Products Export Development Authority. Semi-intensive farming technology has also been introduced in pilot scale MPEDA (Muralidharan, 2019). As shrimp aquaculture spread throughout India, these techniques, along with experiments by other farmers, succeeded and allowed the industry to develop on a large scale.

The Biofloc system was developed to improve the environmental management of aquatic animal production. In aquaculture, feed costs (60% of total costs) and the availability of water and land are the most limiting factors. The principle of this technique is to create a nitrogen cycle by maintaining a higher C/N ratio (the ratio of carbon to nitrogen), stimulating the growth of heterotrophic microbes that assimilate nitrogen-rich waste that can be used as feed by cultivated species. A higher C/N ratio is maintained by adding a carbohydrate source (molasses) and water quality is improved by producing high quality single cell protein. The immobilization of toxic nitrogen species occurs faster in biofloc technology (BFT) because the microbial production per unit of heterotrophs is 10 times higher than that of autotrophic bacteria. Due to the lower habitat and resistance of shrimp to environmental changes, this technique was adopted in shrimp farming (Avnimelech, 1999).

**Physico-chemical characteristics of water and soil in shrimp ponds**

Understanding of physico-chemical characteristics of water and soil in shrimp ponds and growth performance of aquatic animals is necessary to control the disease and prevent the stress to the shrimp. At present, *L. vannamei* is most preferable species for culture by shrimp producers due to short time crop, hardy species and high market value. Water quality in shrimp ponds play a vital role in maintaining aquatic animal health, growth performance and survival rate in the ponds. Due to increased stocking density, feeding rate and pollutants intake, the water seriously faces the risk of water quality issues are common in shrimp ponds but management of the aquatic environment is a challenge. Poor water quality causes disease, mortality, slow growth and low shrimp production. However, the shrimp aquaculture industry has faced various problems such as germplasm degradation, disease outbreaks and water quality degradation, which have seriously hindered its further development (Bachere, 2000; Thitamadee *et al*., 2016). In addition, the high water turnover in the aquaculture process not only causes a loss of nutrients but also seriously pollutes the surrounding environment (Bachere, 2000).

In intensive aquaculture systems, wastes consisting mostly of excrement and unused feed lead to the accumulation of toxic metabolites such as ammonium and nitrite, which degrade shrimp habitat (Avnimelech and Ritvo, 2003; Piedrahita, 2003). Increasing the C/N ratio to promote heterotrophic microbes in pond sewage systems that regulates water quality by removing toxic nitrogen such as ammonia. Heterotrophic microbes are mainly responsible for performing the necessary functions in the biofloc system, and their ammonium fixation by heterotrophic bacteria occurs much faster due to their faster growth rate and higher yield of microbial biomass per substrate unit than nitrifying bacteria. The bacterial protein produced by the assimilation of ammonium nitrogen must have a sufficient content of protein, lipid, carbohydrate and ash as a high-quality aquaculture feed. As organic matter decomposes, carbon breaks down faster than nitrogen, reducing the ratio of carbon to nitrogen. Adding compost or other nutrients can help to find the right carbon-nitrogen ratios. Lot of raw organic matter could be applied to soil, thereby microorganisms multiply rapidly, but in the process of working they consume nitrogen. Dissolved oxygen (DO) is one of the most critical water quality parameters to monitor in aquaculture. Biofloc technology is defined as "the use of matrix-perpetuating aggregates of bacteria, algae, or protozoa with particulate organic matter to improve water quality, waste management, and disease prevention in intensive aquaculture systems" (Avnimelech, 1999). . In addition to the oxygen demand of farmed shrimp or fish, the rich microbial community also consumes DO to a significant extent. The intensity of DO consumption by the microbial community is highly dependent on the feed required for a given stocking density (Boyd, 2009).

The microbial community is responsible for recycling excess nutrients. In such systems, particles are often removed by external filtration such as sedimentation, vortex and sand filters. However, in biofloc systems, particles are allowed to form in a culture system, and part of the microbial community responsible for nutrient cycling is within these particles. The main principle of carbon and nitrogen is to reduce water exchange and encourage the development of heterotrophic organisms using the nitrogen waste absorbed in the pond or tank. The C/N ratio is regularly reduced in the biofloc aquaculture system by adding organic carbon as a source to the animal tank, which inorganic nitrogen uses for microbial aggregation. A balanced ratio of carbon to nitrogen in the feed is important to maximize the growth of heterotrophic bacteria. It is a process used to regulate the nitrogen content of water. The most popular C/N ratios are 10:1 and 15:1, which means that at a C/N ratio of 10:1, 10 carbon sources are needed to destroy one nitrogen. In aquaculture, the C/N ratio is calculated based on the protein content of the feed and the total ammonium nitrogen (TAN). When carbon and nitrogen are in equilibrium in solution, ammonium is converted to bacterial biomass in addition to organic nitrogen containing wastes (Schneider *et al*., 2005). In more populated aquaculture ponds, water quality management is paramount. Degradation of water quality impairs growth and survival. Good water quality usually refers to the suitability or suitability of the water for shrimp survival and growth. By adding carbohydrates to the pool, the growth of heterotrophic bacteria is stimulated and nitrogen assimilation occurs through the production of microbial proteins (Avnimelech, 1999).

