

Original Research Paper

MICROORGANISMS IN SUSTAINABLE AQUACULTURE DEVELOPMENT

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ABSTRACT:

Microbes found in aquaculture are usually from source water, feeds including augmented live foods. These microbes are found in the gills, intestine, muscles and on the surface of fish, shrimps, crustaceans and other aquatic organisms. Microorganisms in aquaculture play several roles including their use as live food, food supplements, probiotics, in pond aeration and in the purification of aquarium. As food, microbes provide the essential nutrients such as vitamins, enzymes, polyunsaturated fatty acids, amino acids, pigments and steroids. They also serve as carbon and nitrogen sources. Microbial diversity in aquaculture depends largely on the quality (physicochemical properties) of the farm water and this has greatly influenced the growth of every aquaculture. Fungi, Bacteria, Protozoa, Helminthes and Microalgae are present in aquaculture, each performing its unique role in the ecosystem. While some are beneficial, others are not. To minimize the use of antibiotics in aquaculture treatment, probiotics are used. These probiotics antagonizes some existing pathogenic species by competing with them for attachment, substrate utilization, lowering the environmental pH (characteristics of lactic acid bacteria) and by production of antimicrobial substances like bacteriocins. Microbial live foods include Bacteria, Fungi, Microalgae and Protozoa. Microbes used as live food include lactic acid bacteria, *Bacillus*, *Chlorella vulgaris*, *Saccharomyces*, *Aspergillus*, *Artemia*, Copepods, Infusoria and Rotifers. These live foods have the advantage of small size, ease of digestion and ability to stimulate enzyme synthesis. Besides, microbes function in pond purification (biofilters) as well as its bioenergetic cycle. Microalgae, specifically aerate ponds through the release of oxygen during photosynthesis. The beneficial roles of microbes contribute to the growth, development and quality of fish, shrimps and crustaceans in an aquaculture by way of better growth rate, survival and disease resistance.

Keywords: *Aquaculture, Ecosystem, Larviculture, Live Feeds, Microorganisms Biofilters, Pathogens, Probiotics, Producers.*

INTRODUCTION:

Aquarium microbiology refers to the population of microorganisms found in aquaculture such as in prawns, shrimps, mollusks, crustaceans, fish, and its associated environment. Microorganisms are found on the gills, intestinal tract, muscles and on the surface of these aquatic organisms. These microbes include bacteria, fungi, algae and protozoa. However, viruses and helminthes have also been isolated from aquaculture. They may be beneficial or non-beneficial to the host, but both play significant role in the quality of the aquaculture. The quality of water in the aquarium determines the diversity of its microbial population. These microbes have functioned as live food, probiotics and have helped in improving the quality of the aquarium water. The beneficial functions of microorganisms have contributed immensely to the growth and the development of fish in fish farms. However, some microorganisms are known pathogens

and have caused diseases and possible death of their hosts. This paper will be limited to discourse on the important role of microorganisms in a fish farm.

MICROBIAL DIVERSITY IN FISH FARMS:

Microbial diversity of fish farm depends to a large extent on its water quality. The water quality of a fish farm is determined by its physicochemical and biological properties. Water is the home of the fish and its quality is one of the most over looked aspect of pond management until it affects fish production. Ehiagbonare and Ogundiran [1] stated that water quality generally means the component of water which must be present for optimum growth of aquatic organisms. These factors include dissolved oxygen, pH, hardness, turbidity, alkalinity, ammonia and temperature. Other parameters such as biological oxygen demand and chemical oxygen demand, nitrate concentration, sulphate concentration and phosphate concentration indicate the pollution level

of a given water body [1]. Productivity, therefore, depends on the Physico-chemical characteristics of the farm's water body [2].

