Thesis for the Degree of Master of Fisheries Science

Microbial Ecosystem Management – a Sustainable Approach to Aquaculture

by

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미생물 생태계의 관리 – 양식을 위한 지속가능한 접근방법

Advisor: Prof. Christopher L. Brown

by

Rhoda Kafui Akpoh

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List of Abbreviations

AHPND: Acute Hepatopancreatic Necrosis Disease

BFT: Biofloc Technology

C/N: Carbon/Nitrogen

DO: Dissolved Oxygen

DOM: Dissolved Organic Matter

EAAI: Essential Amino Acid Index

EMS: Early Mortality Syndrome

FCR: Feed Conversion Ratio

FTS: Flow Through System

PER: Protein Efficiency Ratio

RAS: Recirculation Aquaculture System

TAN: Total Ammonia Nitrogen

WSSV: White Spot Syndrome Virus

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Abstract

Over the years, aquaculture has emerged to be one of the most successful ways to produce aquatic species to make up for the declining capture fisheries production. While increasing production through aquaculture, it is of great importance to consider sustainability and profitability by reducing the use of antibiotics, fishmeal and fish oil. A number of technologies have been developed for sustainable aquaculture. Biofloc technology and green water technology are microbial management methods that can be used to control disease outbreaks while ensuring growth, health and survival of the cultured species. It can also provide nutrition in a sustainable manner. Over a decade ago, the shrimp industry suffered a series of massive outbreaks of disease commonly known as Early Mortality Syndrome (EMS) or acute hepatopancreatic necrosis disease (AHPND), which was devastating and costly. The use of green water for aquatic animal culture greatly mitigated the effect of *Vibrio parahaemolyticus* which causes EMS/APHND as well as white spot syndrome virus (WSSV). White shrimps reared in water containing microbial biofloc produced improvements in growth and survival as compared to those reared in clear or natural pond water. Also, biofloc redirects waste nitrogen into the sustainable biosynthesis of amino acids that are used as a protein source for aqua cultured organisms. In this regard, a formerly problematic waste material – water contaminated with ammonia can serve as a resource in the culture of shrimps, tilapia, mussels and other aquatic organisms. Therefore, biofloc, green water culture, recirculation aquaculture system (RAS) and other microbial management methods could discourage pathogens, reduce stress and contribute to sustainable aquatic ecosystems.

Keywords: Microbial ecosystem, biofloc technology, recirculation aquaculture system, green water culture

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I. Introduction

Aquaculture has roots in ancient Egypt and China, but modern fish and crustacean production has struggled with outbreaks of pathogens. The issue of nutrient discharges, excessive reliance on fish meal, and habitat degradation have also been the subject of concerns for aquaculture sustainability. For example, intensive Penaeid shrimp culture, has been plagued with viral epidemics thereby necessitating the promotion of routine applications of antibiotics and sterilization of culture systems to control pathogens (Wickins and Lee, 2002). Such combative approaches to pathogens were marginally and temporarily effective compared to contemporary alternative methods of managing microbial ecosystems with enduring effectiveness.

Aquaculture has emerged as one of the most successful ways to produce aquatic species to make up for the declining capture fisheries production (FAO, 2019; 2020). A number of technologies have been developed to contribute to the sustainable aquaculture such as biofloc technology among other technologies.

The biofloc technology focuses on the use of nutrient input by recycling nutrient rich water and maintaining the Carbon/Nitrogen (C/N) ratio, which converts ammonia into microbial biomass in order to promote heterotrophic bacterial growth (Avnimelech, 1999). These heterotrophic bacteria form the basis for microbial communities in which many species of cultured fishes and invertebrates can thrive.

The bacteria in the biofloc system could be beneficial or detrimental to the health of the aquatic species being cultured. Hence, it is very important to monitor and manage the microbial community. The microbial biomass together with other suspended particles and microorganisms aggregate into flocs that cultured species can feed on and, in some cases, can be processed for use as feed ingredients (Kuhn *et al.,* 2010). These microbial flocs are highly enriched with proteins which provide an adequate supply of amino acids to support the growth and development of cultured species (Avinmelech *et al.,* 1994; Chamberlain *et al.,* 2001a; Tacon *et al.*, 2002). According to studies done using *Artemia sp.*, (brine shrimp), it has been demonstrated that the biofloc technology (BFT) not only redirects waste nitrogen from constructive use, it can also create an environment that discourages pathogenic bacteria (Crab *et al.*, 2010b).

