AQUACULTURE BIOTECHNOLOGY

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CHAPTER 1

BIOTECHNOLOGY AQUACULTURE: INNOVATIONS, DEVELOPMENT, AND CHALLENGES

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ABSTRACT:

Aquaculture is the practice of raising and cultivating water-based animals and plants such as fish, shellfish, and other aquatic creatures. The global fishing of water organisms seems to have reached its highest point or in some cases have decreased. Aquatic biotechnology has many important uses and can help make aquaculture more productive, efficient, and sustainable. We can use biotechnology to improve various aspects of the culture cycle, which includes growth, nutrition, health, and reproduction. For example, we can enhance the rate at which organisms grow and convert feed into energy. We can also improve their nutrition and the quality of the products they produce. Biotechnology can help in managing stress levels, developing vaccines, and increasing disease resistance. It can also aid in diagnosing and treating diseases more effectively. Additionally, we can use genetic selection and transgenesis to improve the overall outcomes of the culture cycle. The study of genes and proteins in fish can potentially affect how we produce and manage their genetic resources. The process of genetic modification, where genes are manipulated, allows for the introduction of new characteristics such as increased growth, ability to withstand cold temperatures, resistance to diseases, and more in fishes. The freezing of eggs, sperm, and embryos can create new ways to make and raise fish and manage their genes. This could provide more fish to sell and potentially help them be healthier and in better shape. It can also help save the DNA of endangered species in a protected place

KEYWORDS:

Aquaculture, Biofloc Technology, Cell Lines, Fish Cell, Immune Systems.

INTRODUCTION

Aquaculture is a fast-growing industry that helps provide food, nutrition, and jobs for the growing world population. It is still growing faster than other big food production industries, but not as fast as it did in the 1980s and 1990s. The amount of fish farming each year grew by 5. 8% from 2001 to 2016, but some countries in Africa had even higher growth rates during the years 2006 to 2010. Biotechnology is used in aquaculture to help increase productivity, make things work better, and keep things going in a sustainable way. Researchers are working on solving important problems related to nutrition, health, and water quality using biotechnology and advanced technologies. Biotechnological approaches are being used to improve fish production and quality. This includes methods like developing fish that can withstand climate change and diseases, quickly diagnosing and treating fish health issues, providing the right nutrition to fish, and changing fish genes to increase yield. These approaches also involve editing genes and using genomics and proteomics techniques to assess and improve fish genetics and quality. Biotechnology uses molecules and biological materials from living things to make useful new products for people. Aquaculture biotechnology helps fish farmers use genetics, cells, and molecules to make more fish food that is plentiful, strong, and healthy. Biotechnology helps make aquaculture, fisheries, and the food industry more sustainable. More people want seafood but there are fewer places for fish to live. So scientists

are using biotechnology to find new ways to produce more fish and make aquaculture a modern and advanced field [1], [2].

Biotechnology helps scientists improve fish and shellfish by finding and putting together specific traits in them which makes them produce more and become better in quality. Using modern biotechnology to improve the production of aquaculture species has a lot of potential. It can help meet the demand for these species and also make sure that aquaculture is sustainable for the long-term. Using advanced tools in biotechnology and studying proteins, scientists can change the genetic makeup of fish to improve their quality and increase their quantity in aquaculture. One very noteworthy development in recent times is the Aqu Advantage salmon, which is a genetically modified type of Atlantic salmon. The aim is to make the fish grow faster without changing its final size or other characteristics. Biotechnology helps create sterile farmed animals to protect biodiversity from the negative effects of gene escapees. Fish are used in scientific studies to understand how genes work and to develop new techniques in biotechnology for various reasons. Biotechnology is growing quickly and being used more and more in aquaculture. This chapter talks about different ways to improve aquaculture, like changing genes and using special techniques with cells and vaccines. It also discusses using modern technology to have more sustainable fish farming [3], [4].

Scientists have used techniques to change the sex of chromosomes in fish. They have also been able to make fish have more copies of chromosomes than normal. Additionally, they have made it so that fish only inherit chromosomes from one parent. These techniques have been widely used in fish that are grown in controlled environments. These methods are important for improving fish breeding because they help to quickly sterilize the fish's reproductive organs, control their gender, improve the health of hybrid fish, and create genetically identical copies. Most animals with a backbone have two sets of chromosomes in their body cells. Polyploid individuals have extra sets of chromosomes. Triploids have three total sets, tetraploids have four sets, and so on. Induced triploidy is a very effective way to make fish unable to reproduce. It is commonly used in fish farming and fisheries management. The ways to create triploids and other manipulations of chromosomes in fishes are well explained. These biotechnologies are useful in aquaculture and fisheries management. Tetraploid breeding lines can help in aquaculture by making it easier to create many sterile triploid fish.

This is done by crossing tetraploids with diploids. Many fish species have been made to have four sets of chromosomes, but most of these fish did not survive well. For the last few years, people on the West Coast of North America have been growing triploid Pacific oysters for business. Fish breeders are really interested in making gynogenetic individuals because it allows them to create lots of inbred fish in just one generation. Gynogenesis can also be used to create populations with only females in species where females have the same sex chromosomes, and to understand how fish determine their sex. Using all-female gynogenetic progenies instead of normal bisexual progenies is more convenient for sex inversion experiments. New techniques have been developed for breeding certain aquaculture species. These techniques involve using induced gynogenesis and hormonal sex inversion. By using androgenesis, we can create a population of only male fish. This could be useful in aquaculture for business purposes. It can also be used to make fish with the same genes and to bring back lost genes from frozen sperm. Certain types of fish, such as cyprinids, cichlids, and salmonids, have been successfully bred to have both male and female characteristics. The process of meiotic gynogenesis was successfully done for the first time in Indian major carps [3], [5].

A group of scientists started a project called Genome 10K, which aims to study the genetic makeup of 10,000 different types of animals with backbones. This includes about 4,000 types of fish. The goal of the project is to learn more about how these animals evolved over time and

also to help protect species that are at risk of disappearing. The Earth BioGenome Project is a big goal in biology. It wants to study and understand the DNA of all the different living things on Earth. This project will take about 10 years to complete. However, the DNA of every type of fish, which includes over 34,000 different species, will be mapped out soon. The next obstacles involve figuring out how to use this genetic information and apply it to improve fisheries and aquaculture. This can include things like managing genetic resources and selectively breeding fish. We still have a lot to learn and gain from using genomics in fisheries and aquaculture. The use of techniques like genome editing and genomic selection in fish farming will help improve the genes of farmed fish. This, along with the use of new intelligent systems, will have a big impact on aquaculture and fisheries. The first fish to have its complete genetic code sequenced was a Japanese puffer fish called *Takifugu rubripes*. Since then, around 1386 different fish species have had their whole genetic codes sequenced and are now stored in the National Center for Biotechnology Information repository as of December. These genomic resources help scientists study genes in different species and understand how they evolve and are related to each other. They are also useful in finding ways to improve fish farming and fishing practices [6], [7].

New developments in genomics and proteomics have made it possible to study gene expression on a larger scale than ever before. This includes analyzing hundreds of gene products as well as studying patterns of expression across the entire genome. Proteomics is a useful tool for food scientists. It can help them understand what ingredients are in the food, how the quality changes during processing or storage, and how the proteins interact with other ingredients or the immune system after it is eaten. Proteomics research is used a lot in studying humans and animals. After finishing the Human Genome Project successfully, understanding the human proteome became a big challenge. The scientists in India have helped with proteomic research, just like scientists from all over the world. However, there are not many studies on proteins in the field of fisheries science, particularly in India. India needs more research programs focused on this. Because many fish are eaten, it is important to make sure that the fish raised on farms are similar to the fish that live naturally in their original habitat. At first look, this may seem like a strange thing to think about, but there are a few things that could affect how the fish's body works. The food for farm-raised fish is not the same as what wild fish eat, so it makes sense to think that different substances are produced in their bodies. When we think about fish from farms and fish from the wild, we might think they are the same. However, a recent study that looked at the proteins and chemicals in both types of fish found that there are actually important differences. Comparative study of proteins in farmed and wild Sparus aurata has led to the creation of a database of proteins. This information is being analyzed using advanced tools to assess the safety of different seafood products

DISCUSSION

Gene editing is a strong tool used in studying fish health, genetics, and biotechnological research. It has many useful applications. CRISPR-Cas9 has changed the way we edit genes. This technique is mostly used to change genes in mammal cells, but it is still new for changing genes in fish cells. Scientists have used a gene editing method called CRISPR-Cas9 on fish. Recently, scientists have used a method called CRISPR/Cas9 to change the genes of fish commonly found in aquaculture such as Atlantic salmon and rainbow trout. They have also used this method on certain types of carp. Rohita, Ctenopharyngodon idella, and Cyprinus carpio are different types of fish. Siluridae refers to channel and southern catfish, specifically Ictalurus punctatus. Pacific oyster is a type of oyster called Crassostrea gigas. Nile tilapia is a type of fish called Oreochromis niloticus. Gilt-head sea bream is a type of fish, also known as S. Aura is a phenomenon that can sometimes occur in photos when light reflects off a subject,

causing a colorful glow around them. One of the most important recent technological advances for studying diseases in zebrafish is precise editing of their genetic information. CRISPR-Cas9 helps create genetic changes in zebrafish quickly and effectively. It can specifically target and change certain parts of the DNA in specific tissues. The study of DNA barcoding is about identifying different species of organisms by analyzing their DNA [8], [9].

Paul Hebert, a researcher at the University of Guelph in Canada, started using barcoding programs in Canada in 2003. DNA barcoding can change the way we understand and keep track of different species. It will have a big impact on how we deal with things like pests, diseases, food safety, and managing resources. It'll also affect conservation efforts, research, education, and recreational activities. The professor Hebert said that India should join the global Consortium for the Barcode of Life because it has a lot of different plants and animals and is very skilled in science and technology. The purpose of the workshop was to improve India's ability to use DNA barcoding and to find ways to fund current and future barcoding projects for plants and animals. We have created specific molecular signs for important fish and shellfish species. These signs help us identify the species correctly and keep a record of their diversity. Furthermore, DNA barcoding has helped solve legal arguments. This text is about using scientific methods to identify two types of fish: pomfret and whale shark. The whale shark is a protected species that is in danger of dying out. The governments of Maharashtra and Kerala have used barcoding technology to help identify and manage endangered species. DNA barcodes have been used to find out if seafood is labeled correctly and to prove its authenticity. We expect that DNA barcoding technology will be very helpful in identifying animals, ornamental fish, and by-products that are moved across borders.

Researchers are studying cells and tissues outside of the body for scientific exploration. Fish cells that are grown in labs have been used more and more to isolate and identify viruses that cause diseases. This is because there have been many cases of disease outbreaks in fish farms. Fish cell lines have been used as a useful and affordable tool to test the harmfulness of chemicals and environmental samples outside of a living organism. So, it is really important to create and take care of cell lines from fish that people buy and fish that are in danger. This helps with fish farming and also with making sure fish populations are protected. Since 1962, scientists have made about 283 fish cell lines from different types of fish worldwide. In the past, scientists mostly used fish from moderate climates to create cell lines. However, there have been recent reports of cell lines from fish species that live in warm climates, such as Epinephelus coioides and Chanos chanos. Rohita, Puntius sophore, and Schizothorax richardsonii are the scientific names of three different fish species. Various researchers have created several cell lines that resemble embryonic stem cells from different fish species. The crustacean cell culture is being used to create new tools for detecting and studying diseases in shrimp, crayfish, and lobster industries. So far, no successful efforts have been made to create a cell line from crustaceans. We have made a list of fish cell lines, and you can find.

Fish cell lines are very important for studying diseases, toxins, and the immune system. Fish cells can be used for many important things in science, like figuring out how drugs and chemicals affect our bodies, studying how genes work, and moving genes between different organisms. The cultured animal cells make many useful things that companies can sell, like substances that help with our immune system, antibodies, substances that help cells grow, special proteins, and chemicals that our bodies produce. They are used to make vaccines for viruses, medicines like tissue plasminogen activator and interferon, and other things like monoclonal antibodies and tumor specific antigens. The grown cells would also be used as a replacement skin to help heal wounds. Cell culture is useful because it helps scientists have more control over their experiments and makes it easier to understand how fish respond to

stress and their environment. Scientists have made special fish cells that have a specific kind of job. These cells can be like T cells, B cells, or macrophages.

They come from catfish. Punctatus, O can be simplified as O. with dots or spots. Kissing fish have been used to gather important information about the immune system in farmed fish, which can help prevent diseases in them. Scientists have used cells from the gut, skin, and gills to understand how our body protects against diseases. We can use cell lines to study how effective DNA vaccines, recombinant protein vaccines, synthetic peptide vaccines, and immunostimulants are in triggering the immune response. Scientists have used fish cell cultures to learn about cancer development inside the body and how fish's immune systems respond to help make vaccines. They have also used these cell cultures to study the similarities and differences in how the immune system has evolved in different types of fish. Fish muscle cells grown in laboratories have great potential for making seafood meat. In order to keep aquatic animals safe and healthy, we need quick, trustworthy, and very accurate diagnostic tests to identify and treat diseases. Growing pathogens directly in a laboratory is also commonly done. But, these ways take a lot of time and money. And, we have not yet found ways to grow shrimp and crustaceans cells that are good for growing viruses [10], [11].

Efforts to solve these problems have resulted in new ways to diagnose diseases using the immune system and DNA, as well as a technique called polymerase chain reaction amplification. Modern technology allows us to protect ourselves from diseases by getting vaccinated or by boosting our immunity. Fish vaccines created in the past 20 to 30 years have been proven to be effective and affordable in preventing certain diseases in fish that are raised in captivity. Nowadays, there are many vaccines that can be bought to prevent diseases in fish, such as furunculosis caused by Aeromonas salmonicida. Additionally, there are also more vaccines being created to fight diseases like viral hemorrhagic septicemia (VHS). Vaccines not only reduce the seriousness of diseases, but they also decrease the use of antibiotics. They don't leave any leftovers in the product or surroundings and don't cause resistance in pathogens. Vaccines and immunostimulants can be given to animals through additives in their food, by immersing them in a solution, or, for larger animals like fish, by injecting it into their bodies. Scientists are creating vaccines using genetic engineering to keep fish safe from harmful germs.

Recently, researchers found that injecting rainbow trout with a gene from the virus causing VHS can make them highly resistant to the virus. Sahoo and the other authors. Scientists have studied harmful viruses that affect fish in Asia-Pacific. These viruses have been around for a long time and have caused big problems in fish farming and the ornamental fish industry. Some of the viruses are called betanodaviruses and koi herpes virus. The Indian Council of Agricultural Research (ICAR) Central Institute of Fisheries Education in Mumbai has made some important discoveries in their research. They have developed a vaccine to protect shrimp from a virus called white spot syndrome virus (WSSV). They have also created a DNA vaccine to combat an infection called Edwardsiella tarda in carps. These vaccines are currently being tested in the field. Biofloc technology is a way to make the water in fish farms cleaner by adding extra carbon to the system. This can be done by adding carbon from an outside source or by using fish feed with a higher carbon content. This helped bacteria take in more nitrogen, which made ammonium levels decrease faster than nitrification.

Heterotrophic bacteria can immobilize ammonium quickly because they grow faster and can produce more microbial biomass from the same amount of substrate compared to nitrifying bacteria. Biofloc technology helps to reduce the need for changing water and using lots of water in fish farming. It does this by keeping the water quality good in the fish tanks and creating a kind of food called biofloc that is cheap and has lots of protein. This food can be given to the fish. Biofloc technology is a cheaper way to treat water in aquaculture. It can reduce water

treatment expenses by around 30%. It can also help save money on feed because it is more efficient at using protein compared to traditional ponds. This makes biofloc technology a cost-effective and sustainable option for the future of aquaculture. Biofloc technology is a strong, affordable, and easy way to manage and remove nitrogen compounds compared to other methods. To reduce the harm caused by climate change on aquaculture and make the most of new opportunities, we need to learn about and encourage different innovative technologies. For example, combining biofloc technology with greenhouse ponds can be helpful. Animals that live in water farms are more affected by their surroundings compared to animals that live on land. The water that fish need for oxygen and important chemicals also collects their waste and can contain pollution from the surrounding area.

The spread of disease in fish farming is more influenced by the environment than in cattle farming. Another area of biotechnology that has been used in aquaculture is called bioremediation. This process helps clean up any pollution or harmful substances present in aquatic environments. This means using helpful bacteria called probiotics to clean water or food. These bacteria control the growth of harmful bacteria that can make us sick. There has been a big increase in the development of fish cell lines in places called biobanks, where they store and keep track of these cells. This has made it very necessary to save these cell lines in places called cell line repositories and biobanks. Fish cells lines are very important for studying fish in a lab and also for preserving the genetic material of fish. The writers played a big role in creating a modern facility for creating and keeping cell lines at NBFGR, a research center in Lucknow, India. The Indian government in New Delhi, through the Department of Biotechnology (DBT), has been helping with research to develop, understand, and protect fish cell lines [12], [13].

As more fish cell lines are being discovered, the Indian government has understood the importance of preserving them in a cell line repository. DBT provided funding for a project called Setting up a National Repository at NBFGR, Lucknow, for Preserving and Studying Fish Cell Lines. The National Repository of Fish Cell Lines (NRFC), which keeps and protects fish cells, is being run at NBFGR in Lucknow. The authors are leading this project. After the Department of Biotechnology (DBT), Government of India, later provided funds for a new fish cell line repository at location C. Abdul Hakeem College is a school located in Melvisharam, which is in the state of Tamil Nadu. This place offers services for storing, studying, freezing, and sharing fish cell lines with researchers in India. The repository is a place where Indian fish cell lines are stored and shared with people across the country. Right now, the NRFC is taking care of and freezing over 50 cell lines from various types of fish that live in freshwater and saltwater. Different research groups in India, including the researchers at NBFGR in Lucknow, gave these cell lines and stored them. We have studied and labeled all the cell lines using special markers, and we have frozen and taken care of them to ensure their preservation.

Researchers have been given many different types of cell lines to use in their studies. Scientists from Canada, Germany, and the Netherlands have asked for cell samples from this storage place. To send fish cell lines outside India, there are rules to follow. Researchers from other countries need to reach out to the National Biodiversity Authority in Chennai for help. This is related to the idea and announcement of the State Fish Professor. Lakra came up with a new idea of having a special type of fish for each state. He thought of this idea while working at ICAR-NBFGR in Lucknow in 2006. The idea is to choose one important fish species as the State Fish. This will help bring together important people from the state governments to protect and take care of this fish species. The goal of the State Fish is for the state to choose a fish and protect its variety of species and support fish farming if needed. This is a very practical way to protect fish species that are in danger of extinction. Various state governments are now working

together with NBFGR to develop plans to protect and improve their chosen State Fish. They are aiming to achieve success in conserving these fish in real-time [14].

CONCLUSION

The advancements in technology for fish farming are having a noticeable effect worldwide. These include diverse types of fish and farming methods, better ways to identify and treat diseases, improved types of fish, and more intensive farming methods. This progress needs to be expanded with the help of technology, scientists, and industry, and policy support whenever needed. It also requires improving skills, creating new infrastructure, and ongoing communication between those who create things and those who study them. The ability of aquaculture to continue in a good way is the hardest part of this rapidly growing food industry. In simpler terms, the use of new technology and scientific knowledge in farming should be done carefully, with a focus on ensuring that it is sustainable and profitable for farmers. . Nanotechnology has brought new possibilities in studying tiny molecules in our bodies, creating safe ways to treat genes without using viruses, using tiny vehicles to carry DNA, proteins, or cells, delivering medicine exactly where it is needed, diagnosing and treating diseases, and much more. Biotechnology has been very helpful in using natural organisms and helpful bacteria to clean up pollution and manage waste and harmful substances in the environment. Biotechnology could help create smart and high-performing fish in the future. There are also some problems on the way; it is up to the scientists to solve these issues related to the environment and ethics.

REFERENCES:

[1] R. M. Harrell, “Bioethical considerations of advancing the application of marine biotechnology and aquaculture,” *Marine Drugs*. 2017. doi: 10.3390/md15070197.

[2] A. Figueras and B. Novoa, “Marine biotechnology and aquaculture,” *Arbor*, 2014, doi: 10.3989/arbor.2014.768n4007.

[3] R. State, “Biotechnology in Aquaculture: Prospects and Challenges,” *Niger. J. Biotechnol.*, 2011.

[4] N. C. Bols, “Biotechnology and aquaculture: The role of cell cultures,” *Biotechnology Advances*. 1991. doi: 10.1016/0734-9750(91)90403-I.

[5] C. L. Hew and G. L. Fletcher, “The role of aquatic biotechnology in aquaculture,” *Aquaculture*, 2001, doi: 10.1016/S0044-8486(01)00587-7.

[6] L. Krienitz and M. Wirth, “The high content of polyunsaturated fatty acids in Nannochloropsis limnetica (Eustigmatophyceae) and its implication for food web interactions, freshwater aquaculture and biotechnology,” *Limnologica*, 2006, doi: 10.1016/j.limno.2006.05.002.

[7] R. S. Rasmussen and M. T. Morrissey, “Biotechnology in aquaculture: Transgenics and polyploidy,” *Compr. Rev. Food Sci. Food Saf.*, 2007, doi: 10.1111/j.1541-4337.2007.00013.x.

[8] G. L. Fletcher and M. L. Rise, *Aquaculture Biotechnology*. 2011. doi: 10.1002/9780470963159.

[9] J.-K. Lu *et al.*, “The Marine Aquaculture Biotechnology Status and its Sustainability in Taiwan,” *Curr. Biotechnol.*, 2015, doi: 10.2174/2211550104666151016203821.

[10] J. Xiang, "Recent Major Advances of Biotechnology and Sustainable Aquaculture in China," *Curr. Biotechnol.*, 2015, doi: 10.2174/2211550105666151105190012.

[11] J. K. Biswas and P. Maurye, "Aquaculture Biotechnology : Prospects and challenges," *Prog. Biotechnol. India*, 2017.

[12] FAO, "Current Status and Options for Biotechnologies in Aquaculture and Fisheries in Developing Countries," *Biotechnol. Agric. Dev.*, 2012.

[13] J. F. Gui, "Fish biology and biotechnology is the source for sustainable aquaculture," *Science China Life Sciences*. 2015. doi: 10.1007/s11427-015-4812-9.

[14] E. Yamaha, T. Saito, R. Goto-Kazeto, and K. Arai, "Developmental biotechnology for aquaculture, with special reference to surrogate production in teleost fishes," *J. Sea Res.*, 2007, doi: 10.1016/j.seares.2007.02.003.

CHAPTER 2

EXPLORING KEY CHALLENGES IN AQUACUTLUTRAL BIOTECHNOLOGY

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ABSTRACT:
Biotechnology is a scientific field that has been around for many years. It involves changing genetic traits and substances. The Chinese have made different types of goldfish by breeding them for many years. The use of new techniques in molecular biology, specifically genetic engineering, has made the field more interesting. Microorganisms are like new factories that produce drugs. Using them has made many drugs less expensive and easier to get. For instance, insulin for people with diabetes is mainly made by changing the genes of bacteria using a special technique called recombinant DNA technology. Biotechnological tools helped the Green Revolution by creating better types of rice, wheat, and maize that produced more food. Biotech is seen as the driver of a new agricultural revolution that relies less on pesticides and fertilizers. The same tools can also be used to start another revolution, but this time in a different area a Blue Revolution. The world's population is growing quickly, and more people are eating fish. This, along with uncontrolled fishing and bad management, is putting a lot of pressure on fish populations. There are also harmful chemicals polluting their habitats. As a result, global fish production is struggling. Lots of countries are using aquaculture to make more fish for fishing. Right now, aquaculture is the fastest-growing sector for producing food worldwide. The success of fish farming relies on being able to control how fish reproduce and grow throughout their lives. The parent fish's genetic background, finding and stopping diseases, knowing the best conditions for growth, having enough clean water, and using new ways to manage. By making certain improvements, the fish farming industry has made significant advancements in recent years. Using biotechnology can help the industry grow faster.

