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

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**Aquaculture and Shrimp farming**

Looking into the current rate of population explosion and rate of anthropocentric encroachment of land, scientists have put forward a concern of challenges in availability and production of food to meet the global demand. Agriculture sector alone wouldn’t be able to cope with such a huge demand for a global population of 7.6 billion which in no way seems to come down. 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 aquaculture industry’s sub-sectors with the fastest global growth is marine fin/ shellfish cultivation. India’s geographical area boasts an expansive 8,118 kilometers of coastline and a 2.02 million km2 Exclusive Economic Zone (EEZ). The socio-economically underdeveloped artisanal and small-scale fishers, whose lives are intimately entwined with the ocean and sea dominating the marine fisheries sector. 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), during the period from 1961 to 2017, the average annual growth rate of total food fish consumption increased at 3.1%, outpacing annual population growth rate to an extent of 1.6%. In per capita terms, food fish consumption rose from 9.0 kg (live weight equivalent) in 1961 to 20.3 kg in 2017. Aquaculture can control the over exploitation, create employment and supply protein food to the world. The Indian government plans to increase fish production from 137.58 lakh metric tons in 2018-19 to 220 lakh metric tons by 2024-25 under the Pradhan Mantri Matsya Sampada Yojana (PMMSY) scheme of Department of Fisheries, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India.

**Important shrimp species in aquaculture**

Crustacean mainly, Tiger prawn (Penaeus monodon), Indian white prawn (Penaeus indicus), White leg shrimp (Penaeus vannamei), Redtail shrimp (Penaeus penicillatus), Banana prawn (Penaeus merguiensis), Green tiger prawn (Penaeus semisulcatus) are commercially important shrimps used in coastal aquaculture. The classification of the Pacific white shrimp or white leg shrimp, *Litopenaeus vannamei* (Boone, 1931), is under Phylum Arthropoda, Class Crustacea, Order Decapoda, Family Penaeidae, Genus *Litopenaeus* and Species *vannamei* (Perez and Kensley, 1997). It is native to the western Pacific tropical coast of Latin America, from southern Mexico in the north to northern Peru in the south, between latitude 32°N and 23°S. This penaeid is highly abundant along the coast of Ecuador to Esmeraldas (the border Province of Columbia) where gravid females are available year-round (Huang *et al*., 2003). The *Litopenaeus vannamei* is extremely euryhaline, capable of inhabiting low salinity waters of 1 to 2 psu as well as hypersaline waters of 40 psu (Menz and Blake, 1980). Fry and juveniles inhabit muddy bottoms in warm waters (25 to 32°C) having a salinity range from 28 to 34 psu, with a depth of 70 cm, wherein they exhibit burrowing behaviour. Adults prefer higher salinity of 34 to 35 psu and prefer a little deeper water (30 to 50 m). Abundance of juvenile stages presented an inverse correlation with salinity and a positive correlation in a coastal lagoon system in Mexico (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.

In India, shrimp farming was first conducted only on an experimental scale. A major step toward large scale shrimp aquaculture took place soon after the first use of brackish water fish farming was demonstrated in West Bengal by the Central Inland Fisheries Research Institute affiliated to Indian Council of Agricultural Research (ICAR) in 1973. Subsequently, an ICAR coordinated research project on brackish water aquaculture was sanctioned throughout India during 1975 in West Bengal, Andhra Pradesh, Odisha, Tamil Nadu, Goa and Kerala. Simultaneously, successful production of shrimp seed was demonstrated in Narakkal in Kerala by the Central Marine Fisheries Research Institute (Vijayan and Kailasam, 2020). Commercial shrimp hatcheries were established by the Marine Products Export Development Authority. Semi-intensive culture technology was also demonstrated on a pilot-scale project by the MPEDA (Muralidharan, 2019). As shrimp aquaculture spread throughout India, these technologies together with other experimental efforts by farmers were successful and allowed for the large-scale development of this industry.

Biofloc system has been developed in order to improve the environmental control over the aquatic animal production. In aquaculture, the strong influential factors are feed cost (60% of total cost) and most limiting factor is the water and land availability. The principle of this technique is the generation of nitrogen cycle by maintaining higher C/N ratio (Carbon and Nitrogen ratio) through stimulating heterotrophic microbial growth which assimilates nitrogenous waste that can be exploited by cultured species as feed. Higher C/N ratio is maintained by the addition of carbohydrate source (molasses) and the water quality is improved through production of high quality single cell protein. Immobilization of toxic nitrogen species occurs more rapidly in biofloc technology (BFT) as microbial production per unit substrate of heterotrophs are 10 times greater than autotrophic bacteria. Due to bottom dwelling habit of shrimp and its resistance to environmental changes, the technology was implemented 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 production of shrimp. However, shrimp aquaculture industry has encountered a variety of problems such as germplasm degradation, disease outbreaks and water quality deterioration which have seriously hindered its further development (Bachere, 2000; Thitamadee *et al.,* 2016). In addition, the large amount of water exchanges in the process of aquaculture not only causes nutrient loss but also results in serious pollution to the surrounding environment (Bachere, 2000).