The success or failure of the culture system and the aquatic species being cultured greatly depends on species characteristics and on the management of the microbial community. The system must be run effectively to discourage dangerous pathogens which could adversely affect the cultured subjects. The system should be managed in such a way that; it will have benign bacteria in order to establish a matured microbial system devoid of harmful pathogens. Hence, attention must be paid to the type of bacteria present in the rearing tank. The rearing tank must have more k-selected than r-selected bacteria because it provides an environment where important interactions occur between the aquatic organisms, which is consistent with the characteristics of mature microbial ecosystems. This is so because the microbial environment comprises of organisms inhabiting biofilms, live feed, the water column and other colonizing microorganisms (Bourne *et al.,* 2004).

One of the major problems encountered in aquaculture is the low survival of larvae which could be strongly attributed to the rearing conditions. Recent study shows that the poor performance and high death rate of larvae is frequently due to unhealthy larvae-microbiota interactions (Vadstein *et al*., 2013). Aquaculture industries producing on large scale have been reported to incur great losses from low survival rates of 10-15% or lower (Vadstein *et al*., 2013). As one means of addressing this problem, good microbial management techniques can improve the outcomes in the rearing of larvae.

This study seeks to review the effects of using biofloc, green water and Recirculation Aquaculture Systems (RAS). with matured microbial ecosystems as microbial management systems for culturing aquatic species.

1. Overview of microbial management

Pathogens including but not limited to bacteria, viruses and fungi are found in mostly every environment including the aquatic environment. It is crucial to pay attention to the environment of aquatic animals in order to avoid exposure to harmful pathogens that impact aquatic animals negatively. Most aquatic organisms are vulnerable to pathogens that cause high mortality rates, affect growth and compromise the health condition of the animals. The aquaculture industry over some decades has been struggling with control of pathogens and has in the past overused disinfection, sterilization and antibiotics as the best way to control pathogens. It turns out that this way of preventing the spread of diseases and pathogens has done more harm than good. Disinfection creates an unstable environment and gives advantages to the r-selected microbes including many that are pathogenic. Biofloc technology has been recommended as a practical approach to dealing with harmful pathogens. Work done by Wasielesky *et al.,* (2006) confirmed that white shrimps reared in water containing microbial biofloc produced better results as compared to white shrimps reared in UV filtered water and clear water.

II. Biofloc Technology

Biofloc technology (BFT) is an emerging technology that uses sustainable and eco-friendly means to culture aquatic species. This technology is an innovative way of fully making use of wastewater to establish a matured microbial environment which can be used to raise aquatic species while serving as a source of food. The process is based on formation of microbial biomass using Nitrogen from waste that cultured species utilize as feed (Kuhn *et al.,* 2010). Harmless heterotrophic bacteria are formed by manipulating the C/N ratio in the form of adding carbohydrate source to a nitrogen-rich aquatic environment (Avnimelech, 1999).

Figure 1: Biofloc system (nammientrung.com)

1. Biofloc use in the shrimp industry

Biofloc technology even though not well known and has limited usage in the aquaculture industry, it has become very popular in the shrimp farming industry. The emergence of viral diseases and the rising cost of energy makes biofloc technology suitable for sustainable and viable production. Biofloc technology has been used in the nursery, grow-out and breeding phases of different species of shrimps including *L. vannamei* (pacific white shrimp), *P. monodon* (black tiger shrimp) *and F. paulensis* (pink shrimp). The use of this technology has helped manage diseases as it serves as a natural probiotic for mitigating the effect of viral diseases. It has also been applied successfully in aquaponics.

1-1. Nursery and grow-out

It was observed that the application of biofloc with commercial feed in the early post larval stage of pink shrimp increased the body weight by 50% and close to 80% in the final biomass as compared to the conventional clear-water system (Emerenciano *et al.,* 2011). An increasing growth trend was also observed in the culture system where the postlarvae were not fed commercial feed but just biofloc (Emerenciano *et al.,* 2011). The continuous availability of biofloc in the nursery system enhanced growth.

Survival rate is of great importance to every farmer especially in the nursery phase and every farmer strives for crops with survival close to 100%. Biofloc technology is well known to increase survival in a variety of cultured species. In a biofloc technology (BFT) nursery of *L. vannamei*, survival rate was reported to have ranged from 55.9% to 100%. (Mishra *et al.,* 2008).

In comparison with other conventional rearing methods where survival and growth are sometimes dependent on the stock density, with biofloc technology, the stocking density has negligible effect on the survival and growth rate. This gives credence to a study by Arnold *et al.,* (2009) where *P. monodon* postlarvae grown in BFT at stocking densities of 2500 and 5000 shrimp m^{-3} had no effect on the survival and growth rates. Hence, there is the potential of achieving greater production outputs at higher densities.

Fish and other species have also shown encouraging results in the use of biofloc technology. There has been improvement in yields, maximum utilization of feed and lower FCR. For example, Avinmelech *et al.,* (1994) reported a higher feed utilization by tilapia reared in BFT as compared to a water exchange system.