KEYWORDS:

Biotechnology, Fish, Food, Farming, Genetic Engineering, Temperatures.

INTRODUCTION

Even though half of the fish we eat come from the fishing industry, it is not growing anymore. Also, one third of the fish that we catch are being taken too much and may run out soon. This progress has caused a quick increase in global fish farming, which now makes up more than half of the world's food production. In simple words, aquaculture mostly involves raising fish or other organisms in small, controlled areas with lots of them together. This can cause issues like diseases and the use of pesticides and chemicals, which can harm the environment. Aquaculture is the practice of growing fish, crabs, shellfish, water plants, and algae in a controlled environment. It is done everywhere in the world except in very cold areas near the poles. Aquaculture is the farming of fish, and current trends are focused on increasing the amount of fish produced in a specific area. This is done by using new technologies and methods to develop better quality fish, and doing so in a shorter amount of time. These technologies include developing new types of fish food, finding alternative food sources, using better feeding techniques, and improving fish health and breeding methods. Additionally, there is an emphasis on improving fish genetics and using advanced technologies for harvesting and processing the fish after they are caught. Biotechnology in aquaculture involves using living

organisms or biological systems to improve the production of quality food. Over time, our understanding of biotechnology has helped us make better food fish that grow faster, are stronger against disease, and have better meat. We can also make ornamental fish that sell for a lot of money [1], [2].

We use helpful microbes to make fish food and keep the water clean. We can also use certain markers to choose the best fish. We make sure the fish we eat are safe and can breed and produce baby fish all year long. We take care of fish populations and make sure they stay healthy. These are the main reasons why advanced biotechnological tools are now used in aquaculture. In China, scientists have been trying for many years to create different types of pretty fish by breeding them together. They have been experimenting with different shapes, sizes, and colors to make the fish look more interesting and attractive. Recent advances in molecular biology, especially genetic engineering, have allowed us to use microbes like bacteria to produce important medications, such as insulin for people with diabetes. This is made possible by changing the bacteria's DNA through a technique called recombinant DNA technology. Additionally, we can also use genetic engineering to create crops that produce more food, pesticides that are less harmful to the environment, and fertilizers made from living organisms. It has also played a big role in helping the fisheries and aquaculture industry grow to meet the needs of a growing population and a polluted environment. This is especially important because there is less land available and not enough clean water for fish farming. The success of fish farming depends on carefully breeding fish and producing them in a way that is good for the environment and makes a lot of money. The sector has made a big contribution by breeding fish to grow bigger and resist disease. They also use hormones to help fish breed in captivity and make their food healthier. They have developed new vaccines and tests for diseases in fish [3].

They also use helpful bacteria to control the environment and make fish last longer after they are processed. Overall, they have found ways to make fish farming more efficient and productive. Big groups of databases on important types of fish used for food and decoration have been created to find new genes that can be used more widely. In ornamental fish breeding, biotechnological tools such as genetic modification have been used to develop fish with different colors. They have also been used to create fish that can detect pollution in water and to develop genetically modified vaccines. These tools are also used for molecular diagnostics, which help in studying and understanding fish genetics. New developments in biotechnology are creating new chances for aquaculture to become better, kinder to the environment, and able to continue in the long term. However, not everyone in the public agrees with or accepts all types of biotechnological tools. Many consumers are refusing to accept some of the tools or products used in genetic engineering. However, genetic engineering is just one of the many things we can do with biotechnology. Many people have different opinions about the good and bad sides of genetic engineering. The FDA approved the AquAdvantage salmon from AquaBounty Technologies. It grows to market size in 1. 5 years instead of 3 years. This made consumers worried that GMOs will become very common in the markets. People who criticize aquaculture make it seem like the industry only cares about making money and ignores the potential harm it could cause to the environment and people's health [4], [5].

In the past few years, new tools for changing genes have greatly improved scientific research in biotechnology around the world. Several researchers have used the traditional method of selectively breeding animals based on their physical characteristics to develop or change an animal with the most desirable traits. With the advancement of modern biotechnology, we can now move genes from one organism to another, even if they are from different groups of living things. However, the way genes are transferred raises some concerns among the public and

poses ethical problems. This has led to the development of various gene editing technologies. Gene editing is a way to change the genes inside an organism. It can modify the genetic makeup of a species. Different methods are used to add or remove a specific part of a gene. These methods include zinc finger nucleases, transcription activator-like effector nucleases, meganucleases, and the CRISPR/Cas system. Out of all the different gene editing technologies, the CRISPR/Cas9 method is the most commonly used in aquaculture. By using the CRISPR/Cas9 technique, scientists can cut a specific gene in a fertilized fish egg at a particular location. Afterwards, the cell understands there is damage and fixes it by itself. So, after the fix, the current shape of DNA is not the same as the original pattern.

The CRISPR/Cas9 technology has already been successfully used in important fish species like rainbow trout, Atlantic salmon, catfish, tilapia, and carp. In simple terms, scientists have found that modifying certain genes can have specific effects. For example, changing the myostatin gene can make muscles grow more. Eliminating the dead-end gene can make fish unable to reproduce. Modifying the fatty acid elongase gene can increase the amount of omega-3 fatty acid EPA in fish fillets. But we still don't fully understand how these changes happen, and it's important to keep studying this topic. We need to study how different molecules interact with each other. Researchers have noticed that when a specific gene called mstn is turned off in fish, it weakens their immune system. This makes the fish more likely to get sick from various diseases. Even though there are some disadvantages, genetic engineering has many convincing benefits that make it very useful in fisheries and aquaculture for increasing productivity. By turning off certain genes in an animal, it becomes easier to figure out which physical characteristics are affected by that gene. This helps us understand how traits and genes are connected, which improves our ability to breed animals and plants successfully [6], [7].

The most famous biotechnology tool that greatly changed the way fish breeding is done is called gonadotropin-releasing hormone (GnRH). GnRH is important for controlling and starting the reproductive process in all animals with backbones. The making and letting go of GnRH a type of hormone happens in certain brain cells called GnRH neurons. These cells are found in the hypothalamus. GnRH was first taken out from sheep and pigs and was found to be able to make follicle-stimulating hormone (FSH) and luteinizing hormone (LH) be released from the pituitary gland. The amount and frequency of GnRH pulses, as well as feedback from hormones, like androgens and estrogens, determined the release of FSH and LH. High-frequency GnRH pulses make LH be released, while low-frequency GnRH pulses make FSH be released. Most mammals have only found one type of GnRH hormone, except for nonplacental animals excluding guinea pigs, which have 12 different types of GnRH hormones. In fish, scientists have found eight different types of GnRH. Until now, scientists have tested a lot of different chemicals similar to GnRH to help fish breed. Out of all the different ones tested, the salmon GnRH analogue has been the most successful.

DISCUSSION

Transgenesis means putting a gene from one animal into another animal. The animal that gets the new genes is called a transgenic animal. Through transgenesis, animal genetic makeup can be altered to include traits from another organism. Let's rephrase the sentence in simpler words: "Palmiter and his colleagues. A group of researchers created a special kind of animal by adding a gene called metallothionein human growth hormone fusion gene (mT-hGH) to a mouse. This made the mouse grow much bigger than usual. This led to researchers trying to transfer genes to different animals that are valuable for the economy, like fish. The first genetically modified goldfish was created by adding a new gene using a small injection into the fertilized egg. The scientists said that when the fish are 50 days old, around half of them have the desired genetic trait. Since then, there has been a big advancement in the way we do things and it has been

tried out successfully on different kinds of fish. The process of making fish grow faster through genetic modification is showing positive results, especially in salmon. Devlin and his colleagues. It was discovered that genetically modified adult salmon grow 300,000 to 500,000 times larger than regular salmon.

Additionally, in the early stages of growth, the increase in growth compared to the control group is up to 10 times greater. Scientists tried putting genes into fish eggs of different types of fish, including tilapia, rainbow trout, medaka, catfish, common carp, zebrafish, and loach. This was done in research studies. The development of transgenic fish focuses on producing offspring that cannot have babies and are safer for the environment. Extreme cold temperatures in the winter have been a long-standing issue in aquaculture. Researchers always prioritize studying fish that can withstand the cold. In nature, some fish have proteins in their blood that can stop them from freezing. These proteins help lower the freezing temperature of their bodies by stopping ice crystals from forming. The gene that makes liver AFP in winter flounder has been well studied and then put into the genes of Atlantic salmon. The AFP gene was discovered to be fully integrated into Atlantic salmon and passed down to their offspring. In the offspring, there was a significant amount of this gene expressed in their livers. In addition, when scientists put a special gene into goldfish, it made them able to handle cold temperatures better than goldfish without the gene [8], [9].

In the same way, when AFP is injected or given by mouth to milkfish and tilapia, it helps them become more resistant to cold temperatures. So, if we can create fish that can survive in cold temperatures, it will help make temperate regions more productive in the colder seasons. Additionally, a special protein called green fluorescent protein (GFP) was put into fertilized zebrafish eggs to create transgenic zebrafish. Embryonic stem cell (ESC) technology is a new method that scientists are using a lot to develop transgenic fish. ESCs are very flexible cells that can be changed or controlled in a lab easily. The process helps to keep or remove a certain characteristic that has been introduced. To create a genetically similar group of transgenic fish, scientists successfully use a technique called nuclear transplantation. This involves transferring specific genes into the cells of developing fish embryos. Moreover, in certain types of fish like zebrafish and medaka, researchers have successfully shown the presence of stem cell-like lines. Although there has been progress in developing transgenic fish, there are still some problems that need to be solved before these fishes can be successfully sold in the fish farming industry. This is especially true for food fish, where ethical concerns are important.

Chromosome engineering is a way to change the genetic characteristics of a person by altering their original DNA to make them better suited to their natural surroundings. In aquaculture, they use chromosome sex manipulation a lot. It helps to create triploid or tetraploid individuals and also allows for the inheritance of chromosomes from just one parent. These methods help improve the quality of fish breeding by allowing scientists to quickly choose the sex of fish, sterilize their reproductive organs, enhance the viability of hybrid fish, and create clones. Most vertebrates have somatic cells that are diploid, meaning they have two complete sets of chromosomes. However, polyploidy means that some individuals have extra sets of chromosomes. This can make the number of chromosomes three in triploids, four in tetraploids, and so on. In fisheries and aquaculture, induced triploidy is the best way to make sure fishes cannot reproduce. The methods used to create triploid fish and manipulate their chromosomes are well described and have various uses in fish farming and fisheries management. The Pacific oysters, known as Crassostrea gigas, showed improved growth and were sterile when they were triploid have three sets of chromosomes compared to when they were diploid.

This was true not just for fish, but for oysters as well. In addition to triploidy, having tetraploid breeding lines is also helpful for aquaculture. When tetraploids and diploids are crossed, they

can create many triploids that are unable to reproduce. Many fish have been artificially made to have four sets of chromosomes, but in most cases, these tetraploids did not survive well. In fish, using hormones from outside the body can make them unable to reproduce. This is a profitable area of biotechnology. However, the problem with using artificial hormones in fish production is that there are government regulations and many consumers do not like the idea of eating fish that have been treated with hormones. Triploidy can be caused by treating newly fertilized eggs with physical or chemical methods to stop the release of the second polar body. In simple terms, triploid fishes are unable to reproduce because their chromosomes don't pair up properly during a specific division. Triploid induction can happen by treating fertilized eggs with pressure or by subjecting them to extreme temperatures or certain chemicals like cytochalasin B, colchicine, or nitrous oxide. Gynogenesis is a method to create a population of only female individuals in a species where females have the same type of sex chromosomes. It is better to use all-female offspring created through gynogenetic reproduction, instead of normal offspring with both males and females, for experiments on changing sex.

We have developed a method using induced gynogenesis and hormonal sex inversion for several species in aquaculture. Androgenesis helps make fish with the same genes so they can breed with each other. It can also help bring back genes we thought were lost by using frozen sperm. Even though it is difficult to achieve success in androgenesis with only a few species, scientists have tried it out on cyprinids, cichlids, and salmonids. Cryopreservation is a way to store living things in really cold temperatures for a long time. This technique works because when cells are frozen at very low temperatures, their activities slow down and they can be kept alive for a longer time. In fish farming, freezing fish sperm has been used for a long time to keep it fresh. Blaxter discovered a way to freeze and store fish eggs at very cold temperatures. He was then able to use these frozen eggs to fertilize more fish. The text is already quite simple. However, here is a simplified version: herring eggs with thawed fish sperm. The reproductive cells of many fish species that are grown in controlled environments have been successfully frozen for preservation. Cryopreservation helps to solve the problem of male fish maturing earlier than females in breeding. This makes it easier to selectively breed and improve fish stocks. It also helps in conserving fish species. Currently, there is an established method for freezing fish sperm, but there is no reliable technique for preserving fish eggs and embryos.

Surprisingly, it has been announced that shrimp embryos can be frozen. Storing the genes of all the eggs and sperm from commercial fish is really important right now. This will help the fishing industry become more sustainable. Feed is really important for the growth and financial stability of the fish farming industry. It makes up more than 60% of the money spent on producing fish. Because fish meal is expensive or being used less in fish feed, people need to find another source for it. In this situation, people are finding ways to meet the needs of the fish food industry. They are doing this by adding more nutrients to the food, using insect larvae instead of other ingredients, and using fermentation methods. Using pre and probiotics, modified yeasts to make extra carotenoids, and digestive enzymes from outside sources for the young insect's food appear to be very hopeful. Plant-based proteins are being used more often instead of fish meal. However, they have some things in them that can be bad for you if they're not processed properly. Genetically modified organisms (GMOs) can now be used to get rid of harmful substances in food and increase specific amino acids and fatty acids through biotechnology.

Using specially modified microorganisms to create specific enzymes that are of high quality and can withstand different temperatures and pH levels would greatly transform the feed industry. This could be achieved through a process called granulation technology, which helps in creating pellet feed. Trace minerals are now being combined with oligopeptides to make

them easier for the body to absorb. There is now a big demand for feed products that have positive effects on growth and health. These products are made from waste materials. The latest trend is to create diets with manageable amounts of amino acids to lower the need for protein. Lysine and methionine are substances used in fish food. Lysine is made by tiny organisms, while methionine is made through a chemical process. These substances are commonly used in fish feeds. Scientists are using special cells that have been changed at the genetic level to make certain substances like threonine and tryptophan. These substances are being made and sold, and in the future, we might also be able to make other important substances called amino acids. By using these specific nutrients, we can reduce the cost of animal feed by replacing some of the protein. The idea of using a special mix of proteins made from genetically modified plants and adding certain substances to animal food can reduce the amount of nitrogen in their waste.

In the future, there are great opportunities for using toxin removal substances, fish meal additives, and beneficial bacteria to make sure fish are healthy and of good quality. Modern tools like polymerase chain reaction (PCR), biomonitoring, DNA/gene-based diagnostics, HPLC, and ELISA-based tests can be used to find harmful substances like pathogens, toxins, heavy metals, pesticides, biotoxins, and other contaminants in fish food and the environment where they are fed. Biotechnology is really important for keeping fish healthy and making them produce more. Quick diagnosis is important for controlling diseases in aquaculture. Advances in molecular methods, such as PCR, real-time PCR, and nucleic acid sequence-based amplification, have made it possible to quickly detect, identify, and measure small amounts of infecting pathogens. In addition, technologies like microarray provide a new way to test for multiple pathogens and how the body responds to them. The loop-mediated isothermal amplification technique has become popular in diagnostic labs because it is better than PCR. It only requires a water bath to maintain a constant temperature and is more accurate. Immunological tests such as immunohistochemistry, fluorescent antibody technique, indirect fluorescent antibody technique, ELISA, and dot blot or western blot can quickly and accurately detect harmful microorganisms without having to isolate them first. In these tests, certain antibodies are added to the samples and there are many of these antibodies that can be bought commercially.

Because scientists have created shrimp that are free of harmful pathogens and are resistant to certain diseases, there has been a big increase in the amount of shrimp being grown around the world. Furthermore, various vaccines that protect fish from dangerous illnesses are becoming more commonly used in the field of looking after fish's wellbeing. Scientists all over the world are trying to create better vaccines that work really well. Instead of using weakened or killed germs, the new vaccines will use genetically changed microorganisms, special genetic material, small parts of proteins, and pieces of DNA. Although some new-generation vaccines have had success in experiments, only a small number of them are actually being used in aquaculture farms. The future of biotechnology in aquaculture depends on making money from vaccines that can protect many different types of farmed fish from economically damaging diseases. Biotechnology has the potential to help us create useful products from algae, such as oils, pigments, and alginates. Scientists have recently created a potential cancer treatment called CIGB-552.

They discovered this treatment in the blood plasma and tissue extracts of horseshoe crabs. The new peptide is being tested in phase I clinical trials as a drug for cancer. It might also be used to treat other diseases related to metabolism and inflammation. The shrimp's immune system is not as strong as the immune system of vertebrates. However, biotechnologically engineered molecules can be very helpful in boosting the shrimp's immune system. Microbial products

like lipopolysaccharides, peptidoglycans, and glucans have been found to be great at boosting the natural defense system of various types of shrimp. Without a plan to give shrimp vaccines and with the risk of various viruses in shrimp causing problems for the shrimp farming industry, there is a big opportunity to come up with new ways to fight these deadly diseases. Studying the interactions of microorganisms in diseased individuals using metagenomics could help us understand the factors that contribute to disease development. This knowledge has the potential to improve health management and identify ways to create a more favorable environment for beneficial microorganisms.

Traceability is becoming more difficult in aquaculture. Traceability refers to being able to track how food moves from its creation to its distribution. This is used to address food safety concerns and to make sure the food is of good quality. It helps businesses reduce risks and build trust with consumers. The biotechnology industry plays a big role in making sure that the products we consume are safe. They do this by closely monitoring the entire process, from removing antibiotics and other harmful substances from the production line to delivering the final product to the consumer. The new tools used for measuring harmful substances or detecting microorganisms using methods like PCR or ELISA show a lot of potential. Industrial fish farming raises serious worries regarding human health and the safety of the food produced. Using more antibiotics and pesticides in fish farming to prevent diseases can build up in fish bodies. In addition, the food given to farmed fish is made from fish caught from the ocean or from fish that are considered low quality. These fish could have a lot of harmful chemicals in them. Therefore, creating biotechnological tools that can easily detect or screen the organisms would be helpful in providing a safe product to the people who use it. Can you simplify a specific text. Please provide the text you want me to simplify.

The use of genetically modified foods and animals has been a difficult issue. People are worried because the public has many wrong ideas that they believe. The Food and Agriculture Organization, along with the World Health Organization, has created some guidelines to make sure that food made from genetically modified animals is safe to eat. The Codex Alimentarius and the European Food Safety Authority GMO Panel have suggested that when assessing the safety of genetically modified (GM) products, they should be compared to non-GM products that are known to be safe. They also recommend analyzing the genetic material that has been added to the GM product. Other qualities that need to be tested are the possibility of being harmful (of the things that are added or changed), the possibility of unintended effects happening along with the original changes, the transfer of genes between different organisms, and the nutritional value. Over 35 different types of fish, such as salmon, catfish, tilapia, trout, striped bass, and flounder, are being changed genetically all over the world. This is being done to make the fish better for industrial fish farming. They are trying to make the fish grow faster, be resistant to diseases, have bigger muscles, and be able to withstand different temperatures.

These changes are being tested out on a small scale to see how well they work. But in 2015, the FDA said it was okay for AquaBounty Technologies to sell AquAdvantage salmon, despite concerns that it might harm the environment and people's health. It is still a big challenge for the scientists who work with technology and biology to respond to these negative comments while finding a solution to the worldwide need for proteins for the rapidly increasing population. In order for people to be okay with GM and transgenic food, certain negative opinions from society need to be addressed. The group of scientists led by Novoselova et al. More people liked regular pork better than genetically modified pork. People might not like GM pork, but they might change their minds if the animals are healthier and treated better. GM pork may also have fewer harmful substances, be better for the environment, and cost less money. Improving the way animals are treated and providing health benefits is what consumers

prefer the most. On the other hand, making changes to the environment has the smallest impact on their preferences. When it comes to GM salmon, people often say that the biggest benefit they receive is a lower price for the product. Additional benefits provided by GM salmon are the potential for better health due to increased omega-3 levels, as well as environmental benefits such as decreased chemical use and using less feed.

The biggest problem with genetically modified organisms (GMOs) is that they can escape into the wild and mix with natural plants and animals, causing harm to the environment. Researchers are not sure about what environmental problems may happen at the beginning, and it is hard to fix them once they are found. Genetically engineered animals can have negative effects. These can happen either directly, when they have to compete with other animals for limited food, habitat, and resources, or indirectly, when they cause changes in other things that are used or needed by different groups of living things in an ecosystem. To understand the risks of GE fish, we need to consider three things how likely it is for the GE fish to get away and spread to different groups of fish, how the changed genes affect the ability of the GE fish to survive and thrive in the environment where it is released, and how well the existing community of fish can handle the changes brought by the GE fish. It is important to keep both the animals used for experiments and the researchers safe in the laboratory. The laboratory must prevent any genetic material from escaping, as it could cause a lot of problems. Biotechnologists find it difficult to create facilities and model aquatic animals, even though they have access to the genetic information of a few species.

In the future, we will be able to gather detailed information about the entire genetic makeup and metabolism of many different types of fish that are important for both food and decorative purposes. There are ethical concerns for the well-being of animals in every stage of creating and maintaining a genetically engineered aquatic animal. The main things we are worried about are: using invasive methods to create a new genetically modified animal, needing a lot of animals for creating genetically modified embryos, and unexpected problems with the well-being of the animals. Zebrafish were changed using the genes of sea anemones and jellyfish to make them glow in pretty colors. These fish are now sold as pets under the name GloFish. They first started being sold in the United States in 2003. But later on, their sale caused arguments about what is right and wrong in California. Eventually, California decided to be the only state in the US to ban the sale of GloFish as pets. We must share information freely, make people aware, and be clear and honest to solve ethical problems in fishing. Finally, the biotechnology industry is also dealing with a few other difficulties, which include: a. How long it takes to get a patent for a product can be more than a year and sometimes it can even take more than ten years. This causes a lot of pain and stress to both the developer and the investor.

Moreover, the private individuals or businesses involved in aquaculture are not well-established enough to easily sell their products. For instance, when there are no rules about using drugs or checking samples for diseases, or no strong guidelines for keeping things safe in freshwater farming, a lot of the things that are made do not get sold. Once again, many of the products that are being sold are not becoming popular in the industry. There are only a few aquaculture biotechnology companies in India, even though it is the second largest producer in aquaculture. These companies work with fishery technologies. I cannot rewrite the text as you have not provided any text for me to work with. Investments and profits: Fish is a product that is not in high demand everywhere, but it does provide a significant amount of protein that humans need. However, there is not as much focus on all areas of biotechnology, including pharmaceuticals, health products, and tools for breed improvement. Additionally, the wide range of different types of fish that are used for food or as pets also greatly affects how effective certain products are for many different types of fish. There are very few players in the health

or pharmaceutical industry. So, the question is whether technology is worth the investment because there are no clear rules for regularly checking the health of fish farms, making sure pathogens don't enter the country, measuring the amount of antibiotics in domestically consumed fish, and checking for drug residues in food fish. Therefore, no industries are willing to invest in those sectors because there is not enough demand for their products or services.