Bacteria genera such as *Aeromonas*, *Vibrio*, *Staphylococcus*, *Corynebacteria*, *Pseudomonas*, *Acinetobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Proteus*, *Serratia*, and *Bacillus* have been isolated from the gut of fresh water fish [3]. According to Robertson et al. [4], *Carnobacteria*, *Lactobacillus*, *Leuconostoc*, *Streptococcus*, *Lactococcus* and *Vagococcus* have been isolated from fish and fish farm wastewater. Besides, diatoms (chlorella, *Chaetoceros* and *Skeletonema*) and yeasts genera (*Candida albicans*, *Cryptococcus neoformans*, *Saccharomyces*, e.t.c) are found in fish pond.

Fish in farm ponds are constantly exposed to the microorganisms present in water, fish feeds and in pond sediment. These organisms will influence the microflora on external surfaces of the fish, including the gills. Also, the digestive tract will receive water and food that harbors microorganisms. Certainly, colonization may well start at the egg and/or larval stage, and continue with the development of the fish [5]. Thus, the numbers and range of microorganisms present in the eggs, on food, and in the water, will influence the microflora of the developing fish. There are three likely scenarios for the fate of microbes coming into contact with fish: thus; the organisms from the environment around the fish may become closely associated with and even colonize the external surfaces of the fish. There may be accumulation of the organisms at sites of damage, such as missing scales or abrasions [6]. The organisms may enter the mouth with water [5] or food and pass through and/or colonize the digestive tract [6].

MICROBES AS LIVE FOOD IN FISH FARMING:

Generally, bacterial cells have good nutritional value as they contain essential amino acids, protein and polysaccharides [7]. Being a rich source of exogenous enzymes, bacteria also helps in digestion and absorption process in the gut of larvae or food organisms by breaking down the larger particles into smaller ones. According to Wang [7]; and Austin and Austin [6], bacteria can be established in the gut of fish through food or through live food enrichment. Besides the use of bacteria, yeasts and microalgae have also been used as food supplements. Yeast can be directly used as a primary food source for many fish larvae but it is mainly used as a feed for zooplankton [8] which is grown for use in larvae culture.

Bacteria:

Yeasts → Zooplanktons →
Fish
Microalgae

Figure 1: Food chain in Aquaculture

Bacteria, yeasts, algae and zooplanktons are important ingredients in artificial fish diets. Live foods can be cultured easily and economically. Live foods commonly encountered include both phytoplanktonic as well as zooplanktonic organisms. Phytoplankton consist of chlorophyll bearing organisms e.g. *Microcystis*, *Volvox*, *Eudorina*, *Oscillatoria*, etc. and non photosynthetic plants or saproplankton e.g. bacteria and fungi, whereas zooplankton comprise plankters of animal origin. In the tropical areas it mainly comprises protozoans (e.g. *Arcella* sp., *Diffugia* sp., *Actinophrys* sp., *Vorticella* sp. etc.), rotifers (e.g. *Brachionus* spp., *Keratella* sp., *Asplanchna brightwelli*, *Polyarthra vulgaris*, *Filinia opoliensis* etc.) and the planktonic forms of crustaceans (*Artemia* spp.), cladoceran (*Moina* spp., *Daphnia* spp., *Ceriodaphnia* sp. etc.), ostracoda (*Cypris*, *Stenocypris*, *Eucypris* etc.) and copepods (*Mesocyclops leuckarti*, *M. hyalinus*, *Microcyclops varicans*, *Heliodiaptomus viduus*, etc.) and their larvae.

The use of microalgae in aqua culture is not only because of its nutritional attributes but more so for its small size ranging from 5 to 25 microns meeting the feed size requirements ideally well for early stages of various aquatic animals. According to Maruyama *et al.* [9], micro algae is used as an essential food source for rearing all stages of marine bivalve mollusks (clams, oysters, scallops), gastropods (abalone, conch), larvae of fishes (cod, halibut, tilapia) and shrimps (*Penaeus* sp). Micro algae also constitute an important source of food for live food organisms (rotifers, copepods, cladocerans, brine shrimp etc.) used in aqua hatcheries. Micro algae are frequently supplied together with rotifers during first feeding of marine larvae and the technique has enhanced survival as well as growth. Two types of live feeds have been used for rotifer production [9], thus:-

- Live algal cells such as *Nannochloropsis oculata*, *Tetraselmis tetrathele* and *Chlorella vulgaris*,
- and a supplementary food such as baker's yeast.