1-2. Breeding

The shrimp industry suffered great losses due to viral diseases in the 1990's and early mortality syndrome (around 2012-2015). As a result of this, biosecurity was crucial in the breeding of shrimps which encouraged breeding in closed facilities and the use of closed life cycle brood stock. This generated a new focus on disease resistant shrimps and shrimps with good reproductive performance and less focus was put on broodstock nutrition, which remains suboptimal (Wouters *et al.,* 2001). The realization that broodstock nutrition is a major contributing factor to obtaining quality offspring, has brought the importance of proper and adequate nutrition to the fore for farmers consideration.

However, food cost which makes up a big proportion of operating cost is a limiting factor to providing good and adequate nutrition. Alternative nutrition technologies such as BFT, could provide natural food for the shrimps and improve reproductive performance of breeders due to the rich protein and fatty acid constituents (Anand *et al.,* 2014), as well as huge quantities of minerals present in the flocs (Tacon *et al.,* 2002). These nutrients help in early gonad formation and ovary development in young breeders. Studies by Emerenciano *et al.,* (2011) evaluated the spawning performance of shrimps reared in floc and earthen ponds. The results revealed that floc-reared *L. Stylirostris* broodstock performed better than those reared in the earthen pond in terms of spawns per female. Additionally, Emerenciano *et al.,* (2013) reported that pink shrimp broodstock reared in biofloc with fresh food had a higher number of eggs per spawn and good egg size and fatty acid profile of eggs compared to shrimps reared in clear water with fresh food. The better reproductive performance of shrimp brood stock raised in biofloc justifies the application of the technology.

1-3. BFT as a tool for managing diseases

In contrast to conventional approaches such as antibiotic and probiotic application used in the management of disease incidents, BFT is a sustainable method used to prevent or mitigate disease occurrences. It has been used to control viral diseases such as WSSV (Tendencia *et al.,* 2012) and EMS/APHND (De Schryver *et al.,* 2014) which has helped the resilience of the industry. It also has antibacterial compounds that inhibit the growth of harmful bacteria (Kokou *et al.,* 2012). The presence of beneficial organisms makes it possible for BFT to be used in controlling diseases.

2. Aquaponics

BFT application in aquaponics is quite new although it has been used in the commercial production of tilapia and lettuce (Rakocy 2012). Relatively few people are knowledgeable on how to raise aquatic animal and plant species together using BFT. Although Pinho *et al.,* (2017) was successful in improving the yield and visual quality of lettuce using BFT compared to clear-water recirculation system, many attempts by others in using BFT with aquaponics have failed (e.g., Rahman 2010; Pinho, 2018). This could be partly attributed to the presence of high concentration of solids which has been found to damage plants roots resulting into poor growth (Barbosa 2017). The variation in results using this approach suggests that technical refinements in this field will require further investigations.

3. Recycling ammonia into food

The success or failure of a biofloc system largely depends on the ability to control, remove or recycle ammonia in the culture system (Souza *et al.,* 2019). Accumulation of harmful nitrogenous substances even at lower levels could adversely affect cultured species in a BFT system. Accumulations as low as 0.02 mg/l for TAN and 2 mg/l for nitrates has the ability to negatively affect growth performance of species cultured (Bregnballe, 2010). It is therefore crucial to eliminate or recycle ammonia to ensure safety of cultured species, a process successfully done by BFT. Nitrogenous substances as a result of excretory products or leftover feed are usually recycled by microorganisms; heterotrophic bacteria, autotrophic bacteria and algae, which differ in ways of recycling waste (Martinez-Cordova *et al.,* 2015).

Due to the benefits derived from using heterotrophic bacterial method for removal of ammonia in BFT system, this strategy has been the focus of most studies. The role of heterotrophic bacteria to covert nitrogen compounds into protein that serves as a source of food makes it suitable for recycling of waste (Hargreaves, 2013) and the process is largely reliant on the C/N ratio. Mass action effects of an abundance of carbon drive the microbial synthesis of amino acids comprising peptides and proteins. This process results in the reduction in levels of toxic nitrogenous compounds in the aquatic environment.

4. C/N Ratio

Ammonia concentration in fish culture system has been of major concern to most fish farms. Ammonia accumulation is lethal to the health of cultured animals, generally resulting in death and other serious health impairments. The common practice of farmers in dealing with the control of accumulated ammonia in the culture system is to discharge water from the system and add fresh water to reduce the ammonia concentration, which can be very disruptive to the natural water ecology (Crab *et al.,* 2007). Biofloc technology is a water management approach that can be used to solve this problem by manipulating the C/N ratio. Studies have suggested (e.g., Avnimelech 1999; De Schryver *et al.,* 2008) that manipulating the C/N ratio by adding an abundance of organic Carbon will induce the growth of heterotrophic bacteria in the water, which makes use of excess Nitrogen for amino acid synthesis, thereby reducing the ammonia concentration as well as, leading to the formation of microbial biomass which could serve as a source of food to the cultured species. This technique greatly improves the water quality while providing food for the cultured species. Azhar *et al.,* (2016) showed that the use of a higher C/N ratio readily removes greater quantities of Total Ammonia Nitrogen (TAN) from the culture system and improves the conditions and those nuances of this process are affected by the specific type of organic Carbon source.