CONCLUSION

Biotechnology has had a significant impact on the growth of the aquaculture industry. While it hasn't progressed as quickly as it has in land animals or humans, it has played a big role in fish breeding to make them grow better and be resistant to diseases. It has also helped with conserving different fish species, diagnosing diseases on a molecular level, creating vaccines, modifying genes, and editing genes. Moreover, there are many ways that this technology can be used in aquaculture. This includes making new rules to make it easier to trade and sell products, making tools that work better for specific regions, and not relying as much on information about different species when making products. We also need to think about the ethical and biosafety issues that may arise on a worldwide scale. Using research to improve the health and wellbeing of organisms in different aquatic environments and production systems is a big challenge because there are many different species involved. Selective breeding is a way to improve different traits in plants or animals by carefully choosing which individuals to breed. This can include using genetic editing techniques to change specific genes. It is also important to use vaccines and other health tools to keep the animals or plants healthy. This process helps reduce diseases and can increase how much is produced. In order for us to produce things efficiently, we need to understand how living things react to their surroundings. This is important whether we are producing things in closed, high-intensity places with strict safety measures or in open systems. Artificial intelligence is helping us monitor and study animals from a distance. This is creating an interesting and innovative environment for the aquaculture field. The future looks promising.

REFERENCES:

[1] M. Rawat, D. Singh, S. Saraf, and S. Saraf, “Nanocarriers: Promising vehicle for bioactive drugs,” *Biological and Pharmaceutical Bulletin*. 2006. doi: 10.1248/bpb.29.1790.

[2] S. Brooks, “Biotechnology and the politics of truth: From the Green Revolution to an Evergreen Revolution,” *Sociol. Ruralis*, 2005, doi: 10.1111/j.1467-9523.2005.00310.x.

[3] V. Döring, E. Darii, O. Yishai, A. Bar-Even, and M. Bouzon, “Implementation of a Reductive Route of One-Carbon Assimilation in Escherichia coli through Directed Evolution,” *ACS Synth. Biol.*, 2018, doi: 10.1021/acssynbio.8b00167.

[4] R. Suzman and J. Beard, “Global Health and Aging,” *NIH Publ. no 117737*, 2011, doi: 11-7737.

[5] S. Basu, C. Bose, N. Ojha, N. Das, J. Das, M. Pal, and S. Khurana, “Evolution of bacterial and fungal growth media,” *Bioinformation*, 2015, doi: 10.6026/97320630011182.

[6] R. Torres-Ruiz and S. Rodriguez-Perales, “CRISPR-Cas9 technology: Applications and human disease modelling,” *Brief. Funct. Genomics*, 2017, doi: 10.1093/bfgp/elw025.

[7] Y. Mei, Y. Wang, H. Chen, Z. S. Sun, and X. Da Ju, "Recent Progress in CRISPR/Cas9 Technology," *Journal of Genetics and Genomics*. 2016. doi: 10.1016/j.jgg.2016.01.001.

[8] E. Hartenian and J. G. Doench, "Genetic screens and functional genomics using CRISPR/Cas9 technology," *FEBS Journal*. 2015. doi: 10.1111/febs.13248.

[9] S. Saayman, S. A. Ali, K. V. Morris, and M. S. Weinberg, "The therapeutic application of CRISPR/Cas9 technologies for HIV," *Expert Opinion on Biological Therapy*. 2015. doi: 10.1517/14712598.2015.1036736.

CHAPTER 3

USING MOLECULAR MARKERS TO IMPROVE AQUACULTURE GENETICS AND BREEDING

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ABSTRACT:
Having genetic differences within a species helps organisms to be better at adjusting to a changing environment, which is important for the survival of the species. Genetic differences can occur between individuals, which causes them to be different from each other within a population, species, or larger groups of organisms. The information about different genetic traits and characteristics is useful for studying how species change over time, protecting and using natural resources, and improving the genetics of plants and animals. Molecular genetic markers are powerful tools that can be used to study the genetics of individuals, groups, or species. These markers and statistics have greatly improved our ability to study genetic diversity. Molecular markers and analyzing them with statistics greatly improved our ability to study genetic diversity. Different types of markers are being used in fisheries and aquaculture. These markers can be proteins or DNA, like mt-DNA or nuclear DNA such as microsatellites, SNP, or RAPD. These markers are used to collect important scientific information in aquaculture. Some of the things they help with include Identifying different species. Studying genetic differences and how populations are structured in the wild. Comparing wild fish populations to those that have been bred in hatcheries. Assessing if there have been any times when the population got very small, known as a demographic bottleneck. Assisting with programs that help breed and release fish to help the population grow. In this article, we are focusing on the basics of molecular genetics, giving an overview of commonly used markers and how they are applied in fisheries and aquaculture studies. We will also discuss their limitations.

KEYWORDS:

DNA, Fisheries Aquaculture, Genetic Diversity, Genetic Markers, Molecular Markers.

INTRODUCTION

The beginnings of genetic markers can be traced back to the important work of Alfred H. In 1913, Sturtevant studied Drosophila melanogaster while he was a student in Thomas Hunt Morgan's lab. Sturtevant made a map of the chromosomes in a fruit fly. He found six genes related to sex and measured the distance between them. This helped scientists understand how genes are located on chromosomes. Since then, scientists have done lots of research to make maps of genes in plants, animals, and fish. In the 1960s, when electro phoretic studies were introduced, people began to search for markers more actively. At first, scientists did a lot of research on plants, insects, and animals, mainly focusing on protein markers called allozymes. These markers were found using a technique called starch gel electrophoresis. In the early 1990s and beginning of 2000, a scientific technique called polymerase chain reaction (PCR) became very important. This technique made it easier to study differences in DNA. The restricted fragment length polymorphism (RFLP) was discovered when scientists were making a map of how genes are connected in humans. The RAPD, which is a form of DNA testing, was first used in 1990 as a way to identify and study genetic differences. The issue with RAPD was that it was not reliable, but this was fixed by using a different marker called AFLP. We have information about short tandem repeats or microsatellites, which are found a lot in

genomes. Also, we have markers from specific genes in the mitochondrial DNA called mitochondrial markers. Some important molecules in biology are called 12S rRNA, 16S rRNA, cytochrome b, D-loop, and COI.

We used a technique called PCR to study genetic differences. This involved a lot of hard work with electrophoresis. We were able to find some genetic markers, but there weren't enough to study the whole genome. This was a disadvantage of our study. Since the late 2000s, advanced techniques like next-generation sequencing have become very important. These techniques have helped scientists to discover markers called single nucleotide polymorphisms (SNPs), which are very common in the genomes. Breeding programs have been popular for a long time in plants, crops, and animals. It uses usual methods to choose individual organisms with specific characteristics for breeding purposes. The main idea of genetic improvement is to pick and mate individuals that show better growth or production, so their offspring will also do better. Selection is typically done for traits that can be measured and are not just categorical. These traits can vary in a smooth and continuous way. The level of heritability for these traits may range from being low to being high. When artificial selection is used, the number of genes for the chosen trait goes up in the group that was chosen. The growth that happens in one group of offspring is passed on to the next group, so we need to keep selecting the best traits [1], [2].

In aquaculture, breeding only certain types of fish started being done in Norway in the early 1970s. They began doing this with rainbow trout in 1973 and Atlantic salmon in 1975. These programs have made great progress since then and now include disease resistance and other traits like good quality meat. Norway has great programs that breed fish to grow faster and be better at fighting diseases. Some breeding programs for specific types of shrimp, fish, and sea bass are currently popular. These programs have made impressive changes and have had a positive impact on the world. The Pacific white shrimp is now a big part of how much shrimp is produced all over the world. There are 14 countries on 5 continents that are growing and farming GIFT, which is a genetically improved type of tilapia. New sequencing technologies developed since the late 1990s have created new opportunities for genetics research. Population genetics studies how genetic differences are spread among different groups of living things, including species, populations, and individuals. It focuses on how various factors like mutation, natural selection, random genetic changes, and migration influence the distribution of genetic diversity. The differences in genes between populations can tell us about the history and isolation of these populations. The text is saying that genetic differences refer to the differences in the number and quality of different parts of our genes, chromosomes, and how they are arranged.

These differences can be found within and between different groups of individuals. It is important to measure the genetic differences within groups of wild fish or farmed fish in order to understand and manage them better. Genetic diversity has been studied by controlling breeding and observing how animals or plants perform. It can also be analyzed by looking at observable physical characteristics. Different characteristics and methods are used to study how fish populations are organized. These include examining the environment, attaching tags to fish, studying parasites, studying the physical and behavioral traits of fish, measuring the size and shape of fish, studying calcified structures like bones, studying genes and chromosomes, and analyzing blood pigments. Sadly, the connection between genes and how they show up in a person's characteristics is complicated and can be influenced by the environment. Therefore, geneticists who study populations mainly focused on traits that obey Mendel's laws of inheritance. They primarily studied these traits in species that are commonly used in laboratory experiments or in pure breeds of a few species. The techniques used in these studies were not appropriate for wild populations and have only been used a little in fisheries

science and fisheries management. In the later twentieth century, scientists created new ways to study and understand genes [3], [4].

These methods allowed them to identify, describe, measure, and examine genes in more detail. This happened because scientists found out how DNA looks like in the 1950s. This led to the development of molecular genetics, which is the study of genes at a very small level. Molecular genetics is now seen as an important part of genetics. In the 1960s, scientists studied molecules in cells, focusing on proteins like haemoglobin and transferrin. However, they soon started looking at other proteins called enzymes and allozymes. Allozymes were the main method used in research from the 1960s to the 1980s. The PCR technique has made it possible to study genetic changes in fish populations in the last 10 years. Nowadays, there are many scientific techniques that can be used to study different things about wild populations, fish that are being raised in captivity, and how wild and raised fish interact with each other. The use of many different methods and their changes, along with the materials needed for them, are growing. These methods have many uses and can have benefits and drawbacks, depending on how they are used. This can sometimes lead to confusion and misunderstandings, especially between experienced scientists and those who use their research.

Choosing the right markers for specific purposes can be challenging and is often based on the researcher's expertise, the resources in the laboratory, and the amount of funding available. So, sometimes we need to check on the progress of the techniques, applications, and interpretations of the data we collect. The following section of this review focuses on population genetics and molecular genetics. The goal is to give an overview of the molecular markers currently used and how they are applied to issues in fisheries and aquaculture. Assess the advantages and disadvantages of molecular markers in fisheries and aquaculture science. Examine the different ways molecular markers are used in fisheries and aquaculture and how they interact with one another. This research in Chemistry won the Nobel Prize Since then, scientists have learned more about the way DNA and genes are structured and how they work, and they have started using this knowledge to understand the differences and variations in genetics. Ways to copy, read and compare DNA were created in the 1970s, and then in the 1980s, methods to make more DNA and read it automatically were invented. These advancements resulted in different types of DNA markers being made. The allozyme electrophoresis is a common way to study genetic differences at specific genes inherited in a Mendelian way where both versions of the gene have equal dominance [5], [6].

The method was created in the 1960s and was very popular until the early 1990s. In the early 1980s, scientists began studying the genes in our body's energy-producing structures (called mitochondria) to learn about how different populations are related to each other. This was the first time this type of genetic research was done. After the PCR was introduced, many new techniques were developed. These techniques included sequencing DNA and analyzing variations in DNA length, like microsatellites. A molecular marker is a part of DNA that helps us find a specific place on a specific chromosome. A marker gene is a gene that is used by scientists to track or identify certain characteristics or traits in an organism. This is a piece of genetic information. This category includes tests that focus on a specific part of DNA that we don't know what it does. A group of PCR techniques used to find anonymous or random sequences is called multiple arbitrary amplicon profiling or anonymous nDNA markers. The main ways to study DNA are RAPD and AFLP. RAPD uses PCR to amplify random sections of DNA. It has many benefits and has been used a lot in studies about fisheries. The process is easy, fast, and inexpensive. It has a lot of variation, and only a small amount of DNA is needed. There is no need for matching different DNA molecules, and most importantly, you don't need to know anything about the organism's genetic information in advance. known about their

genome. These markers can be used to study the genetic diversity and relationships between different individuals or populations of the species, without having to fully understand the entire genetic makeup of the species. They are a useful tool for genetic research and can provide insights into the evolutionary history and genetic variability of these species [7], [8].

Before we can understand the importance of molecular markers, we need to know what they are first. Molecular markers are specific characteristics of a gene or DNA sequence that can be passed down and observed. They are connected to a particular gene or trait. DNA variations, or changes in our genetic material, can be useful to track certain characteristics or traits. One great advantage of these variations is that they are mostly not affected by environmental factors. However, the main challenge lies in separating these variations from the rest of the DNA material. But now, new sequencing technology has made it easier and cheaper to do this task. This means that there is now a lot of important data available, especially at the molecular level. From a population genetics perspective, it is necessary for the marker alleles to occur in more than 1% of the population. From the point of view of genetic linkage and recombination, it is important for the marker to be close to a gene or trait that we are interested in, and this distance should be less than 5 cM. It is not enough to only find molecular markers. In India, scientists have been working hard to find special signs in fishes called molecular markers. However, unless the identified markers are used to make detailed maps, understand the genetics of a population, determine parentage, and find associations between genes and important traits in fish, this effort will not be very successful. Molecular markers are little indicators in our genes that can help us understand what certain genes do [9], [10].

For example, if we have a known gene that is responsible for a certain function in our body, we can use molecular markers to locate and study that gene more easily. Allozymes and RFLP are known as type I markers. Type II markers include things like RAPD, AFLP, SNPs unless they come from coding SNPs, and microsatellites unless they are linked to genes with known function. All the markers mentioned above come from the nucleus of the cell. In studying aquaculture, scientists use different DNA markers for various reasons. It is important to understand which molecular markers are being used in aquaculture. Single Nucleotide Polymorphism (SNP) markers are playing a big role in creating markers and assisting in the selection of plants, animals, and fish. This is particularly important as sequencing is becoming more affordable and sequence data is easily accessible. The value of single nucleotide polymorphisms (SNPs) for detecting differences in DNA is not as high as that of microsatellites with multiple alleles. However, SNPs are found in abundance throughout the genome, which makes up for this disadvantage. Moreover, in a trial, it was shown that although the low variation in the species is counterbalanced by their large number in the genetic makeup and a set of 700-900 somewhat variable DNA markers is similar to 300-400 sets of repetitive DNA markers. The SNP markers can uncover certain differences in genes that other markers and methods cannot find. This is a very important trait of SNPs. They are also helpful for GWAS, which means scanning the genome for SNPs/QTLs that are connected to economic traits. When we find out that a marker is connected to a certain characteristic, it helps us choose which animals to breed together in order to get better traits. This leads to more improvements in genetics. The SNP markers can be either type I or type II. They work together and follow a pattern of inheritance discovered by Mendel.

DISCUSSION

The discovery of their significance only came about in the late 1990s with the advent of gene chip technology, bringing to light their two different forms.Now, we can make a genotyping tool called a chip that can analyze DNA and give us information about individuals in aquaculture biotechnology. Microsatellites or short sequence repeats are very common in fish.

These markers have many different versions at a specific place in our genetic code and vary greatly between people. They follow the rules of Mendelian inheritance and they both fully express their traits. They can be used to study different types of species and determine who the parents are, as well as for creating maps of genes. Microsatellite markers are helpful in aquatic animals as genetic labels too. Selective breeding means keeping a lot of families together and raising them in the same place so that their genes can be different. In simpler words: If we want to take care of a group of fish together, it would be difficult to physically mark each one. Instead, we can use microsatellites to determine the parents of the fish, as long as we have information about the microsatellites of the parents. Mitochondrial DNA is genetic material passed down from the mother. Aquaculture scientists have often used it to examine the populations of fish. For studies on the genetics of populations, the genes found in the mitochondria, such as.

We use COI, 12s rRNA, 16s rRNA, cytochrome b, and ATPase 6/8. Scientists have noticed that the ATPase 6/8 genes that overlap are very good at detecting the genetic differences in teleosts from different places. RFLP was done using the DNA from two different mud crab species, *Scylla serrata* and *Scylla tranquebarica*. The enzyme called Hind III was used to cut the DNA. This showed that the two species have different patterns. A study compared the mtDNA of Asian sea bass from different places. The findings showed that there are two separate species. One species is found in the Indian subcontinent, Myanmar, and also Australia. The other species is found in Southeast Asia (Singapore, Malaysia, Thailand, and Indonesia) and Australia. The study used three markers: COI, 16S rRNA, and D-loop. Scientists examined tiger shrimp samples from the east and west coasts of India to see how their genetics can vary. They did this by multiplying a small part of the 16s rRNA mitochondrial gene. By analyzing the 520-base pair 16s rRNA mitochondrial gene fragment, it was evident that there were no apparent distinctions and a considerable likeness existed in the sequence of this specific segment. The tiger shrimp is a crucial species in the shrimp industry in India. Before 2009, tiger shrimp was the main type of shrimp being cultivated in India. However, in 2009, a new type of shrimp called specific pathogen-free (SPF) Pacific white shrimp was introduced. Since 2009, the farming of SPF Penaeus vannamei has grown a lot and surpassed the tiger shrimp. This special kind of shrimp now plays a big role in India's exports. Currently, India does not produce a lot of tiger shrimp. However, there is a chance that the production could increase because there are enough SPF seeds available. One big problem for tiger shrimp is their ability to reproduce. The main issue is that female shrimp have a closed reproductive part called the thelycum. Because of this problem, it is now very hard to make a lot of sibling families needed for a selective breeding program.

But now, there are genetic resources available for tiger shrimp. We have made a map using specific markers to help us understand the genetic information of the tiger shrimp. In India, researchers created a tool called Illumina iSelect to study genes. It can look at 6000 different parts of DNA, called SNPs. They used this tool on 1024 children from 7 different families who have the same parents. 3959 differences in DNA were identified in tiger shrimp. The variations were identified in 44 distinct groups of connected genes, mirroring the number of individual chromosomes found in tiger shrimp.The map belonging to the female was 28% longer than the map belonging to the male. In the same research, nine groups were discovered that have something called QTL, which is strongly connected to how long an organism survives after being exposed to WSSV. Another interesting finding was that these QTLs were similar to genes related to the immune system. Once we confirm that the QTLs are effective, we can use them to choose breeding stock that can resist WSSV infection. Additionally, researchers discovered that there were some genetic variations (SNPs) related to the sex of an organism on a specific

chromosome group called linkage group 30. Specifically, they identified three SNPs within a small region measuring 0. 8 centimorgans between positions 43.

These SNPs (genetic markers) have important future uses for the shrimp industry. They can help identify female tiger shrimp that only mate with one partner, which is useful for creating a population of only female shrimp for breeding purposes. The findings from this research can be very useful in the discovery of genetic markers and studying the relationship between important traits in penaeid shrimp. Pacific white shrimp is a type of shrimp that is really important and is the most commonly produced species of shrimp in the world right now. Scientists have studied this species to find ways to help it grow better and to determine its SPF status. As a result, they have spread this type of shrimp to many different countries. Research showed that the growth and resistance to WSSV were genetically linked in a negative way. Another study from India found that the likelihood of tiger shrimp inheriting resistance to WSSV was very low and almost nonexistent. This means that trying to breed shrimp with better resistance to the disease would not work well. In a research project in China, they found 14 different genetic markers that are related to how a shrimp grows. These markers can affect things like how heavy the shrimp is, how long its body is, and the size of certain parts of its body. These QTLs accounted for 2. 62% to 6142% of the differences observed in these traits. At a breeding program in Colombia, they used information from tests with a shrimp virus (WSSV) and marker information to calculate breeding values.

They selected shrimp parents based on these values to produce the next generation. Scientists noticed that the offspring of shrimp with strong genetic traits for resisting WSSV had a better chance of surviving than the offspring of shrimp with weak genetic traits. This method might be helpful in making shrimps stronger against WSSV disease using genetic markers. The benefit of this is that we can choose which shrimp to breed based on their genetic qualities, without needing to test them for diseases. This way, we can make sure there is no risk of spreading infections to the parent shrimp. In research in Australia, scientists used a method called AFLP marker to investigate certain genes that influence body weight, total length, and carapace length in a group of kuruma shrimp called Penaeus japonicus. A surprising discovery was made that two specific genes were found in male shrimp. One gene affected the length of the shrimp's body and shell, and it was located in group 1. Another gene influenced the same traits and was found in group 25. This was the first study to report a gene that affects the growth of penaeid species. The Indian major carp, known as *Labeo rohita*, is a type of fish measuring 3. 2 liters in size.

In 1992, a program to make genes better was started at ICAR-Central Institute of Freshwater Aquaculture in Bhubaneswar. The main goal was to choose fish that grow faster. In 2009, as part of this program, scientists tested how well fish can fight off a harmful bacteria called *Aeromonas hydrophila* that causes bleeding and sores when fish are stressed. Fish that were faced with these bacteria were carefully observed to see how long they survived, and the numbers were recorded in terms of hours. For the first time, scientists made a detailed map of connections in L. Rohita used 3193 markers to study 25 groups of linked DNA. These markers are related to the number of chromosomes in this species. A study found that 21 small differences in our DNA called SNPs were found in 10 groups of closely linked genes and spread across 12 different areas. These differences were strongly connected to how long someone can survive. Many of these SNPs were similar to genes that are already known to play a role in the immune system. A significant finding was that the enzyme ceruloplasmin was 4.

58 times more abundant in rohu fish that were able to resist something compared to those that were easily affected. The amount of cerulo plasmin in the body could be used as a sign to choose A. Hydrophilaeresistant rohu refers to a type of fish called rohu that is resistant to a

bacteria called Aeromonas hydrophila. If we use these QTLs to choose parents for the next generation, the offspring would have genes that make them resistant to aeromoniasis. In addition, it would be interesting and possible to study these genes on other types of carp as well to create carp that are resistant to aeromoniasis. The Asian Sea bass, also known as Lates calcarifer, is a fish that is commonly raised for food in India. Although it has been done on a small scale in the past, it is now becoming more popular. Even though this kind of animal has been successfully reproduced in captivity and good seeds are being made by the government in India, they have not started mixing and matching the best ones yet. However, countries like Vietnam and Singapore already have programs where they breed specific traits in certain animals.

They also discovered that certain differences in genes (SNPs) could be used to choose fish that grow quickly even when they are still young. In the past, a disease called infectious pancreatic necrosis caused more than 90% of young Atlantic salmon to die. This happened especially when they were still in the hatcheries or had just been moved to sea cages for farming. Atlantic salmon goes through two stages in its life. First, it lives in freshwater as babies, and then it moves to the ocean. Scientists found a really big part of the Atlantic salmon's chromosome 26 that has a strong impact on IPN. This part of the chromosome explains a lot of the genetic differences related to this trait in tests done in both freshwater and seawater environments. The need for challenge tests was avoided by using high-throughput sequencing and studying the changes in SNPs that affect IPN. Therefore, a method called marker-assisted selection was used to choose fish with a good gene that made them resistant to IPN. This method helped decrease the number of IPN cases, and in some instances, the disease was completely eradicated. Tilapia is a type of fish that is common in Africa and the Middle East.