DIFFERENT TYPES OF LIVE FEED:

Among the different types of live feed available for use in aquaculture, are the ones discussed below:

Bacteria, Protozoa and Yeasts:

Yeast can be directly used as a primary food source for many larvae but it is mainly used as a feed for zooplankton which is grown for use in larviculture. It is an important ingredient in artificial larval diets. Yeast has also been evaluated as supplement or replacement for algae in the feeding of post larval penaeid shrimps.

In contrast to the use in terrestrial animals, the use of bacteria as probiotics in case of fishes is no longer restricted to feeds only. Their use as a prophylactic measure is gaining momentum day by day in live food production to stabilize and promote larval rearing through a microbiologically balanced system. Probiotic materials have been used as food supplement in fish culture. The fish fed with such probiotics grew well without requiring drugs added [10]. Today commercial preparations of useful bacteria like *Bacillus subtilis*, *B. polyriyxa*, *B. negaterium* etc. are available in ready to use packs. Most probiotics used in aquaculture belong to the genus *Bacillus*, photosynthetic bacteria or yeast, although other genera or species have also been mentioned [11,7]. These bacteria are consumed by several groups of zooplankton like protozoans, rotifers and copepods. *Brachionus plicatilis* can grow by feeding on microorganisms such as yeast and bacteria [8,10]. Generally, bacterial cells have good nutritional value as they contain essential amino acids, protein and polysaccharides. Being a rich source of exogenous enzymes, bacteria also helps in digestion and absorption process in the gut of larvae or food organisms by breaking down the larger particles into smaller ones. After proper selection, the bacteria can be established in the gut of larvae through food or through live food enrichment. In larva culture, probiotic treatment results in better growth rate, survival and disease resistance in larvae.

Micro algae:

Algae of interest are chlorophyll bearing unicellular or multi-cellular plants. When multi-cellular, they may be colonial or filamentous. Most of them are aquatic. Besides chlorophyll, they also show various carotenoid pigments which impart different colours to them. According to the nature of photosynthetic pigments, algae are further classified into three divisions such as Chlorophyta (green algae), Phaeophyta (brown algae) and Rhodophyta (red algae). Chlorophyta (green algae) serve as initial food producers and the first link in the aquatic food chain, both in freshwater and marine ecosystems. In recent years, mass culture of unicellular algae such as diatoms and small phytoplankters has becoming quite popular for feeding larvae of fishes, prawns, shrimps and molluscs in aqua hatcheries.

The nutritional value of any algal species for a particular organism depends on its cell size, digestibility, production of toxic compounds, and biochemical composition. Microalgae grown to late-logarithmic growth phase typically contain 30 to 40% protein, 10 to 20% lipid and 5 to 15% carbohydrate [12]. When cultured through to stationary phase, the proximate composition of microalgae can change significantly [13]. Polyunsaturated fatty acids derived from microalgae are docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA) and are known to be essential for various larvae [14]. The content of vitamins can vary between microalgae. Ascorbic acid shows the greatest variation, i.e. 1 to 16 mg g⁻¹ dry weight [15].

Infusoria:

Infusoria refers to microscopic single celled animalcules belonging to the class – Ciliata of phylum - Protozoa. Besides being small in size, they are soft bodied and nutritionally very rich and therefore, serve ideally as starter diet for early stages of fish larvae. In the early development stages of fish larvae, *infusoria* or small live organisms are indispensable in aquaculture [10]. *Paramoecium* and *Stylonychia* are the most common forms of freshwater infusoria.