The type of carbon source used greatly affects the C/N ratio. The level of C/N ratio influences the microbial biomass and the amount of Total Ammonia Nitrogen eliminated from the culture system. Below is a table of different carbon sources used in a variety of experimental applications to produce biofloc.

Table 1: Different organic carbon sources used in biofloc

Reference	Carbon source	Species
Crab *et al.,* 2010	Acetate Glycerol Glycerol + Bacillus Glucose	Prawn (*Macrobrachium rosenbergii*)
Azhar *et al.,* 2016	Molasses Tapioca Tapioca by product Rice bran	Whiteleg shrimp (*Litopenaeus vannamei*)
Akeem *et al.,* 2017	Sucrose Glycerol Rice bran	African catfish (*Clarias gariepinus*)
Farideh *et al.,* 2018	Corn starch	Common carp fingerlings (*Cyprinus carpio L.*)
Soria Diaz, 2019	Honey	Zebrafish (*Danio rerio*)
Asmaa *et al.,* 2020	Mannanoligosaccharide	Nile Tilapia (*Oreochromis niloticus*)
Guo *et al.,* 2020	Sucrose	Whiteleg shrimp (*Litopenaeus vannamei*)

As Azhar *et al.,* (2016) observed, the type of carbon source used to produce biofloc strongly influences the growth of heterotrophic bacteria in the system. Heterotrophic bacteria play a significant role in removing ammonia from the water to improve the water quality whilst also serving as a source of food for the cultured species. Due to the role of the heterotrophic bacteria, it is necessary to consider the complexity of the carbon sources and their ability to utilize it. Complex carbohydrates require more time for decomposition as compared to simple carbohydrates and the latter is more effective in controlling the total ammonia concentration in a system (Avinmelech 2009). Molasses which is the simplest carbohydrate source used in the experiment showed the highest level of reduction of total ammonia nitrogen at any C/N ratios tested.

Mannanoligosaccharide as a carbon source in biofloc water used to rear *Oreochromis niloticus* readily decreased *Vibrio, Aeromonas* and *Pseudomonas spp*. which was interpreted as an indication of the presence of antibacterial compounds in the carbon source (Asmaa *et al.,* 2020). Mannanoligosaccharide decreased the total ammonia nitrogen and nitrate as well as increasing the volume of the biofloc which served as a source of natural food for the tilapia as compared to the control experiment. Nile tilapia reared in biofloc with mannanoligosaccharide improved performance and immune response as compared to the control. It can be concluded that adding a carbon source can influence the composition of the biofloc and affect the fish health and performance (Asmaa *et al.,* 2020).

5. Nutritional Composition of Biofloc

Biofloc serves as a source of food rich in nutrients and improves the efficiency of feed utilization. Anand *et al.,* (2014) showed that biofloc is rich in protein and fatty acids and improves Feed Conversion Ratio (FCR) as well as Protein Efficiency Ratio (PER) of cultured shrimps. The experiment was conducted using *P. monodon* to measure the effect of dietary supplementation of biofloc on the growth performance parameters and digestive enzyme activities. Experimental treatments supplemented with biofloc outperformed the controls. Ekasari *et al.,* (2014) demonstrated that based on the Essential Amino Acid Index (EAAI) shrimps could utilize biofloc as a good quality protein source; as also observed in tilapia and mussels. Tacon *et al.,* (2002) also discovered high levels of ash in the body of Pacific white shrimp fed biofloc. These findings result from the abundance of mineral elements in biofloc, which can be beneficial by serving as micronutrients to the cultured species.

6. Waste management and reduced water utilization in Biofloc System

The use of biofloc systems has significantly reduced the use of water in aquaculture and has led to near zero water exchange, which is a very effective way to deal with pollution from effluent from aquaculture farms through the establishment of matured microbial ecosystems. This system of production has been used extensively in the shrimp industry to mitigate disease problems as well as pollution problems. Hargreaves (2006) discovered that the use of biofloc system that requires zero water exchange reduced water used to raise 1 kg shrimp in $78m^3$ as compared to the conventional water exchange method. Also, Luo *et al.,* (2014) reported that tilapia reared in Biofloc water used 40% less water as compared to those reared in the Recirculated Aquaculture System (RAS). They also reported that the level of Nitrogen and Phosphorus waste in the RAS system was higher than that of the biofloc system. It can be deduced clearly from these findings that the use of Biofloc systems produced beneficial results that could be highly applicable within the aquaculture industry.