There are many kinds of animals that live in lakes in places like Egypt, Malawi, Ghana, Ethiopia, Zimbabwe, South Africa, Israel, Tanzania, Zambia, and Southern Sri Lanka. Different species are always mixing together and we need to find out which species are involved in this mixing. To tell apart four types of tilapia, scientists studied the mitochondrial gene COI and 24 specific SNPs. Oreochromis niloticus is a type of fish known as Nile tilapia. Oreochromis aureus is a kind of fish called blue tilapia. Oreochromis mossambicus is a species of fish called Mozambique tilapia. Oreochromis urolepis hornorum is a type of fish known as African bream. Moreover, they discovered more SNPs that could differentiate between the pairs of this species within this subset. nioticus, which is a type of fish. TiLV is a harmful germ that can make the fish sick. The niloticus tilapia is a significant species that is widely cultured all around the world. The writers studied information about tilapia fish infected with a virus in ponds. The fish were divided into two groups: those who stayed alive and those who died. They were then tested to determine their genetic makeup using a special technique called a 65K SNP array. A major gene variant that could explain this characteristic was discovered on chromosome Oni22. People who have two copies of either the susceptible or resistant genes can be found. Genes that affect how a person's body responds to a viral infection were found in this part of the DNA called QTL. These markers can be used to choose tilapia fish that are resistant to TiLV. By doing this, we can breed the next generation of fish without having to do any tests on the parent fish.

CONCLUSION

Molecular markers are commonly used in plants, like crops and farm animals, because breeding programs have been popular in these species for a long time. Aquaculture is just starting to see the advantages of breeding programs that select for desirable traits. This is supported by the fact that currently, less than 10% of all the fish and other aquatic organisms raised in aquaculture are from genetically improved stocks. In a good way, people involved in

aquaculture can learn from this because they can find solutions to the problems faced by crop and animal breeders while planning programs to improve aquatic species. Carefully developed genetic improvement programs for important fish species would greatly increase the number of improved fish strains available. This would help boost fish farming and ultimately lead to more fish being produced. At the same time, it would be helpful to discover and confirm molecules that indicate how much a plant grows and its ability to fight off certain diseases. These molecules can then be added to the breeding program to create plants with better genes.

REFERENCES:

[1] G.-F. Zhang and X. Liu, "Theory and method of genetic improvement in mariculture mollusks : a review," *J. Fish. China*, 2006.

[2] A. Cnaani and G. Hulata, "Improving salinity tolerance in tilapias: Past experience and future prospects," *Isr. J. Aquac. - Bamidgeh*, 2011.

[3] D. A. Stick, "Identification of Optimal Broodstock for Pacific Northwest Oysters.," *Diss. Abstr. Int. Vol. 73, no. 10, suppl. B, 224 p. 2011.*, 2011.

[4] K. T. Wada and D. R. Jerry, "Population Genetics and Stock Improvement," in *The Pearl Oyster: A Beginner's Guide to Programming Images, Animation, and Interaction*, 2008. doi: 10.1016/B978-0-444-52976-3.00012-7.

[5] A. F. ZEINA *et al.*, "Enfoque De Los Trastornos Del Equilibrio Acido-Base," *Biol. Eng. Trans.*, 2016.

[6] K. S. Reece, G. P. Scott, R. B. Carnegie, L. R. Calvo, E. M. Burreson, and M. D. Camara, "Molecular markers for population studies, selective breeding and parentage assignments in the hard clam (Mercenaria mercenaria)," *J. Shellfish Res.*, 2006.

[7] G. H. Yue and J. H. Xia, "Practical Considerations of Molecular Parentage Analysis in Fish," *J. World Aquac. Soc.*, 2014, doi: 10.1111/jwas.12107.

[8] D. A. Chistiakov, B. Hellemans, and F. A. M. Volckaert, "Microsatellites and their genomic distribution, evolution, function and applications: A review with special reference to fish genetics," *Aquaculture*. 2006. doi: 10.1016/j.aquaculture.2005.11.031.

[9] M. Alonso, F. C. Lago, J. M. Vieites, and M. Espiñeira, "Molecular characterization of microalgae used in aquaculture with biotechnology potential," *Aquac. Int.*, 2012, doi: 10.1007/s10499-012-9506-8.

[10] J. G. Tong and X. W. Sun, "Genetic and genomic analyses for economically important traits and their applications in molecular breeding of cultured fish," *Science China Life Sciences*. 2015. doi: 10.1007/s11427-015-4804-9.

CHAPTER 4

BREEDING METHODS: AQUACULTURE OF FISH AND THE POSSIBILITY FOR NEW APPROACHES

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ABSTRACT:

Aquaculture is the industry that produces food from water in a very fast way. The sector started to grow in the 1970s because of improvements in how we raise fish in tanks and take care of ponds. The future of fish farming depends on producing species that can grow quickly, adjust to different weather conditions, and handle the challenges of an improved farming system. India has a lot of water inside its country, like rivers and lakes, and it also has a big area of sea around three sides of it. As a result, fish resources that are found in nature are spread out all across the country. Aquaculture has become more important in providing fish, crustaceans, mollusks, and other water animals to the world. In 1970, it only made up 3. 9% of the total production, but by 2000 it had increased to 27. 1%, and then in 2004 it reached 32. 4% Since then, it continues to increase every day. Development strategies should focus on expanding aquaculture by raising fish species that are readily available in the local area. Carp, catfish, prawns, mollusks, and ornamental fish offer choices for raising different types of animals. Being able to successfully breed and produce offspring in captivity is really important for the growth of sustainable fish farming. Fish seed is an important thing needed for fish farming to be successful. In the past, most of the seed needed was taken from rivers. Usually, fish do not reproduce in captivity, so it's important to use artificial breeding techniques in fisheries. This method uses a substance called pituitary gonadotropins or other substances that can stimulate LH, to excite the reproductive fish. The stimulation helps sperm and eggs be released at the right time. This chapter talks about the different substances used to breed fish in fish farms, both in the past and now. It also discusses the possibility of using new substances in the future.

KEYWORDS:

Animals, DNA, Fish, Nuclear Transplantation, Sperm.

INTRODUCTION

Sexual hybridization is a way people have always used to create new types of animals. Based on natural and artificial selection, we can choose and get certain animals with different good qualities from species that are closely related but not the same. To mix sexually and create new hybrids with extra strong characteristics. In simpler words, if animals with stronger traits have babies together, the babies may have even better traits than their parents, which can make the original parents more valuable. Sometimes, animals or plants from different species can have babies together. However, many of them were done artificially. Artificial sexual hybridization is when scientists mix the reproductive cells of male and female animals from different species in a lab. This is done in a way that doesn't normally happen in nature. The goal is to create a new organism called a zygote. Then, sometimes, those 'hybrid zygotes' will grow into hybrids with better traits. In simpler terms, this method is known as combining genes from two different animals who have different genetic backgrounds. The changes in appearance of the mixed species were due to the expression of their newly combined genes. Basically, when different types of animals that are closely related are bred together, the first generation offspring will have strong and advantageous qualities. Their future offspring will be created using Mendel's law of inheritance. In simpler terms, the traits of the male and female parents will separate and

may show up again in their offspring after the second generation when the first generation hybrids are bred together. So, in lots of animals, mating different kinds of species together is helpful in creating improved offspring. However, it will not be a good way to create stable new types of animals. In addition, animals from different species usually cannot mate because their bodies are not compatible [1], [2].

We don't know exactly why this is. But if two species are very similar, they may be able to produce offspring together. When the male and female reproductive cells from different species that are not closely related were used to create a new organism, there might be some instances where these cells cannot join together. In very unusual situations, they can sometimes combine as hybrid zygotes, but these hybrid eggs cannot survive and grow into adults. Alternatively, they may grow into adult organisms that cannot have babies because their reproductive organs did not develop properly. This idea has been shown to be very accurate in nearly all larger animals, from frogs to mammals. A female horse and a male donkey can have offspring called a mule, which is an example of sexual hybridization. It got a few good traits from its mom and dad, but it can't make babies. However, there are a few cases where lower vertebrate species are an exception. In fish, breeding different types of fish together can be done for better hybrid fish in fish farming. This can work even for fish that are not closely related. For instance, we have created adult fish, baby fish, or eggs that come from combining different types of fish from various groups.

This means finding a way for different types of fish to mate with each other, even if it's typically difficult for them to do so due to natural obstacles. However, there are new ways in science to help sperm from one animal enter the eggs of another animal. These methods include microinjection, cell fusion, and electroporation. To achieve this goal, it can be helpful to choose sperm and eggs from different fish that have the same or similar number of chromosomes. We need to look into all those possibilities more closely. The main disadvantage of sexual hybridization to create hybrid animals is that we cannot know in advance what new traits and features will show up in the offspring of hybrid fishes. So, the problem with using this method to meet increasing demands for aquaculture is clear. Therefore, in terms of actually doing it, different ways are necessary for creating new animal breeding plans. Both artificial selection and sexual hybridization are old-fashioned ways of breeding animals without using biotechnology. However, we can use new biotechnical methods to artificially combine the reproductive parts of different animals that are not closely related in order to solve the problem of performing this technique [2], [3].

In the past 20 years, nuclear transplantation has become a way to create new types of fish and mammals. The idea of nuclear transplantation moving a cell's nucleus into an egg cell without a nucleus to study its role in starting embryo growth was initially suggested by a German scientist called Hans Spemann in early 1938. In the 1950s, some scientists found that they could create new types of single-celled organisms by using nuclear transplantation in genetic research. For instance, when the nucleus of one type of amoeba is moved into an amoeba without a nucleus of a different type, new amoebas with traits from both parent strains or in-between ones were created. Since then, other groups of scientists studying early development in animals have also found evidence that when the center of a cell in a growing embryo from one type of animal is put into the empty egg from a different type of animal, a new creature with a mix of characteristics from both species is created. Luckily, just like fish can reproduce by mixing their genes through sex, we can use nuclear transplantation to make fish that can reproduce without sex. This method allows us to create hybrids between different types or species of fish, as well as ones that are more distantly related, like between different subfamilies, families, and orders. Before, scientists would only do nuclear transplantation

between a small number of amphibian species and different types of mammals. Because the nuclei used in nuclear transplantation [4], [5].

Until now, scientists have been able to merge the nucleus and cytoplasm of different species to create hybrid fish with a combined nucleocytoplasmic structure. In the case of fish, it was comparatively effortless to determine the separate genetic responsibilities of the nucleus and cytoplasm, as we could amalgamate them from diverse fish species exhibiting distinct characteristics. Here are some examples of nuclear transplantation in fish. Scientists combined blastula cell nuclei from one type of fish with enucleated eggs from another type of fish. One example was combining the nucleus from a common carp with the cytoplasm from a crucian carp. Adult fish with the basic features of common carp, as well as some mixed and influenced traits at different levels like appearance, body function, and biochemistry, were obtained. We got both boy and girl hybrids and they can have babies. We caught three types of fish: a common carp, a crucian carp, and an NCH fish. A nucleus from grass carp and cytoplasm from blunt-snout bream another type of fish are combined. Adult fish were studied to understand the characteristics of grass carp. Some changes were observed in their blood serum. The male hybrids were able to have babies, but we couldn't find the female hybrids before they were lost or died because we didn't take good care of them in the fish ponds. The female grass carp usually need an extra year to become mature compared to the males. In other words, they require around four years of learning and experiencing different things. We have found other adult, baby fish, and embryos from NCH too.

Nucleus from a type of fish called tilapia (with 44 chromosomes) + fluid inside cells from a different type of fish called loach. We caught NCH baby fish that looked different from tilapia or loach. It is fascinating to note that when the nucleus of a common carp is combined with the egg cytoplasm of a crucian carp which are different types of fish, both male and female hybrid fish were produced and these hybrid fish can reproduce. Besides the physical and chemical changes that happen in this type of mix fish, their worth in terms of money is also supposed to get better. For instance, fish farming companies in various provinces in China successfully bred and raised at least 4 generations of fish in medium-sized farms. Their changed features stay the same. These special NCH fish gain weight faster than regular carp in the same environment. On average, the NCH fish's body weight growth is 22% higher than the growth of common carp. The nutritional benefits of this type of NCH fish are also better. Basically, the protein in fish muscle is 3. 78% more and the fat is 5. 58% less compared to the common carp. This special fish that is a mix of genetic material from a nucleus and cytoplasm is being used in many fish farms in different parts of China and is in demand. Furthermore, the Chang Jiang Fisheries Institute in Shashi, China, achieved the creation of a new type of hybrid fish called 'Ying carp' by breeding male fish of the NCH fish species with female mirror carp which is a mutant of common carp.

DISCUSSION

The findings from nuclear transplantation in mammals are very fascinating. A new report said that when British and American scientists tried making better cows and sheep without mating, they ended up with unexpectedly powerful animals. This surprised the scientific community. A newborn calf usually weighs around 36 kg. However, out of 1000 cows that were manipulated using a non-sexual reproductive method, 1/5 of their fetuses weighed more than 68 kg. Those cows need to have a C-section to help them give birth to their calf. Scientists say that this surprising 'new finding' is the most important thing they have discovered in the past ten years. We need to do more research to find out why those big animals could exist. This event made their program of transferring cow embryos grow. Also, scientists helped female sheep produce a lot of big sheep by using a different way to reproduce instead of having sex.

Scientists have discovered a method called non-sexual reproduction that involves removing the nucleus from an egg and replacing it with a nucleus from another cell using a small surgical technique. Then the egg was put back inside another animal's uterus, and it can keep growing even without being fertilized. This method can create strong animal copies with improved genes and also make a lot of artificially produced living animals. Another benefit of using the nuclear transplantation method is creating hybrid fish with modified traits without the need for sexual reproduction. Some different species of fish can be used as parent fish in certain cases. When mixing genes through sexual reproduction, only closely related species can be used [6], [7].

The offspring produced by mixing genes from distant species are able to reproduce and can be bred as new clones. On the other hand, the offspring produced through sexual reproduction cannot reproduce. Furthermore, when the cell cores of the same embryo were moved into eggs that had their own cores removed, NCH hybrid fish with exactly the same genetic background could be produced. These types of identical fish clones may be hard to find in nature or even in a system where they reproduce with close relatives for a long time. They will be very helpful for using in experiments to study gene transfer and gene targeting. What makes NCH animals change their characteristics from their original fish. We still don't know much about this area and more studies are needed. However, the findings from the nuclear transplantation experiments in fish or other animals can be understood for now, but with caution. We think that when the cell nucleus and cytoplasm from different sources come together in a cell or egg, it can cause the genes in the cell to act differently during the development of animals.

Based on the facts mentioned before, why do we not think this method is useful in biotechnology for creating new animal breeds. Doing nuclear transplantation experiments in fish is much harder than doing them in amphibians or mammals. This happens because in amphibians, we can easily get eggs without nuclei by using UV or laser beams to harm the nuclei of many of their eggs. After the unfertilized eggs were made active, their animal poles automatically turned upwards. To take out the center of a fish egg, you need a more difficult method. In mammals, their eggs are much smaller than those of amphibians and fish. But when looking at them under a microscope to manipulate them, it's easier to see the male and female pronuclei or zygote nucleus. On the other hand, when looking at the unfertilized eggs of amphibians and fish, as well as the male and female pronuclei and zygote nucleus of fertilized eggs, it's impossible to see them under the microscope for the purpose of micro-manipulation. But, the methods for doing nuclear transplantation in fish are already available now. In the near future, it is expected that there will be a big challenge in using this method to do more research with different animals. Lately, people are creating new types of animals using a different method. "Gene transfer" means moving genes from one organism to another organism.

In the 1980s, advancements in molecular biology allowed scientists to separate individual gene sequences and put them together in small DNA structures. They could then increase the amount of these sequences outside of living organisms and put them into tiny living things to study how they work and how they are controlled. Based on their beliefs, scientists hypothesized that by utilizing innovative techniques to incorporate a foreign gene into either an animal cell or a fertilized egg, it would integrate into the genetic material of the cell or egg, consequently influencing the developmental process of the cell. Some people believed that the combined genes could stay in the reproductive cells of animals and be passed down to future generations. They also think that since they know what genes do when they are put into fertilized animal eggs, the transferred genes must be genes that are being aimed for. So, when foreign genes are expressed in animal eggs or embryos, it can cause changes in how the animals look and behave as they grow. These changes can be expected to have different effects in each animal. The

animal that is created in this way is commonly known as transgenic animals. Theoretically, this method appears to be a very appealing and perfect way to create new types of animals. It was first reported that scientists created a special kind of mouse called a transgenic mouse. They did this by putting a gene for growth hormone into mouse eggs. Afterwards, more writers said that some modified genes were found in certain organisms [8], [9].

Because fish eggs are bigger than mammal eggs, it doesn't seem very hard to put genes into fish eggs. In some fish eggs, the covering around them is not tough and can be taken off using tweezers or special chemicals before putting them in. The small needle can go into the egg without taking off the outer layer called the chorion. Fish eggs are usually clear or slightly see-through and their blastodisc is located at the top part of the egg. It is simple to find where the dividing nucleus is positioned. You need to put the medicine in that spot. In many experiments, scientists use a method called micro-injection to transfer genes from outside sources into fish to create transgenic fish. This method is also good for accurately finding the location in the egg cytoplasm that is close to the dividing nucleus. The amount of injected genes can be controlled or changed with precision. However, if we want the injected gene to be combined into our genes more effectively, like it does in mice, we have to inject the genes directly into the center of the mother cell. That's because we can't tell exactly where the center of the cell is in a fish egg that has been fertilized. When this method is used on fish eggs, they must be clear so that we can see where their nuclei are located germinal viscle. Furthermore, we need to develop technology that can help the injected oocytes grow successfully and become fully mature.

We also need to achieve artificial fertilization in a laboratory setting. This method has been successfully developed and is now being tested with goldfish and zebra fish. In certain situations, like with Salmonids, the outer shell of the egg is too tough for injecting with small glass needles. However, micro-injection can be done easily using the micro. Some fish eggs, like Medaka, Fundulus, Tilappia, Chinese bittering, have a jelly-like or delicate cytoplasm and yolk. This is because they have a dense structure between the chorion outer covering and the egg membrane. When the tiny glass needles go into or come out of the eggs, the pressure from putting them in or taking them out can cause the stuff inside the egg to leak out through the hole where the needle went in. This can hurt the egg. In these situations, using electroporation method appears to be a better choice than micro-injection. Inoue and his team examined this idea. Okay They used a method called electroporation to put DNA sequences into Medaka eggs. About 70% of embryos that are given to a recipient do not survive, and only 5% of the embryos that they examined were successful in integrating. This method is simple and can be adjusted to suit different types of fish. This method is also good for transferring genes to a lot of fish eggs [10], [11].

The drawbacks of this strategy is that indeed within the same bunch of angle eggs, each egg may have some physiological contrasts which can cause diverse responses against electric beats. It is additionally troublesome to create beyond any doubt, after electroporation, where is the location of the qualities to be joined into the egg is, which may not closely join to the egg nucleus. Appropriately, the integration recurrence of outside qualities into the genome will diminish. In expansion, when eggs are treated within the electroporation medium, numerous more quality duplicates ought to be utilized for making a appropriate concentration. Though within the micro-injection as it were a little number of quality duplicates is utilized. So, it appears that this strategy isn't a financial way when utilizing limited gene assets. In 1989, detailed that mouse sperm brooded with DNA can carry DNA particles and present them into mouse eggs amid fertilization. Transgenic mice were gotten. Tragically, this result must be re-confirmed. There are too few brief reports which announce that angle sperm can be used as DNA carrier for quality exchange. For case, detailed in a audit that when hGH quality were

carried by sperm and presented into common carp, the expression of hGH quality was as tall as 50% within the transgenic angle embryos and the development rate of the transgenic carp in a few test bunches was approximately 2-fold higher than controls. detailed that zebra angle sperm can carry plasmid DNA PUSVCAT and pxGH5 into fertilized eggs but the presented plasmid DNA was not coordinates into the genome. It endured within the cytoplasm, be that as it may.

Tragically, no point by point exploratory information or portrayals were given in those two brief reports, so it is troublesome to create legitimate comments around those comes about. As of late, our bunch detailed that goldfish sperm hatched with radiator fluid protein (AFP) quality can fertilize eggs and deliver transgenic goldfish. A sperm suspension of 4x10(7-8) cells/ml was arranged by utilizing Niu-Twitty arrangement, containing 4% sucrose, 3% glycerol and 1% DMSO. Straight AFP quality from sea mope was included to the sperm suspension to surrender a last concentration of 3 ug/ml and hatched along with sperm suspension at 4°C for 30 minutes. Polymerase Chain Response (PCR) and Southern blotch hybridization are utilized for recognizing DNA extricate of blood cells of transgenic angle. Three of the 45 grown-up angle tests were found positive. The positive rate is around 7%. In any case, no legitimate strategies are accessible to recognize how can sperm carry the AFP quality into the eggs. It is well known that the physiological behavior of mammalian and angle sperm is very distinctive. When the mammalian sperm are launched out into the female hereditary tract, they can keep up there for a few hours to hold up for fertilization with the eggs; whereas in angle, when the sperm are launched out into the water and do not have the chance to fertilize eggs, they lose their action or kick the bucket inside a couple of minutes. Sperm may carry DNA atoms either by retention on its surface or by entrance into its head parcel. Concurringto previously mentioned reason, to realize the assimilation of DNA molecules by angle sperm inside a or maybe brief period of time in a medium may be exceptionally troublesome.

On the other hand, if the DNA molecules can as it were be ingested on the sperm surface, at that point they will stay within the exterior of the fertilized egg since the sperm layer will be melded with the egg plasma film as before long as they join with each other amid the fertilization process. The as it were plausibility for making sperm-carrying DNA atoms enter the egg is to let the DNA molecules enter the space interior of the sperm layer inside a really short period of time. Likely, electroporation will be accommodating to reach this objective. Be that as it may, from a specialized point of see, it appears pointless to do so in case the exchange of DNA atoms through electroporation into egg will be successfully done. The as it were distinction for utilizing electroporation strategy between the eggs and sperm is that the outside DNA particles can be coordinates into sperm DNA, at that point it can be specifically intertwined into the genome of the zygote, though the DNA particles can as it were be joined into the egg cytoplasm by electroporation. As of late, Vielkind reviewed strategy for making transgenic angle in Medaka and Zebra angle. A arrangement of methods were summarized. They shown that DNA ought to be cytoplasmically infused into fertilized eggs ideally earlier to cleavage of the egg. Infused DNA in direct and super coiled frame or exchanged in phage particles is imitated, degraded but too held in a division of youthful angle. Infused DNA is momentarily communicated, but after a mosaic integration into the germ line of 5% of injected fish, is steadily coordinates into the chromosomes, communicated within the offspring and acquired in a Mendelian design. It too shows up that the correspondent qualities, like CAT quality, are transitorily communicated as well as within the offspring in a tissue particular way.