Rotifers:

Rotifers are popularly called wheel animalcules. They are an important group of live food organisms for use in aqua hatcheries. *Brachionus*, which is the most known form of all rotifers, serve as an ideal starter diet for early larval stages of many fish and prawn species in marine as well as freshwater. Species of the genus *Brachionus* (Brachionidae: Rotifera) are well represented in different water bodies worldwide [8,10]. Depending on the mouth size of the cultured organisms, small (50 to 110 micron length) or large (100 to 200 micron length) rotifers are used. There are about 2,500 species of rotifers known from global freshwater, brackish water, and seawater. *B. plicatilis* is the species used most commonly to feed fish larvae in hatcheries around the world. It is a euryhaline species, small and slow-swimming, with good nutritional value. It is well suited to mass culture because it is prolific and tolerates a wide variety of environmental conditions. The rotifer, *B. plicatilis* and *B. rotundiformis*, have been indispensable as a live food for mass larval rearing of many aquatic organisms [9]. By way of significant developments in larval rearing technology of fishes, demand for rotifer is fast increasing.

Maintaining large cultures of rotifers and their production on a predictable basis is a major problem. The food of rotifers appears to be the key element in their mass production. Presently, fresh baker's yeast is mostly used as the main diet ingredient for rotifers. However, its freshness, a criterion that is difficult to

evaluate, can greatly influence the dietary value of the yeast for the rotifers and as a consequence, determine success of rotifer culture. Several measures are taken to deal with the problem such as supplementation of baker's yeast with micro algae, improving the nutritional quality of rotifers through vitamin C supplementation, treatment with antibiotics to prevent bacterial contamination and use of probiotics, i.e. the addition of beneficial bacteria in rotifer culture.

Artemia:

Artemia commonly known as brine shrimp are zooplankton, like copepods and *Daphnia*, which are used as live food in the aquarium trade and for marine finfish and crustacean larval culture. There are more than 50 geographical strains of *Artemia* identified. Of the live food used in aqua hatcheries, *Artemia*, constitute the most widely used organism. The biggest advantage of using *Artemia* is that one can produce live food on demand from dry and storable powder [16] i.e. dormant *Artemia* cysts which upon immersion in seawater regain their metabolic activity and within 24 hours, release free swimming larvae (nauplii) of about 0.4 mm length. *Artemia* has high nutritive value and high conversion efficiency. All the life stages of *Artemia*, i.e. cysts (after decapsulation), nauplii, juveniles, sub-adults are used as feed for fish.

Today, in majority of the commercial aqua hatcheries, *Artemia* nauplii is virtually used as a sole diet. Frozen adult *Artemia*, are widely used by aquarists, fish breeders and aquaculturists. *Artemia* biomass is also used as food additive for domestic livestock or extraction of pharmaceutical products as also in making protein rich food products. It is even used for human consumption in some countries. Owing to its great utility, *Artemia* trading is a growing business in several parts of the world.

Copepods:

Copepods are common zooplankton of freshwater and brackishwater. They are natural feeds for larvae and juveniles of many finfish and crustaceans and it is generally believed that copepods can meet the nutritional requirements of fish larvae [16]. In the wild, most marine fish larvae feed on copepod eggs and nauplii during the first few weeks of life due to their small size. It contains very high levels of PUFA (Polyunsaturated fatty acids) and other essential nutrients. Consequently, there is considerable interest in the use of copepods as feed sources for small marine larval fish. Herbivorous copepods are primarily filter feeders and typically feed on very small particles. But they can feed on larger particles, which give them an advantage over the rotifers [8, 16]. They can also feed on detritus.