7. Microbial Community in biofloc

Microbial communities comprise primarily of phytoplankton populations mixed with bacteria and some species of algae, as well as living and non-living organic matter. Microbial management creates an unconducive environment for harmful pathogens which have the ability to negatively affect the health and survival of cultured species. A critical study of the r- and K- selected bacteria is crucial in biofloc technology. The r- strategist bacteria include dangerous opportunistic pathogens which have rapid growth rates whiles the K- strategist bacteria are generally harmless, with much slower growth rates. There are numerous interactions of microbes and larval culture subjects, and it is important to strive for a stable and matured microbial ecosystem which limits the propagation of opportunistic bacteria within the system. Meanwhile, it is possible to control and stabilize the microbiota in a rearing tank through a selection regime against some microbes and favouring others (Attramadal *et al.,* 2014).

Larvae actively take up bacteria and algae at rates higher than their drinking rates (Reitan *et al.,* 1998). Hence, particular attention must be paid to the type of bacteria present within a rearing system. According to Hess-Erga *et al.,* (2010) disinfection of rearing water reduces bacterial numbers and the death of organisms leads to an increased dissolved organic matter (DOM) which becomes available to fast-growing r-strategist microbes and can lead to blooms of these harmful bacteria. The reduction in DOM supplied, leads to the selection of K- strategist bacteria due to competition for resources (Rojas-Tirado *et al.,*2017). K-selection in a rearing environment selects against detrimental r-strategist microbes and promotes a healthy microbe larvae-interaction. Changes observed include earlier onset of growth of larvae, faster growth of larvae and higher rate of survival in larvae (Attramadal *et al.,* 2012a). Conversely, r-selected bacteria reduce survival of larvae in the hatchery. This is supported by Attramadal *et al.,* (2012b) who reported a 40% reduction in survival of Atlantic cod larvae as a result of disinfection of rearing water, which led to the selection of fast-growing opportunistic bacteria.

The use of biofloc microbial management approach has been very beneficial to the aquaculture industry but not all aquatic species are comfortable with having increased total suspended solids in their environment. This makes biofloc culture not universally applicable but it has been of huge advantage to the shrimp industry and numerous finfish species.

III. Green water technology

Green water technology is reliant on water rich in phytoplankton and monocellular algae populations for culturing aquatic species including some fish species, molluscs and crustaceans. Shrimp has proven to be the most suitable group of cultured subjects for this culture technique, and tilapia fingerlings also thrive in green water environments.

The green water environment is an enriched ecosystem for larval and other culture subjects, in part because the abundance of monocellular algae shifts the balance of dissolved gases to favor oxygen, and decreases in the concentration of Carbon Dioxide. Microalgae also absorb sulfur and thereby reduce environmental exposure to sulfates (Krishnani *et al.,* 2010), thereby improving water quality for developing culture subjects.