CONCLUSION

In simple terms, there are different ways to transfer genes in fish studies, but none of them are perfect or right for every situation. Generally, using the micro-injection method has several advantages. It can help inject accurately in the right place, control the amount and timing of the injection, and also save the gene source. After the joining of male and female reproductive cells, the nucleus of the egg will keep dividing quickly. If the DNA injection site is near the dividing nucleus, the nucleus will divide faster. This increases the chances of the injected DNA being combined with the chromosomes during the nucleus's division cycles. There is no information available about the difference in how often DNA integrates into the nucleus of an oocyte compared to when it is injected into the cytoplasm of an egg. More research is necessary. There is a big problem where DNA molecules in eukaryote cells often break apart after a few cell divisions, and they can only be inserted into the cell's genome occasionally. It was found that the injected DNA in Medaka started breaking down at an early stage called blastula/gastrula. The ways we treat problems with the pituitary gland have changed over time. In the past, we used a simple pituitary extract. But now, we use hormones and drugs that are sold in stores. This is because we have learned more about how the reproductive system works and have developed new tools to study it. As a result, the breeding methods have become more successful and effective. There are still worries about why fish cannot reproduce naturally when they are kept in captivity. Is it because they feel stressed from being confined, which stops them from reproducing. Two chemicals called kisspeptins and VT have shown to be important in reproduction during lab experiments. Scientists need to do more studies in natural environments to understand how these chemicals may make animals reproduce. By doing more research on these chemicals, scientists may find answers to some of their questions.

REFERENCES:

[1] R. D. Houston, “Future directions in breeding for disease resistance in aquaculture species,” *Revista Brasileira de Zootecnia*. 2017. doi: 10.1590/S1806-92902017000600010.

[2] D. Ye, Z. Y. Zhu, and Y. H. Sun, “Fish genome manipulation and directional breeding,” *Science China Life Sciences*. 2015. doi: 10.1007/s11427-015-4806-7.

[3] C. E. Lind, R. W. Ponzoni, N. H. Nguyen, and H. L. Khaw, “Selective breeding in fish and conservation of genetic resources for aquaculture,” *Reprod. Domest. Anim.*, 2012, doi: 10.1111/j.1439-0531.2012.02084.x.

[4] R. L. Vallejo *et al.*, “Evaluation of genome-enabled selection for bacterial cold water disease resistance using progeny performance data in rainbow trout: Insights on genotyping methods and genomic prediction models,” *Front. Genet.*, 2016, doi: 10.3389/fgene.2016.00096.

[5] V. Martinez, “Marker-assisted selection in fish and shellfish breeding schemes,” *Marker-assisted Sel.*, 2007.

[6] S. Perumal, A. R. Thirunavukkarasu, and P. Pachiappan, *Advances in marine and brackishwater aquaculture*. 2015. doi: 10.1007/978-81-322-2271-2.

[7] G. Liu *et al.*, “Development of Streptococcus agalactiae vaccines for tilapia,” *Diseases of Aquatic Organisms*. 2016. doi: 10.3354/dao03084.

[8] M. Iversen, A. I. Myhr, and A. Wargelius, "Approaches for delaying sexual maturation in salmon and their possible ecological and ethical implications," *Journal of Applied Aquaculture*. 2016. doi: 10.1080/10454438.2016.1212756.

[9] B. Ye *et al.*, "Heritability of growth traits in the Asian seabass (Lates calcarifer)," *Aquac. Fish.*, 2017, doi: 10.1016/j.aaf.2017.06.001.

[10] T. J. Benfey, "Effectiveness of triploidy as a management tool for reproductive containment of farmed fish: Atlantic salmon (Salmo salar) as a case study," *Reviews in Aquaculture*. 2016. doi: 10.1111/raq.12092.

[11] M. A. Jamaludin, S. A. Rahman, and M. A. Ramli, "Fiqh Analysis On The Legal Status Of Coprophagous Animals : A Special Reference To The Malaysian Aquaculture Industry," *Int. Fish. Symphosium*, 2011.

CHAPTER 5

THE FUNDAMENTALS OF BIOFLOC TECHNOLOGY: A BRIEF OVERVIEW

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ABSTRACT:

The biofloc system was created to make it easier to control the environment for raising fish and other water animals. In fish farming, the main things that affect how well it works are how much food costs and whether or not there is enough water and land. When there are a lot of fish or other aquatic animals in a small space, it is important to clean the water they live in. The biofloc system is a way to treat dirty water in aquaculture. It has become very important. The idea behind this technique is to create a nitrogen cycle that helps plants grow better by increasing the amount of carbon compared to nitrogen. This encourages the growth of certain types of bacteria that can use the excess nitrogen and turn it into a food source for the plants. Biofloc technology not only helps clean up waste, but also provides food for aquatic animals. This helps improve the quality of water by producing high-quality protein from single-cell microbes. In this situation, tiny living things grow and act as a machine to control the quality of water and provide food in the form of protein. Toxic nitrogen substances are trapped faster in bioflocs because the microorganisms that consume organic matter multiply and produce more quickly compared to the bacteria that convert ammonia to nitrate. This technology works by making particles come together in the system. The biofloc technology is used in shrimp farming because shrimp live at the bottom of the water and can handle changes in their environment. Research has been done to study how baby shrimps and Nile tilapia fish grow and reproduce. Shrimp raised in the biofloc system had better breeding results compared to traditional methods. We also saw improvement in how the larvae grew.

KEYWORDS:

Aquaculture, Bacteria, Biofloc, Fish, Farming, Water.

INTRODUCTION

The fish farming industry is growing quickly at a rate of around 9% each year since the 1970s. But, people have started to question this industry because it is causing harm to the environment and creating pollution. Therefore, it is still very important to have better ways of managing and practicing culture that are environmentally friendly. Additionally, the growth of aquaculture is limited because it is expensive to obtain land and relies heavily on fishmeal and fish oil. Ingredients like these are important parts of the food for fish farming. Feed costs make up at least half of the total expenses for aquaculture production, mostly because of the expensive protein in the food. People are becoming more interested in closed aquaculture systems because they offer benefits such as better protection against diseases, less harm to the environment, and improved marketing opportunities compared to traditional fish farming methods. When we reuse water, we can reduce or completely get rid of some dangers like introducing harmful germs, releasing foreign species into the environment, and polluting water with waste. Moreover, due to increased efficiency and less water usage, marine creatures can be grown in places that are far from the sea. A good example is the current increase in shrimp farms in the USA, where farmers can sell fresh shrimp in cities and make a good profit [1], [2].

The environmentally friendly aquaculture system called Biofloc Technology (BFT) is seen as a good alternative system because it allows nutrients to be constantly recycled and reused. The sustainable method of this system relies on the growth of tiny living things in the liquid the organisms live in, which is improved by not needing to change the water. These tiny living things called biofloc have two main jobs they keep the water clean by absorbing nitrogen compounds and making protein, and they help with feeding by making it easier for the organisms being raised to eat and reducing the cost of their food. BFT helps prevent the release of water into rivers, lakes, and estuaries, which means fewer escaped animals, nutrients, organic matter, and pathogens get into the environment. In simple words, the nearby areas benefit from the increase in productivity caused by vertical growth. This helps prevent damage to coastal or inland areas, excessive pollution, and loss of natural resources. When we remove water from ponds and tanks, it often has a lot of nitrogen and phosphorous in it. These nutrients make algae grow, which can lead to harmful effects like excessive plant growth and lack of oxygen in natural water bodies.

In BFT, using less water and reusing water helps protect the environment and make the system more environmentally friendly and sustainable. Minimum water exchange means using as little water as possible and only changing it when necessary. This helps to keep the water temperature stable and prevents sudden changes. It also allows tropical species to survive and grow in cold areas. BFT has been given different names like ZEAH or Zero Exchange Autotrophic Heterotrophic System, active-sludge or suspended bacterial-based system, single-cell protein production system, suspended-growth systems, or microbial floc systems. In addition, BFT has been the subject of a lot of research in the field of nutrition as a type of protein used in mixed animal feeds. This kind of source is made as biofloc meal, mostly in bioreactors. Furthermore, because BFT farms are increasing in number and spreading rapidly across the globe, there has been a great deal of research focused on understanding the processes involved in BFT production systems. The purpose of this chapter is to examine how Biofloc Technology (BFT) is used in aquaculture. It also discusses how biofloc biomass, which can be called biofloc meal, is used in making feed. Another objective is to assist students, researchers, and industry in understanding the fundamental aspects of this technology, with the aim of promoting more research in the field [3], [4].

Fish farmers use this method to enhance water quality in fish farming by adjusting the carbon and nitrogen levels. It is a way to change harmful substances in the water into something useful for the fish, like protein-rich food. This method is cost-effective and can help improve water quality while also providing food for the fish. It involves keeping a lot of fish in one place, making sure there is enough air, and using special microorganisms to help clean the water. We also need to make sure there is enough air to keep the microorganisms active. The Biofloc system helps to make the water better for fish and other aquatic animals. It was created to treat the dirty water that comes from raising a lot of fish. This system is important because the cost of food for the animals is a big part of the overall cost of raising them. Biofloc technology helps decrease the need for additional food.

The biggest problem with raising fish in ponds is making sure the water quality is good. Before, the biofloc method used to recycle all of the water frequently. But in certain areas, there is not a lot of water available. Changing the old water in a fish tank can cause the fish to be disrupted and not grow properly. So, biofloc came to solve these problems. Ammonia is the main thing that stops fish from growing. Only a fish farmer knows how harmful ammonia can be. In biofloc fish farming, we usually use probiotic. It uses the ammonia from the tank and increases its population by copying itself. As they prepare their colony, they change the harmful ammonia and other things into proteins. The fish in the tanks consume these proteins.

Therefore, the amount of food given to the fish goes down. They are already giving food to the proteins in the flocs. So, the cost of feeding goes down by 20% to 30%.

DISCUSSION

Animals that live in water, like fish, have important nutrients called amino acids and fatty acids. These nutrients are very important for our bodies. When there are more people but not enough protein, there is a big difference between what is needed and what is made. The difference between fishing and the increasing need for aquatic products can be filled by using aquaculture methods raising fish and other aquatic species in controlled environments. The excessive exploitation of natural aquatic resources worldwide necessitates the advancement of novel fish farming technologies. There are three important objectives that need to be considered when developing aquaculture products. The main aim is to increase aquaculture production without using too much water and land. Due to the lack of enough freshwater, over 41% of people on Earth currently live in areas near rivers that are experiencing drought. Around 70% of the people might not have enough water. The second big aim is to create systems that are sustainable and don't harm the environment. The fish farming industry has caused a lot of pollution in nature lately. It is really important that we focus on creating farming systems that are good for the environment. Furthermore, the commercial fish farming is limited by not having enough space for it and relying heavily on fish oil and meal for feed. In simple terms, the third goal is to make sure that fish production systems are cost-effective and beneficial. The use of new technologies like intensive farming in closed-system aquaculture has helped improve how much fish and other seafood can be produced. These systems have helped decrease the daily amount of water exchanged in fish ponds by around 1% of the total water volume.

RAS systems are getting more attention because they are really good for keeping things safe and clean. Using new and suitable methods and technologies in fish farming, like the biofloc technique, is extremely important for achieving sustainable aquaculture goals. Tilapia is a very popular fish for farming and is the second most important species worldwide. Therefore, it is especially important to use new technologies for growing this species. Tilapia is a type of fish that includes three groups called Oreochromis, Tilapia, and Sarotherodon. They all belong to the Cichlidae family. Nowadays, many types of Oreochromis fish are being grown on fish farms. This is different from many types of tilapia fish. The Nile tilapia, a type of fish, is good for fish farming for three main reasons. It can be grown in crowded conditions. It can survive in different climates, so it can be grown in many places around the world. It is popular because it tastes good, has little fat, and doesn't have any spines in its muscles. There are certain things that can make O grow well or not. In aquaculture, there are several important factors to consider for raising niloticus fish. These include the quality of the food they eat, the variation in their genes, how many fish are in a tank, how often they are fed, and the quality of the water they live in, including temperature, oxygen levels, acidity, saltiness, and ammonia levels. The cleanliness of water also impacts how healthy and strong someone can become, how much they get sick, and how likely they are to survive. To improve the fitness of O and make it grow better. In the culture system, the feed for niloticus fish should have the right amount of protein, fats, carbs, vitamins, and minerals [5], [6].

Making aquaculture production increase by using knowledge and technology better, while using less land and water resources. Intensive fish farming helps to address social and economic problems, like not having enough food, being poor, and not getting proper nutrition. However, increasing the intensity or level of something brings along many difficulties. When more waste from aquaculture is put into the environment, it causes problems for the environment, especially with the water becoming polluted and having too many nutrients. This can lead to

lots of algae growing, which is called algal blooms. Eutrophication can cause a lack of oxygen in water which results in the death of living things in the water. Moreover, harmful substances such as chemicals and antibiotics also disrupt the natural balance of the environment. Water pollution caused by aquaculture has been happening for a while. There is proof that aquaculture activities harm water ecosystems. Managing the stock in a intensive fish farming system can be difficult because it involves keeping the water clean and making sure the fish are fed well. Many things in our environment can get really bad, and when these things are not in balance, it can be really bad for animals. In aquaculture, it is important to carefully monitor and adjust certain factors in order to keep aquatic animals healthy. These factors include the amount of oxygen in the water, the acidity level, the temperature, and the presence of certain chemicals [7], [8].

Regularly changing the water in fish tanks has become unsustainable because it is believed to have negative effects on the environment and can also pose risks to the well-being of the fish. There are many things that can affect the health of animals that are raised in intensive aquaculture. Disease outbreaks in aquaculture happen when there is an imbalance of three things: the environment, the animals' general health, and the number of pathogens present. In intensive fish farming, the environment can change a lot, which makes the fish stressed and causes them to get sick more often. The continuous use of certain medicines and antibiotics has caused a problem called antimicrobial resistance (AMR). This makes antibiotics less effective and greatly affects people's health all over the world. Apart from the danger of water pollution. Aquaculture mostly uses limited water resources to produce food. Around 45 tons of water is used for every kilogram of fish produced through pond farming, which is more than what is used for other types of animals. However, by using filters and recycling water in a recirculating aquaculture system (RAS), intensive aquaculture can greatly reduce the amount of water needed.

Another method is to turn waste materials into protein through efficient bioconversion in a biofloc production system, which can also generate some of the supplementary feed needed for the system. Both of these systems also help to keep the culture stock healthy and well, which is important for the well-being of aquatic animals. The problems in intensive fish farming systems, such as bad water quality, too many nutrients in the water, keeping the fish healthy, and taking care of the animals, can be solved by using biofloc technology. This technology can help improve the way we farm fish in a sustainable way. This chapter explains the theory and practice of biofloc technology in fish farming. It focuses on the advantages and new biotechnological innovations that are improving the use of biofloc in aquaculture. These innovations are helping to solve the problems that come with intensifying fish farming. Biofloc is a bunch of stuff, both alive and not alive, that comes together when uneaten food, fish poop, and little water animals clump together. It's all held together by tiny organisms and is in water in a gooey form. Biofloc is a clump of living things like algae, bacteria, and other tiny organisms grouped together. They all live together in a symbiotic structure. Biofloc technology means creating clumps from solid waste in water, which helps clean the water and make it very nutritious for animals to eat [9].

This is a system that cleans itself and controls the quality of water by getting rid of harmful substances created by microorganisms. In normal pond farming, the water quality is mostly controlled by getting rid of nitrogen waste through microalgae. However, this can sometimes lead to unstable water quality, especially when the pH levels keep changing. But in a biofloc system, bacteria, mostly the type that gets their energy from other sources, keep the water clean with the help of regularly adding organic carbon compounds. The bacteria use the nitrogen from the culture system to grow and multiply. So, the stability of a biofloc system depends on

the ratio of carbon to nitrogen. This ratio helps maintain enough bacteria to keep the rearing environment stable. Biofloc is a type of farming system that is good for raising certain types of fish and shellfish, like tilapia, catfish, and shrimp. We need to have many fish in a small space and make sure there is a lot of oxygen in the water in order to keep the biofloc stable during the entire time the crops are growing. In biofloc production systems, the main bacteria responsible for converting waste into useful substances are the ones that need external food [10].

These bacteria are better at converting waste than bacteria that create their own food. So, in a biofloc system, the water quality is usually more stable compared to an RAS that doesn't have filters or pumps to clean and circulate the water. However, biofloc units require continuous monitoring by knowledgeable staff members to ensure they remain stable and operational in the long run. The RAS system, like the biofloc system, uses microbiology to keep inorganic nitrogen in the water. However, they work in different ways. The RAS's features are similar to a system with clear water, where most of the dirt particles are removed from the system. Strongly mixing air into a liquid removes a type of nitrogen that is dissolved in it. This is done through a series of steps. First, special bacteria change ammonia into NO3eN. Then, another process changes it into a gas called nitrogen (N2). Finally, the nitrogen gas is allowed to escape from the system. In the biofloc system, leftover waste is reused as food for the animals being raised. In the biofloc system, animals are surrounded by a group of tiny living things. These living things are affected by the different kinds of organisms present. In the RAS system, a different method is used to help bacteria grow and break down harmful substances.

Nitrification is when harmful ammonia becomes nitrite and then nitrate. This is done by different kinds of bacteria. The processing of nutrients determines the varying nitrogen removal capabilities among different groups of organisms. This means that the conversion of 1. 0 gram of ammonium nitrogen by microorganisms what is taken in and what is produced. Even though heterotrophic bacteria can remove nitrogen faster than nitrifying bacteria, the nitrifying bacteria still play an important role in the nitrogen cycle in a biofloc system. The first step in changing NH_4^+ to NO_2^- is usually done by Nitro somonas group. Then, Nitrobacter bacteria finish changing it to NO_3^-. There are three types of nitrogen in a water body. NH3eN is the most poisonous, then NO_2eN and NO_3eN. Efficient nitrification can change the nitrogen cycle to have more NO_3eN. However, even though it is not very harmful, a large amount of NO_3eN can make it difficult for fish to take in oxygen. This problem can be solved by microalgae or by the denitrifying process. Zooplankton are usually found in large numbers after microalgae and bacteria are already present. This group consists of tiny creatures like protozoans, rotifers, amoeba, ciliates, and nematodes. They move nutrients to a higher level and eventually become food for animals in captivity. Zooplankton can change the types of tiny plants in the water by choosing what they eat. For instance, rotifers can decrease certain kinds of tiny plants in the water.

They also cause water to become less alkaline as the biofloc grows. How Biofloc is formed? Biofloc is created when small organisms like bacteria, algae, fungi, and other microscopic creatures come together in a group. They all live together and support each other in a way that benefits them all. When microorganisms come together, they make clumps. This process is linked to a substance called extracellular polymeric substances (EPS), which is made by bacteria. The substances in this expanding matrix are mostly made up of polysaccharides, proteins, and DNA. This matrix either surrounds single cells or attracts cells that are not attached to anything. Benthic diatom cells can also help create biofloc in saline biofloc systems. However, the electrical charge of tiny organisms and organic particles can affect how stable bioflocs are. When cells with the same liking for each other are close, they tend to push each

other away. So, it is recommended to add extra ions like calcium and aluminum to keep the bioflocs stable. We need to set certain conditions for biofloc systems to work well. These conditions include things like oxygen levels, acidity, water hardness, temperature, and saltiness. By making sure these conditions are just right, we can make sure that the animals we farm in the water grow as well as possible. Changes in the environment can greatly impact the growth of microorganisms. To ensure their growth is optimal, it is important to carefully maintain the right conditions.

This is very important in biofloc systems because the actions of the tiny organisms can greatly affect the environment. That's why it's important to constantly keep an eye on things. Changes in the group of tiny organisms called microbes can change the way clumps of particles, also known as flocs, behave. This can affect how easily the cultured animal can access these flocs. Scientists use the sludge volume index (SVI) to measure and understand these changes happening in a process called activated sludge. The Floc volume index (FVI) is another measure that helps determine how stable the biofloc system is. This text means that it measures how much space 1 gram of floceVSS takes up by using milliliters per gram. They do this by letting the floc settle in an Imhoff cone for 30 minutes and then measuring the VSS value. In this section, we will talk about the important things that can affect how stable a biofloc system is in the environment. The temperature has a complicated effect on bioflocs because it affects how microorganisms work and how the floc is shaped. At a temperature of 4 degrees Celsius, the activated sludge becomes less solid. At high temperatures between 30 and 35 degrees Celsius, the sludge becomes bulky and has a high SVI (sludge volume index) of over 500 mL/g. This happens because the temperature directly influences how microorganisms work and how they produce EPS. Extreme temperatures can cause bad outcomes. When the temperature is higher, the body's metabolic rate increases. This leads to more growth of living material, which creates more organic matter and solid particles that can settle down. As a result, the SVI values become higher.

Pink shrimp, specifically Farfantepenaeus brasiliensis, tend to have higher survival rates in a biofloc nursery when the temperature ranges from 21 to 27 degrees Celsius. However, during this time, the levels of total suspended solid (TSS) in the water are higher than what is considered ideal, ranging from 855 to 963 mg/L. The best growth performance for the shrimp was observed at a temperature of 27 degrees Celsius. The ideal temperature range for water to form stable clumps of particles, known as flocs, and achieve an efficient settling volume index (SVI) of about 200 mL/g, is between 20 and 25 degrees Celsius. However, thinking about temperature is often too difficult for the farmer. So, the type of species and farming system depends on the current weather conditions. Having enough oxygen in biofloc ponds is important for the microbes living there. It can affect how the flocs are made up and how they are structured. In plain language, the text means that when the dissolved oxygen (DO) levels in water are high (between 2. 0 and 50 parts per million), the bioflocs are more tightly packed and bigger. The SVI value, which measures the compactness of these bioflocs, is around 100 milliliters per gram. On the other hand, when the DO levels are low (between 0. 5 and 20 parts per million), the bioflocs are looser and smaller. However, the SVI value is higher at around 250 milliliters per gram.

It looks like the types of tiny organisms in the water are changing. When there is a lot of oxygen, certain bacteria form groups called flocs. But when there is less oxygen, a different type of bacteria takes over. These bacteria are better at using oxygen, but they can't group together organic matter as well. When there is less oxygen in the water, the amount of suspended solids increases. This is good because it means the solids sink slowly and can be consumed by animals without collecting on the bottom of the pond. However, if there are too many suspended solids,

it can cause clogging in the gills of animals, in addition to the negative effects of low oxygen levels, such as reduced feeding.

Biofloc systems are beneficial because they help save water and make the water better in a model where a lot of organisms are raised. You can reduce the amount of water you need to change while still keeping the water clean and healthy if you follow a certain way of managing it and keep checking different things regularly. Making water better involves reducing ammonia, nitrite, and nitrate. But, at the same time, it can cause problems like lowering alkalinity and increasing suspended particles. To fix this, we can add chemicals like sodium bicarbonate or add carbon sources like wheat flour in a controlled way. Bioflocs help provide extra food for the fish and shrimp being farmed, which means they can eat less and grow faster compared to traditional systems. The particular group of tiny organisms in a biofloc system help fish feel less stressed and also provide some protection against harmful germs. The quality of meat from farmed fish can be influenced by the conditions in which they are raised using biofloc farming techniques. This section talks about the many advantages of biofloc technology in taking care of water quality, giving extra nutrition, managing health, and improving product quality. A biofloc system in aquaculture helps save water, reduce waste, and make water better. It keeps the water quality good for the entire farming season. Many tiny plants and bacteria work together to clean up the main pollutants, like organic matter and nitrogen, in the biofloc system.

But, there are some problems with this process. Scientists are currently trying to fix these problems in biofloc systems. These problems include reducing high alkalinity and a lot of solid particles in the water. Testing three different sources of carbon, such as sugarcane molasses, tapioca flour, and wheat flour, in a trial involving white shrimp L. Vannamei, Rajkumar, and others. Researchers discovered that wheat flour is better than other substances in several ways, such as lowering ammonia and nitrate levels. However, the amount of alkalinity in the wheat flour with the supplement decreased more quickly, so we needed to add NaHCO3. Although the water quality was not great due to high BODs in all treatments related to biofloc, the oxygen level was still good enough for the shrimp to survive at a concentration greater than 4 parts per million. The use of wheat flour can help keep the pH and alkalinity stable. Even without using sodium bicarbonate, it can reduce TAN and nitrate-N levels. Additionally, it can even undo environmental damage in treatments where wheat flour was not used before. In environments with enough sunlight, small plants called microalgae, specifically Cyanobacteria, grow and multiply.