**Carbon and Nitrogen ratio (C/N ratio)**

The Carbon to Nitrogen ratio (C/N ratio) is a process of controlling the amount of Nitrogen in water. Most popular C/N ratios are 10:1 and 15:1, which means in C/N ratio 10:1, it requires 10 Carbon sources to kill 1 Nitrogen. Avnimelech (1999) put forth his views that, in aquaculture, C/N ratio has been calculated based on protein percentage of feed and total ammonia nitrogen (TAN). If carbon and nitrogen are well balanced in the solution, ammonium in addition to organic nitrogenous waste gets converted into bacterial biomass (Schneider *et al*., 2005). In intensive aquaculture systems, waste generated during the course of culture period, primarily, faeces and unconsumed feed induce the accumulation of toxic metabolites like ammonium and nitrite thereby spoiling the living environment of the shrimp (Avnimelech and Ritvo, 2003). In more populated aquaculture ponds, water quality management is paramount. Degradation of water quality impairs growth and survival. Good water quality is usually the condition or suitability of the water for shrimp survival and growth. By adding carbohydrates to the pool, the growth of heterotrophic bacteria is stimulated and nitrogen assimilation occurs through the production of microbial proteins (Avnimelech, 1999). Wujie *et al*. (2016) investigated the effect of C/N ratio on biofloc development and subsequent water quality and efficiency of L. vannamei refrigeration in biofloc-based high-density zero-exchange external tank systems. They reported that volatile suspended solids (VSS) and turbidity values ​​were better quantitative parameters for biofloc quantification than suspended solids (SS) or total suspended solids (TSS). TAN and NO2 concentrations can be effectively controlled by heterotrophic assimilation. Autotrophic nitrification that helps maintain shrimp concentrations within acceptable ranges for shrimp farming even at high stocking densities. Microalgae and autotrophic bacteria are more beneficial to shrimp performance in high-density zero-transformation culture systems than heterotrophic bacteria. Muthusamy *et al*. (2016) reported a reduction in ammonia total nitrogen content while maintaining good water quality in shrimp culture in biofloc culture systems. Water quality and shrimp production were monitored in extensively managed ponds with or without carbohydrate based diet to shrimps (Hari *et al*.,2006). Jaganmohan and Leela (2018) reported that all the tested parameters such as pH, Salinity, Carbonates, Bicarbonates, Total alkalinity, Calcium, Magnesium, Total hardness, Total ammonia and Nitrite maintained under optimal conditions were suitable for *L. vannamei* farming. According to Islam *et al*. (2004), salinity fluctuated from 3.0 to 15.0 ppt in the southwest, whereas it was between 2.5 and 20.0 ppt in southeast region and total ammonia-nitrogen higher than the recommended level for shrimp farming in Bangladesh. Claude and Gross (2014) studied use of probiotics for improving soil and water quality in aquaculture ponds and reported very few positive benefits of probiotics to water and bottom soil quality.

Panigrahia *et al*. (2018) studied Carbon : Nitrogen (C:N) ratio influenced microbial community of the system and growth as well as immunity of shrimp (*L. vannamei*) in biofloc based culture system. The trend of *Vibrio* dominance decreased with the increase in C:N ratios and thus confirming the dominance of heterotrophic bacteria in high C:N ratio groups. Upon challenge with pathogens, shrimps from C:N10, C:N15 and C:N20 groups showed significantly higher survival (p<0.05) compared to the C:N5 and control groups. Sheng *et al*. (2021) studied performance of *Platymonas* and microbial community analysis under different C/N ratio in biofloc technology aquaculture system. *Platymonas* sp. and C/N ratio significantly affected species diversity and stock richness. *Platymonas* sp. and related bacteria in bioflocs had a beneficial effect on water quality by reducing nitrogen compounds, providing a favorable environment for certain groups of bacteria, reducing dependence on higher concentrations of carbon sources. Fontenot *et al*. (2007) reported the effect of temperature, salinity and carbon:nitrogen ratio on a sequential reactor treating shrimp aquaculture wastewater. The results showed that a salinity of 28-40 ppt, a temperature range of 22-37oC and a C:N ratio of 10:1 gave the best results in terms of maximum nitrogen and carbon removal from wastewater. Asaduzzaman *et al*. (2010), C/N ratio and substrate addition in natural trophic communities in freshwater shrimp ponds, increased C/N ratio was significantly associated with biomass of plankton, periphyton, heterotrophic bacteria, and benthic macroinvertebrates. However, pond communities were underutilized by freshwater shrimp. Therefore, it is recommended to further investigate the possibility of reducing the artificial feeding or increasing the shrimp stocking density. Chakrapani *et al*. (2020) conducted an experiment for the Pacific white shrimp *Penaeus vannamei* with three different C:N ratios under practical conditions that considered estimated growth efficiency, immune response and metabolic pathways. However, the results showed that shrimp grown in C/N 10 (630 mg) and C/N 15 (646 mg) biofloc systems showed significantly faster growth compared to C/N 20 (528 mg) and the control group (374 mg). Regarding extracellular enzyme production, protease, lipase and xylanase were found to be mostly present in colonized bacteria isolated from biofloc treatments, while amylase was commonly found in all treatments.

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