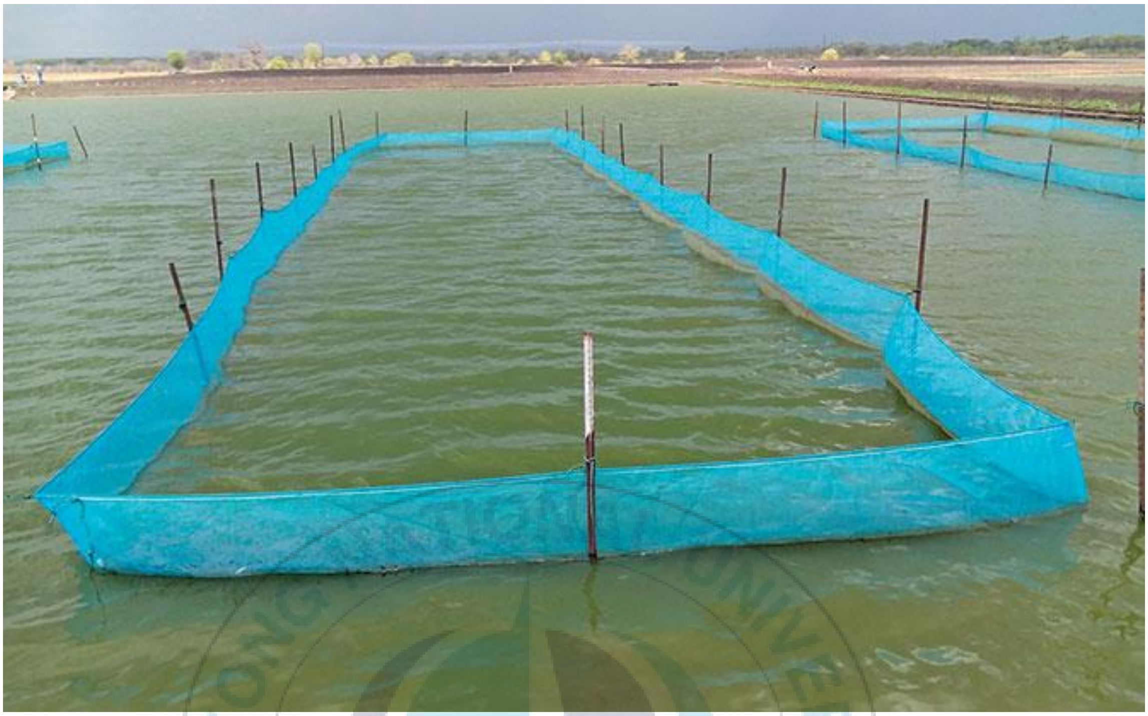

Figure 2: Green water fish culture (farmersweekly.co.za)

Table 2: Experiments using green water culture technology

Reference	Effect of green water
Tendencia *et al.,* (2012)	Controlled the effect of White Spot Syndrome Virus (WSSV) in tiger shrimp (*Penaeus monodon*)
Kokou *et al.,* (2012)	Antibacterial compounds
Appenteng (2019)	Improved larval rearing conditions of zebrafish (*Danio rerio*)
Lio-Po *et al.,* (2005)	Prevented the outbreak of luminous vibriosis among tiger shrimp (*Penaeus monodon*) and reduced mortality.
Cadiz *et al.,* (2016)	Controlled the growth of green colony-forming *Vibrios* and *Vibrio parahaemolyticus* in the intensive tank culture of whiteleg shrimp (*Penaeus vannamei*)

1. Use of green water in the shrimp industry

Over a decade ago, the shrimp industry suffered an outbreak of a disease commonly known as the Early Mortality Syndrome (EMS) or acute hepatopancreatic necrosis disease (AHPND) which was devastating to the industry. It was first reported in Southern China and then subsequently reported in Vietnam, Thailand and Malaysia (FAO 2013). EMS was eventually found to be caused by a bacterium, specifically

Vibrio parahaemolyticus (Tran *et al*., 2013). The bacteria are known to affect shrimp post larvae and can cause up to 100% mortality, with estimated shrimp industry losses amounting to USD 1 billion (GAA 2013). De Schryver et al., (2014) argue that the use of green water for microbial management could be a key factor in solving the outbreaks of EMS/APHND. The normal practice of pond disinfection prior to stocking does more harm than good to the cultured species. Disinfection disturbs the microbial community and produces a lot of nutrients which are utilized by the fast-growing opportunistic bacteria such as the *Vibrio spp.* which recolonize the system (Attramadal *et al*., 2012). This practice of pond disinfection has affected the shrimp industry greatly by causing proliferation of the infectious *Vibrio parahaemolyticus* which causes EMS/APHND. Growing aquatic species in a green water ecosystem and applying biocontrol strategies have been found to be a highly effective method to mitigate disease outbreak (De Schryver *et al.,* 2014).

As Tendencia *et al.,* (2012) showed, green water was effective in controlling white spot syndrome virus (WSSV) in *Penaeus monodon*. The experiment was conducted to investigate the effect of different culture systems against WSSV. Results led to the conclusion that WSSV decreased from an original load of (1.40×10^1) WSSV/mg sample to (7.0×10^0) WSSV/mg in shrimp cultured in green water, as compared to the control and other treatments in which the viral load quadrupled. The rate of WSSV infection was significantly lowest in *Penaeus monodon* cultured in green water.

Kokou *et al.,* (2012) also reported that microalgae in green water have antibacterial compounds that inhibit the growth of bacteria. Different microalgae cultures (*Chlorella minutissima*, *Tetraselmis chui*, *Nannochloropsis* sp., *Arthrospira platensis* and *Isochrysis* sp.) were used in the experiment to test their antibacterial activity on six *Vibrio* bacterial strains (*V. parahaemolyticus, V. anguillarum, V. splendidus, V. scophthalmi, V. alginolyticus and V. lentus*). Results obtained indicated that the colonies of four bacteria strains including *Vibrio parahaemolyticus* were not detected in the culture system whereas; in the control groups the bacteria load kept increasing till the end of the experiment. *V. alginolyticus* and *V. anguillarum* were not completely eliminated but their population in the green water culture system was lower as compared to the control. The authors concluded that green water helps eliminate harmful bacteria and reduces their population in a culture system.

Green water environments are effective in the hatchery production of wild-type zebrafish (*Danio rerio*). Appenteng (2019) reported that larval rearing in green water presents a cost-effective alternative to conventional zebrafish hatchery technology, which frequently involves the relatively costly and labor-intensive culture of live feed organisms such as rotifers (*Brachionus plicatus*).