This has been discovered to have certain benefits. Biochar made from Cyanobacteria can create a biofloc system that has less TSS (particulate matter) compared to using only glucose as a carbon source. The biochar also keeps its ability to remove nitrogen from the system. Combining glucose and biochar together has a positive effect on the microbes in a community. This is because both glucose and biochar have different types of carbohydrates that can be used by the microbes at different speeds. This combination helps keep the microbial community healthy. Another way to decrease the problem of too much TSS might be to combine the biofloc production system with grazers like mullets. In a pond fish farming system, the amount of water used to include evaporation, seepage, and exchange is estimated to be 16. 9 cubic meters for every kilogram of fish produced. Reducing the amount of water being changed in a biofloc system can greatly decrease water usage. The daily exchange rate can be as low as 1% or even 0. 5%, which helps to save a lot of water. The amount of water used in a biofloc system for raising Nile tilapia fish was greatly reduced by 186 times compared to a normal fish farming system. A lot of different types of bacteria like Proteobacteria, Bacteroidetes, Actinobacteria,

Planctomycetes,Verrucomicrobiota, etc. have been found a lot in the water where animals are raised and in their digestive tracts.

The types of microorganisms present can be influenced by different things like the type of food, the amount of carbon compared to nitrogen, how many microorganisms are in one place, the conditions in the environment, and how long they have been growing Complex carbohydrates often lead to more diverse bacteria in biofloc systems. Among the bacteria commonly found in the biofloc system, Proteobacteria is important for the nitrogen cycle and getting rid of organic matter. There are a lot of certain bacteria in the gut of aquatic animals that can help keep them healthy. Actinobacteria are a type of bacteria that contain many good species, and when it gets very hot, there may be a lot of them. The presence of Bacteroidetes tends to go up when there is more organic matter. This suggests that they help break down organic material in aquaculture systems. Certain bacteria from a group called Flavobacteriaceae in the Bacteroidetes family can cause diseases, especially in cold water. On the other hand, some of these bacteria that live freely can actually be helpful in forming bioflocs by producing sticky substances outside their cells. Chloroflexi is found a lot in hot temperatures and they help break down organic matter. However, Planctomycetes are more common in colder temperatures.

CONCLUSION

Biofloc technology (BFT) is a new and important advancement in the field of aquaculture. This technique uses microorganisms to do three important things: keep the water clean by taking in nitrogen compounds to make microbial protein, help the culture grow by reducing the amount of food needed and the cost of food, and fight against harmful bacteria. The clusters of organisms (bioflocs) found in nature provide a lot of protein and fat-rich food that is available all the time. This happens because of a complicated process involving organic matter, physical surfaces, and many different types of microorganisms. This natural process is important for reusing nutrients and keeping water clean. This chapter will talk about how microorganisms are important in BFT. It will discuss the main water quality parameters and why it is important to have the right balance of carbon and nitrogen in the culture media. It will also explain how to calculate the carbon-to-nitrogen ratio and describe different types of media. Additionally, it will cover metagenomics of microorganisms and what might happen in the future. Biofloc technology will help aquaculture become more environmentally friendly and safe for the fish. Eating microorganisms in BFT helps decrease FCR and therefore saves money on feed. In simpler terms, the tiny organisms in the water can use the nitrogen from the waste of shrimp and fish, as well as leftover food, and turn it into their own protein, which helps to keep the water clean. The things that happen in biofloc systems are complicated and involve different types of interactions: things happening with the physical properties, the way things react chemically, and the way living things interact. More research can help us understand exactly what is happening and how it could be useful in other areas of biotechnology.

REFERENCES:

[1] I. Ahmad, A. M. Babitha Rani, A. K. Verma, and M. Maqsood, “Biofloc technology: an emerging avenue in aquatic animal healthcare and nutrition,” *Aquaculture International*. 2017. doi: 10.1007/s10499-016-0108-8.

[2] J. Y. Jung *et al.*, “Autotrophic biofloc technology system (ABFT) using Chlorella vulgaris and Scenedesmus obliquus positively affects performance of Nile tilapia (Oreochromis niloticus),” *Algal Res.*, 2017, doi: 10.1016/j.algal.2017.09.021.

[3] S. M. Zhu *et al.*, "Applications of computational fluid dynamics to modeling hydrodynamics in tilapia rearing tank of Recirculating Biofloc Technology system," *Aquac. Eng.*, 2016, doi: 10.1016/j.aquaeng.2016.07.005.

[4] WIDANARNI, J. EKASARI, and S. I. T. I. MARYAM, "Evaluation of Biofloc Technology Application on Water Quality and Production Performance of Red Tilapia Oreochromis sp. Cultured at Different Stocking Densities," *HAYATI J. Biosci.*, 2012, doi: 10.4308/hjb.19.2.73.

[5] S. Deb, M. T. Noori, and P. Srinivasa Rao, "Experimental study to evaluate the efficacy of locally available waste carbon sources on aquaculture water quality management using biofloc technology," *Aquac. Int.*, 2017, doi: 10.1007/s10499-017-0180-8.

[6] B. W. Green, "Performance of a temperate-zone channel catfish biofloc technology production system during winter," *Aquac. Eng.*, 2015, doi: 10.1016/j.aquaeng.2014.11.001.

[7] S. Hastuti and Subandiyono, "Application of biofloc technology in intensive farming affected production and blood performances of the catfish [Clarias gariepinus (Burchell, 1822)]," *J. Teknol.*, 2016, doi: 10.11113/jt.v78.8212.

[8] N. Taw and D. Ph, "Future of Biofloc Technology," *Aquaculture*, 2012.

[9] A. Braga, D. L. A. Lopes, V. Magalhães, L. H. Poersch, and W. Wasielesky, "Use of biofloc technology during the pre-maturation period of Litopenaeus vannamei males: Effect of feeds with different protein levels on the spermatophore and sperm quality," *Aquac. Res.*, 2015, doi: 10.1111/are.12352.

[10] W. Liang, G. Luo, H. Tan, N. Ma, N. Zhang, and L. Li, "Efficiency of biofloc technology in suspended growth reactors treating aquacultural solid under intermittent aeration," *Aquac. Eng.*, 2014, doi: 10.1016/j.aquaeng.2014.02.001.

CHAPTER 6

UNDERSTANDING THE MONOSEX POPULATION IN AQUACULTURE

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ABSTRACT:
Monosex means having either all male or all female populations, which is a popular method in aquaculture. This section discusses the benefits of having a population made up of only one sex and how to achieve it using various methods to determine sex and sexual development. A recent study looked at how to use biotechnology to control the reproduction of crustaceans in aquaculture. The study helps us understand what things are important for this technology to work well. This app uses an important hormone to help fish and other farmed water animals grow in an eco-friendlier way. This chapter talks about how people manipulate sex in fish and other aquatic animals. It starts by looking at fish and then looks at crustaceans. It also talks about how these techniques can be used for other animals too.

KEYWORDS:

Fish, Female, Male, Species, Sex.

INTRODUCTION

Aquaculture is a way of farming aquatic animals in order to make more food. It involves using technology and management skills to help the animals grow. Aquaculture is growing quickly and makes up half of the world's fish production. Fish and shellfish are the most common types of animals raised in water for either eating or decoration. Fish farming has become more popular because new types of fish and advanced farming methods are being used to increase the amount of fish that can be grown in a specific area. Freshwater fish farming is growing quickly because it is easier to get land and water for it. There are also many different kinds of fish that can be raised in captivity, and technology for breeding and growing them is available. Compared to farming fish in the ocean, freshwater fish farming is easier to do. Out of the top 10 groups of animals that are farmed, more than half (57. 5%) of the total production of fish is freshwater species. Carps are one of the most popular fish in aquaculture. They are grown in over 92 countries and make up about one fourth (25. 7%) of all fish produced through aquaculture worldwide. Tilapias and other cichlids make up 5. 25% of the world's fish farming, and they are grown in 127 countries. Catfish make up 4. 93% of fish farming and are grown in 86 countries. Salmons, trouts, and smelts, which are types of fish, are the only ones that migrate in the top 10 lists. They are ranked at number nine in terms of how many there are, with a total of 3. 5 million tonnes or 3. 11% of all the fish. They are also ranked third in terms of value, with a total of USD 22 billion or 8. 94% of all the fish [1], [2].

Some types of fish that are raised for food in ponds, like tilapia and common carp, have some problems. These fish can mature and have babies very early, and they can have a lot of babies. This can be a problem because it can lead to too many fish in the pond, which can stop the fish from growing well. Some species, like tilapia and freshwater prawn, have a difference between males and females in terms of their traits or value. For example, male fish and prawns may grow bigger or be more valuable in markets. Researchers in aquaculture are focusing on studying sex manipulation to deal with issues related to early sexual maturity and differences in physical characteristics between male and female in species that are important for

aquaculture. It is very important to understand how animals develop and reproduce in order to keep them safe and healthy in captivity. Controlling sex and reproduction is key for the mass production of aquaculture species that are sold worldwide. One way to deal with problems like early sexual maturity, excessive breeding, and physical differences in traits in aquaculture is by creating populations with only one sex. This chapter talks about the benefits of having a single sex in aquaculture and different methods to achieve it in fish. It also discusses the process of producing a single sex population for commercial purposes, along with some successful examples and the current use of this technique [2], [3].

Sexual dimorphism means that there are differences between males and females in certain animals. This is common in animals that reproduce sexually. The triplewart seadevil anglerfish has a big difference between males and females. The female can be up to 30 cm long, but the male is only 1 cm long and lives as a parasite. The male does not have a digestive system, but it has a very good sense of smell. The male anglerfish thinks the pheromones released by a female that can reproduce are like food. He quickly goes to eat her flesh and, in the process, releases enzymes that eat him and part of the female. Only the male's testes remain, which are connected to the female's body. If people wanted to raise this species for people to eat, it would be much better to only have female populations instead of having a mix of males and females. Although there may not be a big difference in physical characteristics between males and females in certain species that are valuable in the trade industry, there are still benefits to having a population that consists of only one gender either all males or all females in different species and markets. There are several reasons why having a population with only one sexual orientation is seen as a positive thing in society. In many species, there are noticeable differences in certain desirable traits between males and females. In the older poultry industry, it is preferred to have only female chickens for egg production and only male chickens for meat. The same thing happens with milk and meat from cows and bulls. Usually, different fish species grow at different rates. In some types of fish, males grow faster than females [4], [5].

This is true for species like tilapia and catfish. However, in other types of fish like grass carp, certain salmon species, and cyprinids, females tend to grow faster. The population of one gender is increasing quickly, so their culture helps them make more things. When we culture one gender, we adjust the culture conditions to better match the energy needs of that gender. Also, because there is no unintentional breeding, the genetic breeding program is handled in different sections and is more regulated. If accidental breeding happens in the ponds where fish are grown, it would create too many fish and make them too close together. This would also waste energy because the fish have sex instead of using that energy to grow. The outcome of a culture that focuses on only one gender will be more similar, which can be very beneficial for marketing. Some countries do not accept or resist introducing new exotic species because it can harm the environment. One example is the Mozambique tilapia, which is an important fish bred in many places. It has invaded waterways in Queensland and is seen as a harmful species. Being able to separate male and female genders could help prevent the escape of harmful substances into rivers and other natural water sources. Even if there is a leak, it will not last for more than one generation. Lastly, it is not okay to steal genetically improved lines, especially when they are only being sold to one gender [6], [7].

DISCUSSION

First, we need to figure out the genetics Are males different from females because they have two different sex chromosomes (XY) or because they have two of the same sex chromosomes (ZZ). Which gender is preferred. This preference not only depends on the species, but also on the market. Some markets may want more males while others may want more females. What are the small parts inside our bodies that decide if we will be a boy or a girl, and how we

become different from each other. These parts work differently in different types of animals, so it is difficult to find the main influences that can be changed. However, there are certain important parts that remain similar in different species and can be focused on. When do sex traits show up and when can someone successfully change their sex. Usually, when sexual traits appear, it is too late to change someone's sex. Because of this, sex markers are important to tell the difference between someone who has successfully changed their sex and someone who has been mistakenly identified as a different gender.

Sex determination and sexual differentiation are two processes that happen at the same time. Usually, a boss controls these processes. This boss controls a main part that starts a whole chain reaction of genetic communication which causes the characteristic traits to show up. Sex determination is how an organism's sex is decided based on their genes, except for some cases where temperature or other factors like social structure can influence it. Sexual differentiation is when physical changes occur in the body as a result of the sex determination process. These are processes that overlap with each other. You can control them using the right timing and tools, if they are available. Throughout the process of evolution, the ways in which organisms determine their sex and develop sexual differences have changed many times. To achieve the same results in species that reproduce sexually, there are many ways in which this is done. Actually, in the animal world, sex can be flexible. Some species begin as males and later become females or start as females and later become males. In addition, some types of animals can reproduce by themselves and have both male and female reproductive organs. They are called hermaphrodites. The different fish species that are important for aquaculture and how they determine their sex were talked about in great detail. To make it easier to talk about only one sex, we will be looking at how genes determine sex and species that have only two sexes [8], [9].

The way an organism decides its sex is controlled by different factors like temperature for some species. However, for most species, it is determined by their genes. In some species, a special gene controls how the gonads develop. This gene starts a process that leads to other genes being expressed at different times, which causes the gonad to change and become different. In mammals and cartilaginous fish, the area where the gonad grows is called the urogenital ridge. This region is important because it develops both the gonads and the urinary system. At first, the gonad is not yet developed and is able to develop in two different ways depending on certain factors. The bipotential gonad has two groups of tubes called the Müllerian ducts, which can become oviducts, and the Wolffian ducts, which can become sperm ducts. During a specific stage, the reproductive cells begin to multiply and produce hormones called anti-Müllerian hormone and testosterone.

They also have certain proteins called Dmrt1 and Sox-9 that help with the production of Amh. All of these factors work together to help the development of testes. When the signal is not very strong, other signals will be sent after meiosis. These signals cause the hormone aromatase to change testosterone into estradiol. As a result, the ovary forms. In teleost fish, the undeveloped reproductive organ called gonad does not have two sets of tubes, but only one set of tubes known as Wolffian. These tubes can develop in different ways, depending on certain genetic factors. Because teleost fish do not have Müllerian ducts in their developing gonads, it is not known what the purpose of Amh is. This is a basic explanation of the process. There are a lot of differences between species in 6. 1 The active form of Amh is a big protein made up of sugars. It has two identical parts and weighs 140 kDa. It is held together by special bonds called disulfide bridges. In most animals, a gene encodes this trait, but in Nile tilapia it is encoded by a gene on the Y chromosome. Amh or Müllerian-inhibiting substance is a protein made by cells in a growing baby. Amh is a good option to use for changing the sex of animals because it has

specific DNA sequences for each species. Testosterone is made in the body from a process called steroidogenesis. It is found in a wide variety of animals. Using testosterone, estradiol, or similar substances is harmful to the environment [10], [11].

Steroidogenesis is the ongoing process of converting cholesterol, a starting molecule, into different steroid hormones using different enzymes. The process of steroid production happens in both males and females, not just in their reproductive organs but also in other tissues. Various enzymes change cholesterol into substances called progestogens. Then, another group of enzymes converts these progestogens into androgens. Finally, a different set of enzymes converts the androgens into estrogens. This process is different for each species and involves different male and female hormones. Androgens determine male characteristics, while estrogens determine female characteristics. It's not surprising that one important enzyme in the process of sex differentiation in animals with backbones is called aromatase. This enzyme changes the hormone testosterone, which is responsible for male characteristics, into the hormone estradiol, which is responsible for female characteristics.

Aromatase helps determine someone's gender by being more active in females and can be found in body parts on the outside of the body. However, it is not as dependable as genetic DNA markers, and scientists are still looking for these markers for many important species in aquaculture. To create groups of only males or females in fish farms, scientists often use man-made hormones like estrogens or androgens to change their sex. This process is not good for the environment because these substances end up in water sources. In some animals, changing the temperature is somewhat helpful, but it doesn't work for most animals. Feminization can happen in two ways. One way is by giving a hormone called estradiol directly. Another way is by giving a different hormone called androgens. Both ways can make all females, whether they have WW or XX chromosomes, become neo-males. These neo-males are functional males but carry genes that are usually found in females. In simpler words, masculinization can happen in two ways: by giving someone male hormones directly or by giving them female hormones which then leads to the development of female bodies with male characteristics.

We can see the different ways sexual manipulations can happen based on how genes are inherited in the picture. 62 can be rewitten as "six point two. " You don't need to know how genes are inherited to do direct manipulation. So, it is easier. The drawback is that this method is more expensive and not as good for the environment. This is because all individuals in the group have to be treated, compared to the indirect method where only the parent group needs to be treated. In species with XX/XY chromosomes, indirect feminization is easier because it directly results in populations with only females. This happens because the Y chromosome is removed from the lineage. In ZZ/ZW systems, it is more complicated for a population to become all female. This is because male birds (ZW) need to mate with female birds (ZW), and only after two generations can all-female birds (WW) be found. In ZZ/ZW species, it is easier to make males by breeding ZZ neo-females with ZZ males. This leads to a population with only male offspring.

In XY/XX species, indirect masculinization is more complicated because neo-females have XY chromosomes and mate with XY males. If the YY individuals are viable, then the ratio of males in the population will be about 75%. If the YY individuals are not viable, then the ratio of males in the population will be about 66. 7% If they are present, 75% of the group will be males. Then, these male individuals can be separated and bred with females to create populations with only male offspring in the next generation. To remove the X chromosome from the family line, you need to continue feminizing YY males indirectly. In simple words, the easiest way to get populations of single-sex aquatic animals in fish farms is less environmentally friendly. In rainbow trout, they use direct feminization because female fish

take longer to reach sexual maturity than male fish, grow faster, and have better quality flesh. However, there is a problem with incomplete sex reversal that can sometimes result in hermaphrodites who have both male and female reproductive organs. In order to make male Nile tilapia grow faster, they are given certain hormones called androgens. The grass carp is becoming more like females because they are growing faster in this species. Instead of giving grass carp hormones through their food, scientists implant them with slow-release hormone implants to change their sex. Female salmon grow faster than male salmon, so in salmon farming, they use a method called indirect feminization.

In recent years, aquaculture has been expanding rapidly, while the amount of fish caught from the wild has stayed about the same. This means that there is a greater need for improved techniques in aquaculture to meet the increasing demand for fish in the market. In the large freshwater prawn *Macrobrachium rosenbergii*, there is a bigger need for a quick fix to improve aquaculture techniques because the problem is getting worse quickly. M M is a letter in the alphabet. It is the thirteenth letter and usually has a round shape. It is commonly used as the first letter of names or words. Male Rosenbergii shrimp grow faster and become bigger than female shrimp when it is time to harvest them. This means that males are organized in a system where some are bigger and some are smaller, while females tend to all grow at a similar rate. This species shows that it is better to have populations that are all male or all female, depending on what the market wants. In 1988, It was proven that if you choose to harvest only males or only females in a population of prawns, you will get more prawns and make more money. This shows that farming only one gender of prawns is a good idea economically. Manual separation is a usual process in crustacean farming where males and females are put into different ponds. Although it makes money, this process requires a lot of physical effort and is very boring. A new method using biotechnology has been created. It uses what we know about how organisms develop into males or females. In crustaceans, the hormone from the male-specific androgenic gland determines whether they become male or female. This hormone is called the circulating androgenic gland hormone. This gland controls the growth and upkeep of manly traits.

Rosenbergii mating usually results in an equal number of males and females. If you remove the AG from males when they are very young, they can become fully functioning neo-females. Since M Male rosenbergii shrimp have two identical sex chromosomes called ZZ. When neo-females are bred with these males, all of the resulting offspring will be male. This process takes a lot of time, needs expert doctors, and has very few successful outcomes. To put it simply, when AG tissue or cells are put into female organisms at a young age, it can turn them into neo-males. These neo-males are able to create populations that are entirely female. Until recently, the chances of success were very low in both situations. However, they improved when scientists discovered genetic markers for determining sex. The sex markers are important because if we want to change something about a person's sex, we have to do it when they are still developing, before their sexual characteristics develop.

This means that half of the people being treated are males and the other half are females. After the process of manipulation, such as giving AG cells, we predict that more than half of them will become male. That means half of them are males and the others are neo-males. How can we tell the difference between them. If we don't have any signs of what gender they are, we will have to wait and see if they become males or females. Then, we will get rid of the ones that don't develop into the desired gender specifically females, as we want to create male individuals. We will then mate the suspected males with females, which will take a lot of time and resources, possibly up to a year. After that, we will have to wait for the offspring of each mating to grow and show their sexual characteristics, which might take another 6 months. Depending on the ratio of females to males, we can determine if the original individual is

indeed a male with ZZ chromosomes or a specialized male with ZW chromosomes. Using the newly discovered indicators of biological sex, there is less need to go through the long and tiring process of confirming their accuracy. You just need a small piece of tissue from each person, and within a day, we can accurately separate all the neo-males.

Researchers found a gene called Mr-IAG that is similar to insulin and is only found in a specific part of the body called the AG. They discovered this gene by studying a library of cDNA from the AG. In the year 2009. Like other similar peptides, it is believed that mature Mr-IAG consists of a B chain connected by two disulfide bridges to an A chain with another disulfide bridge. Mr-IAG is a gene that becomes active before sexual traits develop. This helps determine the right time for successful sexual manipulation, as discovered by Ventura and others. Repeatedly injecting male prawns with a certain type of genetic material called dsRNA caused the interference and reduction of a specific gene called Mr-IAG, which caused the male prawns to fully change into females in both appearance and function.

CONCLUSION

Monosex means either all males or all females in a group, which is a popular method in aquaculture. This section talks about the benefits of having a population with only one gender and explains how it can be achieved using various methods of determining gender and sexual development. In this chapter, a recent study on using biotechnology to control the sexual behavior of crustaceans in aquaculture is discussed. The study helps us understand the important factors needed for a successful implementation of this technique. This app uses a special hormone to help fish and other aquacultured species. It can be used to make environmentally friendly changes in these organisms. This chapter talks about how scientists are studying ways to control the reproduction of underwater animals. They first look at fish and other creatures with backbones, then they study crustaceans like crabs and lobsters. They also discuss how the methods they use in these studies can be used on other animals too.

REFERENCES:

[1] T. Ventura and A. Sagi, “The insulin-like androgenic gland hormone in crustaceans: From a single gene silencing to a wide array of sexual manipulation-based biotechnologies,” *Biotechnology Advances*. 2012. doi: 10.1016/j.biotechadv.2012.04.008.

[2] C. Palaiokostas *et al.*, “Mapping the sex determination locus in the Atlantic halibut (Hippoglossus hippoglossus) using RAD sequencing,” *BMC Genomics*, 2013, doi: 10.1186/1471-2164-14-566.

[3] S. K. Omasaki, H. Charo-Karisa, A. K. Kahi, and H. Komen, “Genotype by environment interaction for harvest weight, growth rate and shape between monosex and mixed sex Nile tilapia (Oreochromis niloticus),” *Aquaculture*, 2016, doi: 10.1016/j.aquaculture.2016.02.033.