In intensive aquaculture systems, waste generated mainly faeces and unconsumed feed will induce the accumulation of toxic metabolites like ammonium and nitrite, spoiling the living environment of the shrimp (Avnimelech and Ritvo, 2003; Piedrahita, 2003). Increasing the C/N ratio to promote heterotrophic microbes in pond wastewater systems that controls the water quality by removal of toxic nitrogen like ammonia. Heterotrophic microbes are primarily responsible for carrying out the necessary functions in biofloc system and their ammonium fixation by heterotrophic bacteria occurs much more quickly due to their faster growth rate and higher microbial biomass yield per unit substrate than that of nitrifying bacteria. The bacterial protein produced by ammonia nitrogen assimilation should be an adequate protein, lipid, carbohydrate and ash content as a quality aquaculture feed. When organic matter decays, the carbon is dissipated more rapidly than the nitrogen, thus bringing down the carbon-nitrogen ratio. 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 be monitored in the cultivation of aquatic organisms. Biofloc technology is defined as the ‘use of aggregates of bacteria, algae or protozoa, held together in a matrix along with particulate organic matter for the purpose of improving water quality, waste treatment and disease prevention in intensive aquaculture systems’ (Avnimelech, 1999). In addition to oxygen requirements of shrimp or fish being cultivated, the rich microbial community also consumes DO at a significant rate. The intensity of DO consumption by the microbial community is largely a function of feed inputs required for particular stocking density (Boyd, 2009).

The microbial community is responsible for cycling excess nutrients. In such systems, particulate matter is often removed by external filtration such as sedimentation, vortex devices and sand filters. However, in biofloc systems, particles are allowed to form within the culture system and a portion of microbial community responsible for nutrient cycling is contained within those particles. The primary principle of carbon and nitrogen is to reduce water exchange and promote heterotrophic organisms by utilising waste nitrogen that is assimilated within the pond or tank. The C/N ratio regularly fallowed in biofloc aquaculture system by addition of organic carbon as a source to the animal tank which is utilized by the inorganic nitrogen to aggregate the microorganisms. A balanced carbon to nitrogen ratio in the feed is essential to maximize the growth of heterotrophic bacteria. It 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, wherein 10 Carbon sources are required to kill 1 Nitrogen. In aquaculture, the C/N Ratio is 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 will be converted into bacterial biomass (Schneider *et al*., 2005). Management of water quality is of primary consideration in aquaculture ponds with higher stocking rates. Degradation of water quality is detrimental to growth and survival. Good quality water is usually defined as the fitness or suitability of water for survival and growth of shrimp. By adding carbohydrates to the pond, heterotrophic bacterial growth is stimulated and nitrogen uptake through the production of microbial proteins takes place (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). Management of water quality is of primary consideration in aquaculture ponds with higher stocking rates. Degradation of water quality is detrimental to growth and survival. Good quality water is usually fitness or suitability of water for survival and growth of shrimp. By adding carbohydrates to the pond, heterotrophic bacterial growth is stimulated and nitrogen uptake through the production of microbial proteins takes place (Avnimelech, 1999). Wujie *et al*. (2016) studied effects of C/N ratio for biofloc development followed by water quality and performance of *L. vannamei* juveniles in a biofloc-based high density, zero-exchange, outdoor tank systems. They reported that, the volatile suspended solids (VSS) and turbidity values were superior quantifying parameters for quantitative determination of biofloc than suspended solids (SS) or total suspended solids (TSS). The TAN and NO2 concentrations could be effectively controlled by heterotrophic assimilation. Autotrophic nitrification that helps maintain their concentrations at acceptable ranges for shrimp culture even at high stocking densities. Microalgae and autotrophic bacteria are more beneficial for shrimp performance in high density zero exchange culture systems than heterotrophic bacteria. Muthusamy *et al*. (2016) reported reduced total ammonia-nitrogen while maintaining good water quality for shrimp culture in biofloc farming 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. Addition of *Platymonas* sp. and C/N ratio affected significantly the species diversity and richness of the flocs. The supplementation of the *Platymonas* *sp*. and its associated bacteria in the bioflocs performed beneficial effects on the water quality by reducing the nitrogenous compounds providing a favorable environment for certain bacterial groups thereby reduced dependence on higher content of carbon sources at the same time. Fontenot *et al*. (2007) reported effects of temperature, salinity and carbon: nitrogen ratio on sequencing batch reactor treating shrimp aquaculture wastewater. The results indicated that the salinity of 28 to 40 ppt, temperature range of 22 to 37oC, and a C:N ratio of 10:1 produced best results in terms of maximum nitrogen and carbon removal from the wastewater. According Asaduzzaman *et al*. (2010), the C/N ratio and substrate addition on natural food communities in freshwater prawn monoculture ponds, an increased C/N ratio with biovolume of plankton, periphyton, heterotrophic bacteria and benthic macroinvertebrate had significant relations. However, the availability of pond communities noticed to be under-utilized by the freshwater prawn. Thus, suggesting further investigation on the possibility of decreasing artificial feeding rate or increasing in stocking density of prawn. Chakrapani *et al*. (2020) conducted experiment on three different C : N ratios for Pacific white shrimp, *Penaeus vannamei* under practical conditions where the evaluated growth performance, immune response and metabolic pathways were considered. However, the results showed that shrimps reared in C/N 10 (630 mg) and C/N 15 (646 mg) biofloc systems showed significantly higher growth when compared to C/N 20 (528 mg) and control (374 mg) groups. With regard to the production of extracellular enzymes, protease, lipase and xylanase have been found to be predominantly in colonized bacteria isolated from biofloc treatments, whereas amylase was commonly found in all the treatments.

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