Experiment by Lio-Po *et al.,* (2005) showed that the use of green water for culture of *Penaeus monodon* was effective in preventing the outbreak of luminous

Vibriosis among tiger shrimp, resulting in reduced mortality. From the study, it was observed that the green water in the grow-out pond used for the culture had bacterial, fungi and phytoplankton populations that included anti-luminous Vibrio factors. The presence of these factors in the green water reduced the population of the luminous *Vibrio* population and prevented the infection of the cultured tiger shrimp with the disease.

Tilapia green water controlled the growth of green colony-forming *Vibrios* and *Vibrio parahaemolyticus* in the intensive tank culture of *Penaeus vannamei* (Cadiz *et al.,* 2016). The tilapia grown in green water had lower densities of attached culturable *Vibrios* including *Vibrio parahaemolyticus* as compared to the other treatments. The findings in this study provide the basis that the proliferation of potentially pathogenic bacterial species could be controlled using green water to culture aquatic species. Nile tilapia (*Oreochromis niloticus*) fingerlings produced in green water in the Philippines sell for higher prices than those grown in conventional culture systems (Brown, C.L.; personal communication).

IV. Recirculation aquaculture system (RAS)

Recirculation aquaculture system (RAS) which is a new and emerging technology has the potential to contribute to the establishment of a matured microbial system used for the rearing of aquatic species. Properties such as their long water retention time and the large surface area of biofilters for bacterial growth stabilizes the microbial system which eliminates the harmful opportunistic bacteria.

Figure 3: Recirculated Aquaculture System (niwa.co.nz)

1. Importance of RAS

RAS technology has the potential to contribute to tackling constraints that result from traditional aquaculture. Common practices used by people in rearing aquatic species has posed many negative impacts on the environment as well as contributing to unsustainability. The use of this technology helps mitigate some of these effects as well as ensuring sustainability. Although constraints and drivers change from region to region, Table 3 outlines the common constraints.

Table 3: Constraints and potential drivers for the aquaculture industry

Constraints	Solution (RAS)
Demand for sustainability Pollution from carbon	RAS controls every input into the system, rearing conditions and discharge of waste. Rearing under controlled conditions ensures less or zero use of antibiotics and other medications. Facilitates the combined use of renewable energy. RAS has the ability to reduce carbon by establishing production units close to consumption areas.
Diseases and disease management	Due to the controlled environment and water treatment in an RAS, diseases can be completely avoided or better controlled.
Protection of the environment	RAS makes it almost impossible for escape and ensures effluents are well controlled to avoid pollution of the environment.

Global warming	Unlike in a traditional aquaculture, in an RAS, the temperature of the water environment can be controlled to suit the requirement of the culture subjects.
Availability of areas and shared use of water bodies	RAS facilities are not restrained by access to water bodies and are not affected by or affect wild stocks.
Constricted supply to regions closer to production areas	Production can be done anywhere hence can be closer to consumers and meet their demand.
Higher consumption of freshwater	Reuse of water which ensures lower consumption of freshwater

2. r/K selection

Vadstein *et al.* (1993) used the ecological theory of r/K- selection to explain the microbial maturity of the water in which the aquatic species are reared. A matured microbial water contains K- selected bacteria close to the carrying capacity of the system which improves the health of cultured species. Meanwhile, a system comprised of low microbial maturity produces fast-growing r-selected opportunistic bacteria which could adversely affect the cultured species (Vadstein *et al.,* 1993). K-selected bacteria are K-strategists that are larger in size and occupy stable environments with a longer life expectancy while r-strategist are smaller, inhabit unstable environments and have the ability to reproduce rapidly and abundantly with a short life span.

RAS is a form of intensive system that uses disinfection to get rid of harmful bacteria, which are relatively rare in a stable, well-established RAS. This process kills the pathogens but also destabilizes the culture system and also produces organic matter which are usually remains of dead organisms, waste materials or decomposing food. The organic matter is easily utilized by the fast-growing opportunistic r-selected bacteria which reduces competition in the culture system and leads to their proliferation (Hess-Erga *et al.,* 2010). Having a microbially matured aquatic ecosystem ensures stabilization of the culture environment in the presence of mostly benign microbial species, thereby improving the performance of the cultured species (Vadstein *et al.,* 1993, Skjermo *et al.,* 1997, Salvesen *et al.,* 1999). It is important to minimize or avoid the use of disinfection methods in order to minimize the organic matter load in a culture system.