[4] K. Arai, “Significance and prospect of chromosome manipuation in aquaculture of salmonids,” *Fish. Sci.*, 2002, doi: 10.2331/fishsci.68.sup1_734.

[5] T. Arslan and R. P. Phelps, “Production of monosex male black crappie, Pomoxis nigromaculatus, populations by multiple androgen immersion,” *Aquaculture*, 2004, doi: 10.1016/j.aquaculture.2003.12.007.

[6] N. Shpak, R. Manor, E. D. Aflalo, and A. Sagi, “Three generations of cultured prawn without W chromosome,” *Aquaculture*. 2017. doi: 10.1016/j.aquaculture.2016.06.008.

[7] M. Chiasson, T. J. Benfey, and D. J. Martin-Robichaud, "Gonadal differentiation in Atlantic cod, Gadus morhua L., and haddock, Melanogrammus aeglefinus (L.)," *Acta Ichthyol. Piscat.*, 2008, doi: 10.3750/AIP2008.38.2.07.

[8] B. Chatain and H. Chavanne, "La génétique du bar (Dicentrarchus labrax L.)," *Cah. Agric.*, 2009, doi: 10.1684/agr.2009.0296.

[9] M. A. Mirando and D. L. Hamernik, "Funding priorities in animal reproduction at the United States Department of Agriculture's Cooperative State Research, Education, and Extension Service," *Biology of Reproduction*. 2006. doi: 10.1095/biolreprod.105.048686.

[10] M. Ovidio, D. Desprez, C. Mélard, and P. Poncin, "Influence of sexual genotype on the behaviour of females (genotype WZ) and pseudofemales (genotype ZZ) in the tilapia Oreochromis aureus," *Aquat. Living Resour.*, 2002, doi: 10.1016/S0990-7440(02)01168-3.

[11] A. Sagi *et al.*, "Culture of the Australian red-claw crayfish (Cherax quadricarinatus) in Israel: II. Second growout season of overwintered populations," *Isr. J. Aquac. - Bamidgeh*, 1997.

CHAPTER 7

APPLICATIONS OF CELL LINES AND CELL CULTURE SYSTEM: FINFISH AND SHELLFISHES

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ABSTRACT:
Precision in all operations, including farm preparation, brood stock growth, maturity and spawning, nursery rearing, feed formulation and feeding, culture environment quality management, and timely harvest, is required to achieve sustainability in aquaculture. The system is under danger of collapse at every point of the cultural cycle for a variety of reasons, and farmers are constantly under the strains of uncertainty. Precision aquaculture tackles all of these difficulties and attempts to make the farmer's life easier. Cell lines and cell culture systems are critical components of precision aquaculture. This chapter examines the areas where it may be used and discusses how it can be incorporated into system management to make it failsafe. The host specificity of viral infections necessitates the use of specialized cell lines with pathogen receptor sites. As a result, the sensitivity of fish cell lines to the virus differs depending on the species and the tissue from which the cell line is produced. Because most viruses retain very high degrees of tropism, the circumstance necessitates the creation of species and tissue-specific cell lines from all cultivated species.

KEYWORDS:

Cells, Culture, Fish Cell, Study, Viral.

INTRODUCTION

The most regularly used fish cell lines, providing a simple reckoner for selecting a suitable cell line for viral isolation and investigation. Meanwhile, experienced virologists have repeatedly realized that detection and isolation of an unknown virus may not be possible using an established cell line because the cells may have modified the receptor sites during in vitro transformation, rendering them undetectable by the virus in question. Primary cell culture from a particular organ of a certain fish species is the best alternative in such cases. As a result, the capacity to retain established fish cell lines and use them for virus isolation is critical. Furthermore, one should be able to generate primary cell cultures from any species of fish on demand in order to investigate fish viruses and do actual viral isolation. This capacity must be part of a fish virology laboratory and be customizable.

The cell suffers specific biochemical and morphological alterations during viral multiplication, known as cytopathic effects (CPE), indicating that the causative virus is cytopathogenic. The degree of apparent cell damage induced by viral infections varies depending on the kind of virus, host cell type, multiplicity of infection (MOI), and other parameters. Some viruses induce very little or no CPE in their natural host's cells. In such cases, their presence can only be detected visually through hemadsorption or interference, in which an infected cell culture with no CPE inhibits the replication of another virus introduced later into the cultures or in situ by viral antigen or nucleic acid detection. Some viruses, on the other hand, induce full and fast death of the cell monolayer following infection. The CPE induced by certain cytocidal viruses may be sufficiently distinctive to enable tentative identification of unknown viruses, which has great practical implications. Erica and Carol have described a simple approach for detecting CPE in cell lines that may be used in a fish virology laboratory. It should be noted that a

particular virus may cause different CPE in various host cell types, hence it is critical for a technician to employ the same cell type consistently for a certain virus in a laboratory. Control and infected cells should constantly be monitored to differentiate between normal cell alterations that occur as the cells mature and CPE. The rate of CPE appearance is another trait that may be utilized to identify recognized viruses. A virus is termed sluggish if CPE develops after 4–5 days in culture infected with a low multiplicity of infection (MoI). It must be noted that with a high MoI, all CPE may happen quickly. As a result, the rate of CPE manifestation should be determined by the lowest MoI that causes CPE [1], [2].

Cell fusion must be differentiated from cell clumping or clustering, which retains the plasma membrane. Inclusion bodies are patches of abnormal staining in cells that are not normally visible in living cells. Single or many inclusion bodies, big or tiny, spherical or irregularly shaped, intracellular or intracytoplasmic, eosinophilic or basophilic are all possible. In certain circumstances, chromatin margination may be present, causing a thin stained ring to appear around the nucleus's perimeter. Inclusion bodies can form for two reasons: in one case, they indicate areas of the cell where viral protein or nucleic acid is being synthesized or where virions are being assembled, but in other cases, no virus is present and inclusion bodies indicate areas of viral scarring. In aquatic animal health management, initial isolation of viral pathogens from ill fish is always a difficulty. If one intends to produce a real isolation of the viral etiology, numerous parameters must be accomplished, including pathogen viability, sensitivity of the utilized cell line to the virus, visible CPE, and the capacity to transmit to new bottles. However, on numerous instances, the fish arrive to the laboratory in a putrefied form, making the process seem unworkable. Despite the fact that the viral particles are extremely well protected inside a cell, regardless of the type of nucleic acid (RNA or DNA viruses), the tissue may be utilized for trying to isolate the pathogen correlating with clinical indications of the illness, external, internal, and behavioural [2], [3].

If seeking for a recognized virus based on clinical indications, PCR on tissue would be very helpful. The viral extraction from tissue is used to extract the virus from the afflicted tissue is more important in isolating the virus. Once the samples are obtained from the field, they must be transported to the laboratory in chilled ice, dissected and the organs are stored at 2^0 C for short term and 8^0 C for long term, thawed only once, that too for macerating the tissue with glass wool in the respective tissue culture medium at aseptic conditions using a tissue homogenizer at 4^0 C in an ice bath, centrifuged at 10,000 rpm at 4^0 C, filter sterilized using polyvinylidene difluoride membrane having 0.6. The absolute requirements the situation necessitates that the technicians involved be well trained and have an adequate knowledge base on fish disease in general, in addition to having a laboratory well equipped for the purpose with PCR primers, positive controls, the required cell lines in liquid nitrogen, media, antibiotics, and stains. Living cell culture combined with molecular tools like PCR and serological procedures like ELISA would greatly aid in the identification of the virus recovered. Meanwhile, histology and electron microscopy must be used to look for new viral etiologies. To get the most out of the fish cell lines available, the absolute requirements are as follows well-informed manpower with holistic knowledge and mindset to approach the problem, a standard animal cell culture laboratory, molecular biology laboratory, serology laboratory, histopathology laboratory, access to electron microscope facility, and bioassay systems with biosec. Fish cell lines for the research of intracellular bacterial pathogens It is worth noting the use of fish cell lines in the isolation and investigation of intracellular bacterial infections. Bacterial diseases of fish, such as *Rickettsia spp.* Cell lines were used to extract *Renibacterium salmoninarum* from fish [4], [5].

The first Gram-negative intracellular bacterial pathogen identified from fish using the CHSE-214 cell line is Piscirickettsia salmonis. It was proven as the causal agent of the sickness in Coho salmon by fulfilling Koch's postulates. P. salmonis was cultivated in two cell lines generated from Atlantic salmon kidney, salmon head kidney (SHK-1) and anterior salmon kidney (ASK), by Ortiz-Severn et al. Gentamycin was used to destroy extracellular bacteria at a final concentration of 50 mg/mL. The susceptibility of two fish cell lines to three chlamydia-related bacteria: Waddlia chondrophila, Parachlamydia acan thamoebae, and *Estrella lausannensis*. *Yersinia ruckeri* invasion and replication in fish cell cultures (CHSE-214, ASK, and SHK). The above description clearly implies the presence of many intracellular bacterial infections in fishes, as well as the employment of cell lines in their research aiming at integrated health management at the field level. Fish cell lines for viral vaccine manufacture According to the literature, periodical illness outbreaks in fish culture are caused by 54.9% bacterial pathogens, 22.6% viruses, 3.15% mycotic agents, and 19.4% parasitic agents [6], [7].

The Office International des Epizooties (OIE) has listed certain important diseases caused by DNA viruses such as epizootic hematopoietic necrosis (EHN), koi herpesvirus disease (KHVD), and red sea bream iridovirus disease (RSID) and RNA viruses such as infectious hematopoietic necrosis virus (IHNV), infectious salmon anemia virus (ISAV), spring viremia of carp (SVC), and viral hemorrhagic septicemia (VHS) causing major catastrophes in aquaculture. Vaccination is the sole approach for controlling viral infections at the appropriate life stage. In aquaculture, many types of vaccinations have been reported, the most common and least costly of which are conventional or traditional vaccines, which are inactivated entire viruses. These viruses must be grown in specific cell lines, extracted, and inactivated with b-propiolactone, binary ethylenimine, formaldehyde, or at high temperatures, with BPL inactivation providing the greatest efficacy, as reported in IHNV. However, certain fish viruses, such as the Iridoviridae family's lymphocystis disease virus, are difficult to replicate in cell cultures. As a result, the development of inactivated vaccines for such viruses remains a challenge. This situation necessitates enhanced attenuation to either test current cell lines for viral susceptibility or develop fresh cell lines for the purpose. Among the three types of vaccinations, inactivated whole vaccines, subunit recombinant vaccines, and DNA vaccines, the inactivated vaccine, when combined with proper adjuvants, will be the most ideal for oral use in aquaculture [8], [9].

Fish cell lines for antiviral chemotherapy A systematic development of antiviral drugs for preventative and therapeutic applications is an important prerequisite in the control of viral infections in aquaculture. Aside from employing fisheanimal models to screen such compounds, particularly those derived from natural bioresources, cell lineevirus complexes are effective tools. While doing so, two types of screening may be performed: one for studying virucidal properties in vitro, which would kill the virions, and the other for interfering with the viral multiplication cycle inside the cell. When screening antiviral drugs in cell lines, molecular techniques to measure viral gene expression by cDNA, cellular health via trypan blue exclusion test, MTT assay, and bromodeoxyuridine incorporation assay are advised. Furthermore, viral replication may be inhibited by passing them on to other cell lines and measuring infectivity in vitro and in vivo by introducing them to animal models. Cell lines from the respective fish serve as an effective in vitro model for studying nutritional intake and digestion. In order to achieve this goal, intestinal cell culture systems must be established. Primary cultures of adipocytes, hepatocytes, and myoblasts are widely employed to investigate the molecular processes underlying fish feeding [10], [11].

To study nutrient-sensing signalling, a rainbow trout hepatoma-derived cell line was used to address nutrition-related questions based on major pathways such as macroautophagy, general

control nonderepressible 2, and mechanistic target of rapamycin that regulate cell homeostasis through amino acids. Similarly, RTH-149 cells have been employed to investigate the kinetics of starvation-induced autophagy. Several fish cell lines have been employed as in vitro models to examine the elongation and denaturation of various polyunsaturated fatty acids (PUFA), which is important for understanding the proinflammatory processes underlying the link between dietary PUFA and heart pathologies in salmon. The aforementioned occurrences indicate the need for additional such cell lines to be used in fish nutrition research, as well as the creation of precise experiments including particular such cell lines in place of animal models. When functional feed and feed components are addressed, the need for cell line-based nutritional assays becomes highly significant, implying accelerated development of fish cell line technology in support of fish nutrition.

Aquaculture environment quality evaluation for sustainable aquaculture production Environmental quality is an important component of monitoring. Animal models are no longer utilized for measuring environmental quality owing to different restrictions and animal-to-animal variance to a given circumstance, in addition to ethical concerns. Many international regulatory organizations in Europe, as well as the Food and Drug Administration in the United States, have chosen fish cell lines as an in vitro method for ecotoxicological assessment of substances. Embryonic stem cells and transgenesis have been the subject of active study since the formation of the first embryonic stem (ES) cells in mice due to their immense potential for fundamental research, medicine, and animal biotechnology. Several studies have recounted the thirty-year history of fish stem cell cultivation, demonstrating the discipline's methodical evolution. Germ cells, particularly primordial germ cells (PGCs) and gonadial germ stem cell spermatogonia in the testes and oogonia in the ovaries, have piqued the attention of researchers working on germline competent stem cell cultures. PGCs populate the gonad purely for egg or sperm production and have the potential to evolve into pluripotent stem cell cultures. Since the discovery of zebrafish vasa as a molecular marker of fish germ cells, the vasa promoter has been utilized effectively to generate transgenic lines in a variety of fish species, including medaka and trout.

Future research is required to evaluate if fish PGCs can generate stable cell cultures while retaining the capacity to transmit germ lines. Fish stem cells have the potential to be used in gene targeting, germ cell transplantation, and nuclear transfer semi cloning. These techniques, in conjunction with CRISPR-based genome editing, will open the way for increased strain development initiatives to improve fish output, disease resistance, and environmental resilience. There are several benefits to using ES cellemediated transgene insertion over traditional approaches. It guarantees stable site-specific transgene integration/deletion, in vitro selection of clones with desired genotypes, germ line transgene transfer into progenies, and economic feasibility. Another appealing feature is that when ES cells are put into a host embryo, they may engage in normal development and contribute to numerous tissues of the host, including germ line cells. Furthermore, ES cells are becoming as promising candidates for biodiversity cryopreservation. Furthermore, Blastula cell transplantation (BCT) may achieve high fertility restoration efficiency for the experimental examination of these biological parameters toward BCT-mediated surrogate production of high yielding, genetically enhanced fishes for biodiversity conservation.

Fish pituitary organoid culture and tissue engineering. The pituitary gland, a key endocrine gland, regulates the growth, development, and function of the other endocrine glands. It generates gonadotropic hormones, which are required for fish development and spawning. These gonadotropins include follicle-stimulating hormone and luteinizing hormone, which are released and linked to gonadal maturation cycles. Other than hypophysation, commercial

hormones such as human chorionic gonadotropin (hCG) (hCG alone or in combination with fish pituitary [hCG 70% þ PG 30%]), luteinizing hormoneereleasing hormone (LHRH), progesterone (17a-hydroxyprogesterone and 17a-hydroxy-20b-dehydroprogesterone), antiestrogens (clomiphene citrate and tamoxifen), and Ovaprim (Linpe method) (LHRH-A and the drug domperidone) are used in fish breeding with varying level of success. If the pituitary gland can be kept in an organoid culture state, it opens the door to producing hormones in vitro and regulating production by tissue engineering. Organoids, spheroids, and the research of 3D cell culture models indicate significant promise in having an organoid pituitary culture in situ, which opens up enormous options in fish endocrinology, breeding, and controlled seed production.

Crustacean cell line creation has a 35-year lengthy and laborious history. It remained elusive owing to a disregard for the know your animal mentality, since the successful history of insect cell lines began with in-depth information obtained on insect biochemistry, which allowed for the development of an adequate and unique insect cell culture medium. Meanwhile, primary cell cultures of crustaceans with established viral susceptibility have been successfully developed from lymphoid organs, hemocytes, and heart tissue. Despite modifications to commercially available media based on hemolymph analyses, a unique medium for the in vitro growth and development of shrimp cells has not been developed. Despite the fact that Wyatt et al. were not completely successful, their contribution was critical to Grace's eventual success in the invention of Grace's insect cell culture medium, which resulted in the establishment of over 500 insect cell lines. With the creation of a medium special to shrimp cell culture by Jayesh et al., such a scientific temper may be instilled in shrimp cell culture research for the successful production of a continuous cell line. The next attempt was to induce in vitro transformation, which included the identification of a putative promoter system to construct transformation and transduction vectors specific to shrimp, and the use of oncogenes was proposed.

Furthermore, transgenic expression of oncogene and telomerase reverse transcriptase may result in the effective establishment of legitimate shrimp cell lines. To summarize, crab cell line generation remains a grey area that needs collaborative efforts from all of the aforementioned viewpoints in support of sustainable aquaculture. Meanwhile, PmLyO-Sf9 might be used as an in vitro model to tackle a variety of viral-related challenges in shrimp cultivation. Molluscan cell lines Despite efforts dating back to the 1960s, no verified cell line of marine molluscs has been made accessible. Given the importance of marine bivalve aquaculture to the global economy, the current viral threats, and the fast breakthroughs in molluscan virology, a molluscan cell line is required. There might be many problems impeding the creation of a molluscan cell line, and it is critical to address each one in order to achieve a satisfactory conclusion. To maximize the development and division of molluscan cells in vitro, correct cleaning techniques, tissue selection, and the creation of an appropriate cell culture media must all be addressed. To get a successful conclusion, the right intervention of gene transfer and cell hybridization procedures may also be used.

DISCUSSION

Cell cultures are helpful tools for studying how fish and shellfish protect themselves against diseases. This information can be used to keep these animals healthy in aquaculture farming. Scientists have created special cells called fish leukocyte cell lines and macrophages from different fish species such as carp and catfish. These cells are used to study the immune system. Some cell lines similar to monocytes have been created using white blood cells from channel catfish. Cell lines made from the gut, skin, and gill of fish are good to use in the lab for studying how fish defend themselves. These fish cell lines can be used to see how well DNA vaccines,

synthetic peptides, immunostimulants, and other products boost the immune response. Scientists created a constant line of blood cells from a certain type of fish called Cyprinus carpio. This helped them learn more about how fish's immune systems work. Fish macrophage cell lines are really helpful for studying the immune system and other research purposes. Two types of macrophage cells, called CTM and CCM, were created from a type of fish called Catla catla. These cells can be used to study how they interact with other immune cells and their role in helping fish develop and stay healthy. The SHK-1 cell line, made from Atlantic salmon, reacted to certain antibodies against white blood cells from Atlantic salmon.

The cell line was also able to eat bacteria. The researchers created a type of immune cell called RTS11 in rainbow trout. This cell line was used to study how immune cells respond in a lab setting. Saprolegniales are a type of fungi that are known for causing diseases in freshwater fish. They are considered to be the most significant or important fungi when it comes to fish diseases in freshwater environments. We do not fully understand how the fish hosts respond at a cellular level. The RTS11 cell line, which comes from rainbow trout, was used to see how macrophages react to water molds called Achlya and Saprolegni. Fish cell lines are helpful for studying how nutritional problems lead to diseases. Researchers used fish cells to study how dietary polyunsaturated fatty acids (PUFA) can cause inflammation and heart problems. They specifically used cells from chum salmon to investigate this mechanism.

Genetically modified fish cells have many uses in biotechnology and medicine. CRISPR is a tool that scientists use to change genes. It has changed the way we edit genes. Using the CRISPR-Cas9 technology to create better fish cells can help with research in aquaculture, specifically in studying fish diseases. Using genome editing technology, we can modify cell lines in fish to improve their ability to transfer genes. These edited cell lines can then be used to produce viruses more quickly and efficiently for making vaccines. This method has been mainly used for changing genes in mammal cells, but using gene editing for fish cells is still in the early stages. Scientists have discovered a new way to edit genes in fish using a method called CRISPR-Cas9. This method has been used before, but researchers have now found a more effective way to edit genes in a specific type of fish called Chinook salmon. They used a fish cell line called CHSE, which was developed from Chinook salmon Oncorhynchus tshawytscha. The cell line was modified to produce more of different types of CHSE cells. Even though people have tried, no good method to knock out fishes in a controlled environment has been created yet.

We used a type of cell from a trout's head kidney that stays the same, and we added different plasmids that make proteins called cytokines. These cytokines are called Interleukin-6 and macrophage colony-stimulating factor (MSCF). Scientists created a line of cells called rainbow trout head kidney cells and stable RTG-2 cells. They manipulated these cells in a special liquid to produce certain proteins called interleukin (IL-2), IL-6, and macrophage colony-stimulating factor (MCSF). The liver cells of the greasy grouper fish were changed using genetic modification to study how well the anti-apoptotic protein Bcl-xL works. Fish cell lines are used in labs to study and change the number of chromosomes in fish embryos. Polyploidization was achieved in a crucian carp by using a chemical compound, resulting in the development of a cell line with four sets of chromosomes.

The use of genetically modified fish cells has the potential to greatly help with studying fish health, genetics, and biotechnology research. Creating a steady group of cells is super important right now for studying fish genetics and health in functional genomics. As gene delivery methods have gotten better, more fish cell lines that have been genetically modified are staying stable. Not many attempts have been made to understand how immortal fish cell lines work so we can develop better methods for genetic engineering. Scientists have changed the genetics

of goldfish, Chinook salmon embryos, and rainbow trout liver cells to learn more about fish diseases and the immune system. A changed cell line from a type of fish called tilapia was used in an experiment. The cell line had a special gene that made it produce a green glowing protein when the cells were under stress. The fish cell lines can be used for studying diseases that affect fish and also for studying how climate change impacts their environment.

Advancements in cell culture techniques make it easier to target and transfer genes for producing transgenic fish. Scientists have been able to create special fish by modifying the genes of male fish cells. This has helped in creating new kinds of animals for research. A new type of cell culture was made by changing the genes of a trout fish. The genes made the fish have a special protein that made it glow green. We can create the transgenic line by using special cells called primordial germ cells. The Vasa marker helps identify and study specific cells involved in reproduction in fish. Tanaka and their colleagues Scientists created a special type of fish called medaka that has a gene called GFP in its reproductive cells. The successful transfer of reproductive cells in fish showed that we can produce fish babies using a different parent fish. For the first time, scientists successfully used intraperitoneal transplantation of PGCs to create seedlings in rainbow trout. The advancements in growing stem cells and using them in laboratory experiments and fish farming will greatly change the fishing industry to achieve a major revolution. Spermatogonial stem cells transplantation can be very useful for breeding fish in captivity. A special type of cell called SG3, which comes from the mature testis of a fish called medaka, was able to make sperm. The creation of fertile medaka fish using ES cells showed that it is possible to make copies of fish using cells from their embryos. We need to do more research on using ES cells in aquaculture species. Using embryonic stem (ES) cells to transfer genes is a hopeful method for creating transgenic animals. Scientists have created a way to put genes into rainbow trout using stem cells. This allows them to make transgenic rainbow trout. ES cells, PGCs, and nuclear transfer help make it easier to create transgenic fish.