Importance of Live Food Organisms in Aquaculture:

Success of aquaculture depends on healthy cultured stock. A disease free healthy stock can be maintained by feeding live food to the cultured stock along with supplemented artificial feed. Supplemented artificial feed cannot meet all the elements required for the growth of fish. So, fish and shellfish must be fed with live food. For getting good return from rearing of fish and shellfish they should be fed with nutrient rich diet. Zooplankton is required as a first food for many cultured fish; for others it contributes to faster growth and higher survival. Larvae of fish and shellfish cannot feed on artificial supplemented feed because of size. They require small size live foods for their nutrition. Live foods are easily digestible protein rich diet for fish and shellfish. Importance of microalgae as larval food is also because it stimulates enzymatic synthesis and on-set of feeding in young larvae. Larval rearing is one of the riskiest phases of aquaculture, but it could be one of the most profitable ventures. Special planning and strategies are required to overcome the risk of high mortality during this phase of culture.

Microalgae are utilized as live feed for all growth stages of bivalve molluscs (e.g. larval/early juvenile stages of abalone, crustacean, some fish species and zooplankton) in aquaculture food chains. Over the last four decades, several hundred microalgae species have been tested as food, but probably less than twenty have gained widespread use in aquaculture and they must possess a number of key attributes to be useful as aquaculture species [17]. Microalgae have an important role in aquaculture as a means of enriching zooplankton for feeding fish and other larvae and also help to aerate the pond. In addition to providing protein (essential amino acids) and energy, they provide other key nutrients such as vitamins, essential polyunsaturated fatty acids (PUFA), pigments and sterols, which are transferred through the food chain. In nature, zooplankton is one of the primary foods of fish larvae. Two of the dominant zooplankton groups are Rotifera (rotifers) and Copepoda (copepods). These two groups are the preferred prey for shrimp and fish and they are the most widely used live feeds by aquaculturists. The intensive larval culture of most marine fish depends on a large supply of zooplankton.

MICROBIAL PROBIOTICS/ EFFECTIVE MICROORGANISMS:

According to the currently adopted definition by FAO/WHO, probiotics are: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host [18]. *Lactobacillus* and *Bifidobacterium* genera have been used almost exclusively for the competitive exclusion of pathogenic

bacteria from the gut of humans, farmed animals and most recently aquaculture species. The flora also acts as a barrier to gut pathogens by blocking their attachment to gut binding sites which is the first step of pathogenicity [19]. It can also play an important role in maintaining immune function. Thus, members of the natural aquatic microflora are effective at inhibiting fish pathogens [20]. However, the utilization of probiotic to applications other than pathogenic bacteria exclusion are now been develop in several areas like; immune system stimulation, vaccine production, bioremediation and functional feed development [21]. It is possible to manipulate the microflora of the developing fish by use of probiotics, i.e., live microbial food supplements, which may colonize the digestive tract for short or prolonged periods [4]. These bacteria genera are mainly lactic acid bacteria. The lactic acid producing microorganisms have the ability to deliver antagonistic properties against undesirable pathogens by inhibiting the growth of such pathogens, competing for substrate and generating a non conducive acid environment (by changing the pH to more acidic values of 4 to 4.5). The antagonistic properties of some lactic acid producing microorganisms are partly due to their ability to produce other metabolites like enzymes, toxins, carbon dioxide, peroxides or antibiotics, also known as bacteriocines [22, 23]. The use of bacteria as a prophylactic measure is gaining momentum day by day in live food production to stabilize and promote larval rearing through a microbiologically balanced system. Probiotic materials have been used as food supplement in fish culture. The fish fed with such probiotics grew well without requiring drugs added [10]. Today commercial preparations of useful bacteria like *Bacillus subtilis*, *B. polyrixa*, *B. negaterium* etc. are available in ready to use packs [11, 7]. *B. subtilis* is not harmful to mammals, including humans, and is commercially important as producer of a high and diverse amount of secondary metabolites like antibiotics, fine chemicals and enzymes, as well as heterologous proteins, antigens and vaccines [7].