The carrying capacity (K) of a system also plays an important role in establishing a matured microbial environment. K-selected bacteria have the ability to adapt well in crowded systems with density of bacteria close to the carrying capacity, and they compete successfully in such environments. Adjusting the biomass of the microbial community to the carrying capacity encourages the proliferation of beneficial K-selected bacteria (Skjermo *et al.,* 1997). RAS has the potential to produce a microbially matured aquatic community at a carrying capacity equal to that of the rearing tank because water flowing into the rearing tank has microbial population comparable to those in the tank (Attramadal *et al.,* 2012c). The biofilters in the RAS provide a suitable substrate for heterotrophic bacteria which are typically K-selected bacteria, for them to proliferate and consume organic matter thereby reducing the amount of organic matter that enters the tank which in turns influences the microbial load as well (Michaud *et al.,* 2009).

3. Nitrogen recycling

Nitrogen processing bacteria also become established in biofilters, in what normally consists of a two-stage conversion of ammonia first into nitrite and then into nitrate. These chemical conversions are typically carried out by *Nitrosamonas spp.* and *Nitrobacter spp.* bacteria, respectively, resulting in detoxification of the culture water. Flow through systems (FTS) as compared to RAS have lower microbial carrying capacity which encourages the proliferation of r-selected opportunistic bacteria hence, RAS should be considered for microbial management (Attramadal *et al.,* 2012c).

4. Importance of biofilters

Biofilters used in RAS play important roles in protecting microbes within the biofilms and their absence can lead to disease infestation in cultured species considering the high level of resistance of biofilms to antibiotics (Kimberly 2004). Although it is difficult to prevent pathogens from propagating in an aquatic environment, a bio-secure RAS has the potential to reduce disease outbreak largely due to the presence of neutral bacteria that compete for resources and thereby prevent the proliferation of harmful opportunistic bacteria in the rearing unit (Michaud *et al.,* 2009). As Attramadal *et al.,* (2012a) maintained, competition for space and nutrients in the biofilters discourages blooms of opportunistic bacteria within the culture system.

The authors (Op. Cit.) performed an experiment where the same group of Atlantic cod, *Gadus morhua* were grown in a recirculation aquaculture system (RAS) with moderate ozonation and a flow-through system (FTS). Results showed that the RAS had a stable microbial community as compared to the FTS and that the fish grown in the RAS outperformed those in the flow-through system.

A recent study by Chaeyoung *et al*., (2018) conducted to evaluate the effect of dietary microbial probiotics on growth and disease resistance of olive flounder reared in RAS and still water showed that RAS reared olive flounder performed 1.5 to 2.5 times higher. This is largely attributed to the presence of the biofilter in the RAS which maintained the highest microbial diversity as well as containing microbial communities which were very effective in denitrification, ammonium oxidation and suppression of the effects of fish pathogens. Water in a stable RAS environment is generally inhospitable to pathogens and other r-selected or opportunistic microbes.

In as much as the microbial communities play an effective role in ensuring fish health, some processes, inputs or interactions in the culture system affect the microbial communities and are determinants of their impact. The composition of a microbial community in an aquaponic system has the tendency of being affected by temperature, DO, carbon dioxide and nutrient levels among others (Junge *et al.*, 2017). It is of great importance to look out for the quantity and type of input in a culture system.

V. Conclusions

Intervening in the microbial ecosystem of aquatic species does not only help mitigate or eliminate disease outbreaks but also can contribute to the enhancement of production, ensuring sustainability as well as protecting the environment which is crucial to the aquaculture industry. The use of these microbial management approaches has contributed significantly to the improvements of key issues pertaining to shrimp culture such diseases outbreak. Studies have shown that the presence of opportunistic pathogens in the aquatic environment induces stress which influence the immune system of the cultured species and make them more susceptible to infectious diseases. Establishment of a matured microbial ecosystem discourages the proliferation of opportunistic r-selected pathogens which reduces the risk of disease incidence hence decreases the use of antibiotics which is the normal unsustainable way to deal with diseases. Meanwhile, routine use of antibiotics persists in South Korea, alternating with probiotics application in an effort to alter the constitution of the microbial ecosystem as a microbial management system.

The use of biofloc and green water technique provides flocs and microalgae respectively which serve as a good source of food and provide adequate amount of nutrients to the species being cultured. Microbial activities such as denitrification also gets rid of nitrogen and ammonia which is toxic and unhealthy for the species being cultured. Channeling Nitrogen into amino acid biosynthesis eliminates sources of ammonia that usually ended up as environmental contaminants, and contributed to eutrophication in estuaries and coastal areas.

For further expansion of the aquaculture industry, it is recommended that particular attention should be paid to microbial management strategies for the consideration of their integration. Further studies should be commissioned to find different techniques for analysing microbial communities as well as changes in structure and function of the community over time. The type of input into the system should also be critically studied since input have a major impact on the microbial community and how it interacts. There should be maximization of nutrient recycling where biofloc technology and other equally vital approaches can contribute substantially. Considering the positive feedback of microbial management technique, more target aquaculture species that respond well should be reared using the approach.

VI. References

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