POND WATER QUALITY:

This is reviewed under the following subheading:-
Microbes as wastewater purifiers in Fish Farm:

According to Ringo et al. [25], there are numerous microorganisms that are beneficial to fish by restoring water quality and creating a conducive culture environment. These microbes, act as water conditioner by stripping off the nitrogenous substances. The enzymes produced by these microorganisms degrade organic accumulated debris from shrimp/fish cultures inducing ponds bioremediation and consequently the prevention of viral and bacterial diseases [24,26]. This biological filter (biofilter) is the heart of the recirculating aquaculture system (RAS) and it is a living filter usually on a medium upon which a film of bacteria grows. The

bacteria provide the waste treatment by removing pollutants. The two primary water pollutants that need to be removed are (1) fish waste (toxic ammonia compounds) excreted into the water and (2) uneaten fish feed particles. The biofilter is the site where beneficial bacteria remove (detoxify) fish excretory products, primarily ammonia [27].

Aeration of ponds by the aquatic green algae:

In addition to toxic ammonia, carbon dioxide tends to concentrate in intensive fish production systems. As carbon dioxide increases, the pH of the water decreases, and fish respiration is affected [28, 27]. Carbon dioxide levels should be maintained at levels less than 30 mg/l for good fish growth [12]. Some carbon dioxide is beneficial since it reduces pH and mitigates ammonia toxicity. Carbon dioxide removal can be accomplished by the photosynthetic activities of aquatic green algae. These algae utilize carbon dioxide in the synthesis of plant carbohydrate and in turn release oxygen into the aquatic system. The release of oxygen ensures continued aerobic state of the pond and maintains healthy state of fish in the aquarium.

MICROBIAL PATHOGENS OF FISH:

Microorganisms are not only beneficial in aquaculture; there are some with negative impact on fish and other aquarium species. Although this paper is not interested this group of microbes, there is the need to discuss a few of them. This is because their presence in aquaculture prompts the need for use of probiotics and some other antimicrobial agent due to their ability to cause diseases. As many pathogens naturally occur in aquatic environments, all forms of aquaculture are prone to disease outbreaks which are largely determined by host susceptibility [24, 25]. Also, physiological stress contributes to diseases and increase mortality in aquaculture. It leads to decreased disease resistance, impaired reproduction and reduced growth. It could also lead to intestinal microbiota disorders which decrease the level of beneficial micro-organism and thereby giving room to invasion from bacteria disease; a significant cause of mortality in most fish hatcheries [24, 31, 32] and high mortalities especially during transition from the yolk sac to the first feeding stage of development [24]. Reason may be because the larvae stage and fish generally, are highly exposed to gastro intestinal microbiota associated disorders [33]. Bacteria, fungi, protozoa and helminthes are known pathogens of fish. Bacteria genera such as *Aeromonas*, *Vibrio*, *Staphylococcus*, *Corynebacteria*, *Pseudomonas*, *Acinetobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Proteus*, *Serratia*, and fungal genus *Cryptococcus*, protozoan(*Ichthyophthirius multifiliis*) and helminthes (*Huffmanella huffmanii* and *Clinostomum marginatum*)

have been incriminated in fresh water fish diseases [3] and are described as common pathogens in fish farm

SUMMARY AND CONCLUSION:

Microorganisms have been used to improve the growth and development of aquaculture business in Nigeria. They are used as food by zooplanktons which in turn serve as food for crustaceans and fish. They also function as food supplements for enzymes, amino acids and vitamins needed for growth and development of these aquatic lives. Microorganisms occupy central position in the bioenergetic cycle in aquaculture. They have been used in degradation of wastes generated by these aquatic organisms, utilizing them as carbon and nitrogen sources, and detoxifying some generated toxic wastes. Consequently, they purify the aquarium wastewater by acting as biofilters. Finally, probiotic activities of microorganisms have earned them good use in aquaculture and this has helped in preventing and controlling aquatic life diseases. Although they have these beneficial qualities, some have also been associated with fish diseases. Since microbes are organic, their use in aquaculture creates a more ecofriendly environment and, therefore, must be encouraged.

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