Fish cell lines have great potential to be used as model systems for studying fish disease, immune systems, biotechnology, nutrition, and testing the safety of chemicals and medicines used in fish farming. They are a good alternative to studying the entire fish, which can raise ethical concerns. Scientists have used a laboratory model to study how viruses grow and reproduce, as well as to create experimental vaccines for fish farming. Organ culture was made using glands from tilapia, eel, and trout. This culture was used as a model to make the growth hormone called prolactin. Fish cell lines were used to study how fish develop and understand how certain communication pathways are involved in their growth. Cell cultures made from fish can be used to study how fish eat and use nutrients. But not many people have used these cultures to study fish nutrition in that way. This also means we need to create and understand cell cultures of the intestines to help with these studies. A cell line made from the fish intestine is helpful in studying how certain ingredients in fish food, like probiotics, and exposure to chemicals in the water, affect aquatic systems. The researchers studied how fish's intestines process and break down harmful substances in the environment using a cell line called RTgutGC from rainbow trout.

Langan and his colleagues Examined how spheroid size affects the way propranolol is processed by studying a specific type of fish cell line that mimics the intestinal system in a 3D structure. The cells in the intestine of rainbow trout act as a blockade for studying how the body's immune system works, how the body reacts to things like illness and injury, how nutrients are taken in, and how harmful substances affect the body. The RTgutGC cells were compared to new cell lines from different parts of a rainbow trout's intestine. These new cells were called RTpi-MI and RTdi-MI. When these cells were together, they created a protective

barrier that didn't let bigger molecules pass through. They also absorbed glucose and proline. The RTgill-W1 cells were used in a lab setting to test how harmful certain chemicals are to the environment when they are released into water. This testing included both saltwater and freshwater conditions. Food and water are important for survival. To keep track of how our body is working, we can monitor its functions in real-time. In labs, it is very important to use models to study how the body makes and releases collagen in humans and other animals with backbones. Not many studies have been done on how fish make and release collagen. Lee and Bols looked at how fish cell lines could be used to study collagen as a model in a lab. They wanted to see how various factors affect the production, release, and placement of collagen.

Cell-based aquaculture refers to the process of growing seafood, such as fish and shellfish, from individual cells in a controlled environment. This method eliminates the need for traditional fishing or fish farming, and can help meet the growing demand for seafood in a sustainable and ethical way. Aquaculture has been growing quickly and facing many difficulties in order to meet the increasing demand while also guaranteeing the safety and quality of fish products. The idea of making seafood from cells is becoming popular as a new way to make animal protein options. This new way of producing animal protein from fish could help solve some important problems faced by traditional fish farming and decreasing ocean fishing. This new way of producing fish will help protect our natural resources and environment by putting less strain on them. So, the whole world is working towards making production systems that can handle the effects of climate change. One area of research that is very important and advanced is making meat in a lab instead of from animals.

This could make producing fish meat from these cells cheaper than producing meat from animals. Using tissue engineering and aquaculture techniques, we can use marine cell culture to make fish meat in a laboratory. Fish muscle cells grown in a lab can be used to make fish meat. This is possible because these cells have certain qualities that make them capable of surviving in low oxygen conditions, have the ability to adjust to changes in acidity levels, and can withstand colder temperatures. Fish muscle cells can survive and grow better in lab settings compared to cells from mammals. Because of this, it will be easier to produce meat in labs using fish muscle cells. We need to do more research and investigation to find out how to grow fish and shellfish cells in a lab to make fish meat. The best way to make fish meat in a lab quickly is by starting with zebrafish for studying and improving the process.

CONCLUSION

Cell lines and cell culture systems are really helpful tools to find and separate viral diseases, understand how viruses multiply, make medicines to fight viruses, study harmful bacteria in cells, make vaccines, learn about what fish eat and how their bodies work to make fish food better, test the water in fish farms, make genetically modified fish, and make hormones in a lab. Their participation helps with precision fish farming, which is an important step towards making the industry more sustainable. But the most important thing is to correctly combine the tools in order to make them work in the right place at the right time to solve specific problems. In this situation, it is very important for the people in charge of human resources to have the right skills and knowledge to use the tools. This is because their skills and knowledge are very important in properly using cell culture and cell lines. The education system needs to include enough time in the curriculum to teach students how to use cell culture systems correctly and well.

REFERENCES:

[1] M. J. Goblirsch, M. S. Spivak, and T. J. Kurtti, "A Cell Line Resource Derived from Honey Bee (Apis mellifera) Embryonic Tissues," *PLoS One*, 2013, doi: 10.1371/journal.pone.0069831.

[2] M. Čuperlović-Culf, D. A. Barnett, A. S. Culf, and I. Chute, "Cell culture metabolomics: Applications and future directions," *Drug Discovery Today*. 2010. doi: 10.1016/j.drudis.2010.06.012.

[3] N. Desai, P. Rambhia, and A. Gishto, "Human embryonic stem cell cultivation: Historical perspective and evolution of xeno-free culture systems," *Reproductive Biology and Endocrinology*. 2015. doi: 10.1186/s12958-015-0005-4.

[4] Y. Lei and D. V. Schaffer, "A fully defined and scalable 3D culture system for human pluripotent stem cell expansion and differentiation," *Proc. Natl. Acad. Sci. U. S. A.*, 2013, doi: 10.1073/pnas.1309408110.

[5] Y. Chen, W. Zhou, T. Roh, M. K. Estes, and D. L. Kaplan, "In vitro enteroid-derived three-dimensional tissue model of human small intestinal epithelium with innate immune responses," *PLoS One*, 2017, doi: 10.1371/journal.pone.0187880.

[6] V. C. Chen *et al.*, "Scalable GMP compliant suspension culture system for human ES cells," *Stem Cell Res.*, 2012, doi: 10.1016/j.scr.2012.02.001.

[7] H. Bahadar, F. Maqbool, K. Niaz, and M. Abdollahi, "Toxicity of nanoparticles and an overview of current experimental models," *Iranian Biomedical Journal*. 2016.

[8] D. D. Allen, R. Caviedes, A. M. Cárdenas, T. Shimahara, J. Segura-Aguilar, and P. A. Caviedes, "Cell lines as in vitro models for drug screening and toxicity studies," *Drug Dev. Ind. Pharm.*, 2005, doi: 10.1080/03639040500216246.

[9] R. C. Rowland-Jones, F. van den Berg, A. J. Racher, E. B. Martin, and C. Jaques, "Comparison of spectroscopy technologies for improved monitoring of cell culture processes in miniature bioreactors," *Biotechnol. Prog.*, 2017, doi: 10.1002/btpr.2459.

[10] R. Graepel *et al.*, "The virtual cell based assay: Current status and future perspectives," *Toxicol. Vitr.*, 2017, doi: 10.1016/j.tiv.2017.01.009.

[11] A. Simcox *et al.*, "Efficient genetic method for establishing Drosophila cell lines unlocks the potential to create lines of specific genotypes," *PLoS Genet.*, 2008, doi: 10.1371/journal.pgen.1000142.

CHAPTER 8

AN OCEAN OF OPPORTUNITY: OBSTACLES TO THE DEVELOPMENT OF CULTIVATED SEAFOOD

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ABSTRACT:

All around the world, there is a pressing need for new ways to support current efforts in order to fulfill the growing global need for seafood. Cultivating seafood is a way to produce marine animals for commercial purposes. This can help reduce the strain on wild fisheries and aqua culture systems. It is a promising approach if done with the right policies in place. Cultivated meat is much more efficient compared to using animals to produce food. It also provides a way to balance the food chain in seafood production. In simpler terms, the materials and resources needed to make farmed tuna are the same as those needed for other types of farmed fish. We should focus on making and selling seafood that is grown instead of caught from the oceans. This seafood should taste good, be affordable, easy to get, and be healthy. This is important to keep our oceans healthy and alive. The process of making cultured meat involves giving animal cells signals that tell them how to grow into muscles, fat, and connective tissues. No matter what type of animal, these cells all have similar basic needs because their development is controlled by the same biological processes that have been preserved over time in various species. So, the way to make cultivated meat from marine animals should be similar to how it's done with other animals like mammals or birds. Changes to the usual process and improvements for each end product will be needed because the types of substances that help cells grow and the specific nutrients, they need may be slightly different.

KEYWORDS:

Climate Change, Fish Populations, Fisheries Management, Sustainable Development.

INTRODUCTION

The objective to develop produced seafood items has distinct benefits and disadvantages when compared to developed meat from terrestrial farmed species. The most significant problem is that cells from fish and other aquatic species are not frequently cultivated in most research facilities, therefore procedures tailored for these cell types are often unavailable. Furthermore, resources for seafood-relevant species, such as sophisticated genome annotations, are limited when compared to common laboratory species or even common livestock species, and species specific reagents, such as validated antibodies, are not generally commercially available for most aquatic animals. As a result, the goal to develop cultured seafood items has distinct benefits and obstacles in comparison to produced meat from terrestrial farmed animals. The most significant problem is that cells from fish and other aquatic species are not frequently cultivated in most research facilities, therefore procedures tailored for these cell types are often unavailable. Furthermore, resources for seafood-relevant species, such as sophisticated genome annotations, are limited when compared to common laboratory species or even common livestock species, and species specific reagents, such as validated antibodies, are not generally commercially available for most aquatic animals [1], [2].

As a result, this industry lacks established processes and a wealth of scientific literature from which to draw, necessitating large upfront investment in fundamental R&D for enterprises entering this market. Cultivated seafood may provide various benefits over mammalian or

avian cell culture. Cells generated in culture work best when the growth settings resemble the animal's natural environmental parameters. In contrast to human cell culture, which is normally done at 37 degrees Celsius, fish cell culture may be accomplished at much lower temperatures of 4e24 degrees Celsius for saltwater species and 15e37 degrees Celsius for freshwater species. Many fish species have muscular hyperplasia as juveniles, resulting in rapid increases in muscle cell number and biomass. When applied to a grown meat production setting, this quick development capacity may give a larger output of skeletal muscle tissue in a shorter period. Furthermore, many fish and crustaceans retain high levels of telomerase expression in multiple tissue types, which may allow for long-term proliferative capacity or the establishment of immortalized cell lines for research and, eventually, commercial cultivated seafood production [3], [4].

Cultivated seafood may potentially provide benefits as well as distinct problems in terms of producing tissues that mimic the structural patterns observed in fish muscle. Meat in finfish has a basic structure in comparison to muscle tissues in many terrestrial animals, which have complicated and stochastic vasculature and marbling. This shows that creating scaffolds to stimulate muscle and fat separation in specific patterns may be easier for grown fish than for meats like beef or pig. At the same hand, more regular fish structure may highlight any discrepancies in farmed fish, demanding better control over the structure. Scaffolds for fish must also mimic the features of fish connective tissue, such as its lower melting temperature as compared to terrestrial flesh. Much of the allure of pursuing farmed seafood stems from customer concerns about how fish is eaten, as well as business reasons, since many species of seafood fetch very high costs per pound. When compared to meat from other animals, the flesh of aquatic species, such as oysters and tuna steaks, is more likely to be eaten raw or slightly cooked [5], [6].

Aquatic animals, as previously stated, contain a range of germs, viruses, and parasites that commonly cause foodborne sickness. Given that raw consumption increases the risk of foodborne illness and that these raw dishes command relatively high price points from consumers, these items may present an ideal entry market for introducing high-end, contaminant-free, cultivated seafood products prior to the introduction of mainstream seafood products, which may take longer to achieve price parity at scale. Finally, although many customers value fresh seafood that has not been frozen, product loss due to rotting is a serious problem in the seafood sector. Because cultured seafood would be produced in aseptic cultivators, product shelf life might be significantly increased without the need for freezing. Industry requirements While farmed seafood items will be produced and supplied by the private sector in the end, the underlying technologies and their road to commercialization will need a healthy innovation ecosystem. Given that very little funding has been expended in this area outside of a few companies' R&D budgets, and that the estimated total global R&D expenditure to date across all forms of cultivated seafood is on the order of $100 million, this industry has tremendous potential to benefit from concerted public and private resource allocation. Activities must be coordinated across startup companies, multiple sectors of established industries, private and public funders and investors, governments, trade associations, and academic and other research institutions to accelerate the process from early product development to widespread market adoption. All of these businesses, as well as every person who sees a future with abundant and healthy seas, should see this as a call to action to contribute to the creation and expansion of the cultured seafood sector [6], [7].

DISCUSSION

Oceans and coastal ecosystems play important roles in human life, subsistence, and economic development. Fisheries are an essential part of the world economy, with about 3.2 billion

people relying on them for approximately 20% of their protein consumption. Millions of people, particularly in developing nations, rely on the industry for food, nutrition, work, and money. Furthermore, fisheries are an important element of many nations' cultural and traditional history. Simultaneously, oceans and coastal regions face substantial problems as a result of climate change consequences such as rising seas or storm surges, illicit fishing, and inefficient resource use, all of which create a strain on marine ecosystems and aggravate existing policy and governance issues.

As the world's population continues to rise, providing food security is the primary motivator for both traditional fisheries and the rapidly expanding aquaculture industry. The ultimate goal is to offer people with safe, nutritious, and cruelty-free food sources in a sustainable manner that does not impact the marine environment or ecosystems. However, half of the world's marine and coastal ecosystems are presently overfished. According to FAO's State of World Fisheries and Aquaculture report, overall annual fish output will grow to 204 million metric tonnes by 2030. This would not only put strain on the fisheries industry, but it might also contribute to environmental degradation. Overfishing may have a direct influence on fish biomass, biodiversity, and fisheries viability, as well as worsen the effects of damaging fishing gear on marine ecosystems. Overfishing's indirect impacts include habitat damage caused by harmful fishing gear and contamination from microplastics or oil [5], [8].

Aside from the destructive impacts of overfishing on fish populations, illicit, unreported, and unregulated (IUU) fishing impedes national and global conservation efforts and progress toward achieving'sustainability' in the fisheries industry. According to studies, at least 7.3 million tonnes of dead or dying fish are dumped from maritime fisheries each year due to large-scale industrial fishing. According to the FAO, the average edible yield of the roughly 60 million metric tons of fish captured yearly for human use is between 50 and 60 percent, leaving an estimated 25 million metric tons of potential byproducts. At the same time, the expected output of aquaculture byproducts exceeds 60 million metric tons. The bulk of this lost fish and other marine fauna might be utilized as starting materials for other industrial processes such as animal feed, fertilizer, fish oil, fish-skin leather, gelatine, or chitin/chitosan from crab shells/shrimp trash. If the present catch was used more completely, it may boost the sector's viability and give extra livelihood chances. Furthermore, by-catch accounts for almost 40.4% of total capture. Furthermore, recreational fishing is not included in national catch data. As open seas and coastlines grow more popular for tourists and tourism-related activities, a rise in recreational fishing may have an impact on the sustainability of fisheries stocks.

There has been much debate about the extent to which overfishing and IUU impact ocean ecosystems, which is also known as top-down forcin" because such changes occur when predators at the top of the food web are removed, versus the availability of nutrients and other resources in an ecosystem, which is known as bottom-up forcing. It is critical to identify a threshold at which humans may benefit from aquatic biodiversity and ecosystems without jeopardizing their long-term viability. The degree of pollution stress on marine ecosystems varies across industrialized and developing nations, but it places significant strain on the fishing industry. The primary repercussions are the discharge of effluents from industry, agricultural fields, and sewage systems into the open ocean without treatment, which has a broad range of effects on fish populations.

Fertilizer residues from agricultural runoff induce eutrophication nutrient enrichment, notably nitrate and phosphate, lowering water quality and impeding oxygen dissolution, perhaps leading to the extinction of many marine animals. Chemical components, notably heavy metal residues from industrial effluents, may accumulate in fish bodies, causing metabolic changes, and will then move down the food chain to reach humans. Apart from these effects, the

degradation of coral reef and mangrove ecosystems, as well as the disruption of fresh/marine water bodies, will affect reproduction, resulting in a drastic reduction in fisheries stock; additionally, ocean-borne sources of marine litter and oil pollution have the potential to influence fisheries decline while also raising concerns about ocean health. Finally, plastic pollution, particularly micro- and nano-plastics, poses the greatest threat to the survival and sustainability of fisheries stocks. Scientists have reported plastic pellet ingestion, entanglement, and suffocation caused by microplastics and marine litter all over the world, and the problem is getting worse by the day. Their effects not only affect species longevity but also deteriorate the health of the ecosystem [9], [10].

Climate change, in addition to the anthropogenic influence on the decline and disruption of fish stocks, plays a significant role in determining the sustainability of marine and coastal fisheries. Changes in temperature can affect an organism's physicochemical and biological processes, which can influence growth, reproduction, and survival. Ecologically, these effects on a specific species can spread across various taxonomic groups through the trophic levels to alter population dynamics. Climate-assisted factors such as sea-level rise, temperature fluctuations, disruptions in the water cycle and altered precipitation patterns, increased heat, expansion of minimum oxygen levels, increased intensity and frequency of storms, freshwater flow and rainfall, ocean acidification, and salinity changes could all have an impact on fish production, biology, size, and reproductive efficiency, potentially affecting faunal biodiversity and the ocean-based economy.

Apart from these, climate change-induced natural disasters could cause extensive damage to ecosystems such as mangroves or coral reefs, which are critical for fish reproduction, while also destroying fishery-based economies through property damage or the death of fishermen. More than 850 million people live within 100 kilometres of the coast and are being impacted by changing coastal systems. The impact of climate change on the fisheries sector has significant economic consequences, including the loss of fishery-related jobs and returns. Because the social and environmental stakes surrounding the future of fisheries management are high, traditional techniques centred solely on protecting fish populations have repeatedly failed across both social and environmental dimensions. In 1971, oceanographer Jacque-Yves Cousteau spoke of the need for us to "plant the sea and herd its animals using the sea as farmers instead of hunters." Almost 50 years later, the world is still struggling to adopt the 'sea farmer' lifestyle as we continue to search for the elusive 'sustainable' equation that allows for thriving marine ecosystems and a thriving fishing industry in parallel. There are several aspects that are essential to achieving sustainable fisheries management: scientifically based stock assessment and management advice, regulation and enforcement of access to fisheries and catch restrictions, and regulation enforcement. Drawing the line between sustainable use and overuse in the fisheries sector is a complex undertaking, but countries with fishing waters are responsible for maintaining and sustainably managing their fishing stocks.

If current trends in income and population growth, urbanization, and diets continue, the demand for seafood is expected to rise significantly by 2050, prompting researchers to consider the future role of aquaculture in meeting demand and supporting nutrition needs. For aquaculture, this means a food system that supports public health through the production of diverse seafood, provides multiple, rich sources of essential nutrients, and promotes equity. Aside from food production, rearing endangered marine faunal populations has become increasingly important for these culturing practices. Conservation of their genetic stock, regeneration, and enriched rearing have all become popular aspects of aquaculture. Seafood can also be grown locally to reduce our carbon footprint. If done correctly, ocean farming can be accomplished without the use of electricity, fresh water, or antibiotics.

While many parts of the ocean and coast have been severely degraded as a result of human actions such as coastal development and urbanization, by acting now and becoming 'ocean-responsible,' humanity can turn the tide. Improving aspects of ocean health such as the condition of marine habitats such as corals, seamounts, mangroves, and seagrass beds can benefit other components of the ocean ecosystem including fish stocks and increase resilience to other pressure. There is also a need to vastly improve monitoring to discover the true state of fisheries around the world, as well as better enforce management and regulations that prevent overfishing or illegal activities. Current ocean governance recognizes economic and ecologic objectives, but more attention should be given to social objectives and the benefits that fisheries could provide. With its key stakeholders spread across the globe, finding a point for inclusive participation is critical. This poses a challenge for governments in terms of policy coherence, institutional coordination, and collaboration, to ensure that issues such as decent catch, social protection for fishers, and ocean protection are addressed. All partners and actors should therefore strive to seize those opportunities and rethink fisheries.

According to conservationists, while efforts to reduce overfishing and promote ocean biodiversity through the establishment of marine reserves have generally yielded positive results, the period leading up to policy implementation can be particularly vulnerable. For vast swaths of the ocean, no single owner has exclusive rights and thus must compete against others for extraction. An estimated 7% of the world's ocean is currently classified as marine protected areas (MPAs), a marine spatial management technique that limits human activity. Many of these areas are also declared as marine reserves, a sort of "strict" MPA that forbids any extractive fishing. MPA numbers and coverage have increased during the previous several decades. While the trend of growing MPA declarations has lately slowed, this involves extensive agreements among numerous parties ranging from fishermen to government authorities. The numerous and diverse reasons for adopting MPAs, particularly no-take marine reserves, include habitat conservation, increased abundance of fish inside the marine reserve, and increased productivity and overflow of fish beyond the marine reserve. MPA supporters claim that marine reserves are an important tool in contemporary conservation efforts, and that protecting huge areas of the world's oceans from commercial fishing pressure would allow fragile fish species and whole marine ecosystems to heal and replenish. However, defining the borders of an MPA without jeopardizing fisher livelihoods may be difficult.

The value of marine conservation and the sustainable use of its fishery resources has been recognized as a critical component of sustainable development since it contributes to poverty reduction, food security, and sustainable livelihoods. As part of the UN 2030 Agenda for Sustainable Development, a stand-alone Sustainable Development Goal (SDG) on the protection and sustainable use of oceans, seas, and marine resources for sustainable development (SDG 14) was adopted. The fisheries and aquaculture industry can help secure all of the SDGs, but it is central to SDG 14. Conversely, the Convention on Biological Diversity (CBD) was mainstreamed into SDG 14 implementation by incorporating biodiversity and ecosystem services into the fisheries sector, including as part of the ecosystem approach. The planned effects of this synergy are primarily centred on the creation of new MPAs.

Restricting access to marine resources that provide food or income without considering alternatives or transitional livelihood arrangements contributes to conflict over use of contested marine space. Failure to achieve people's participation in management decisions can lead to a growing divide between those prioritizing marine conservation goals and those prioritizing, for example, food security resilience from the same marine resources. This is a demand for new methods to enable the successful implementation of policy and management rules for

sustainable fisheries and ecosystems, with economic and human conservation as the only way to guarantee that fisheries throughout the globe are sustainable.

Historically, fisheries studies were accomplished using single-stock evaluations, with only modest data necessary. However, with rising demands and possible impact on fishing habitats, as well as climate change challenges, research trends in fisheries should take numerous detours. Protecting the fish population while meeting the planet's protein demands has prepared the way for significant differences in fisheries research messaging. Every day, scientists try to better understand fish ecology, migrations, invasions, and thermal responses as a result of ocean warming, fish dynamics, and other variables. Furthermore, research programs that examine the contributions, implications, and drivers of change for small-scale fisheries are crucial to providing policymakers with policy-relevant understandings and arming activists with vital facts.

The ocean is a three-dimensional volume that changes across time and space as the fourth dimension. An interdisciplinary ocean connection research program is necessary, including greater understanding of the functional relationships that connect components of the marine system, such as physics, chemistry, biology, geology, ecology, and people. As a result, fisheries scientists must approach fisheries management holistically, bringing together the views of various disciplines that have hitherto operated independently of one another. To comprehend, monitor, anticipate, and manage various stresses, extreme events, and a safe operating area for human usage of the four-dimensional ocean, simulation-based ocean technologies, modelling, local data management, and artificial intelligence are required.

Monitoring and monitoring fisheries status on a global/regional/national scale is critical to ensuring their sustainability or, in the event of depleted fisheries, supporting their recovery. Such monitoring and information gathering would aid in educating fishermen and other fishing stakeholders about the repercussions of overfishing and IUU fishing. Government officials can offer enough information on how to use suitable fishing gear while causing little or no harm to the environment. They should be informed about the effects of plastics and hazardous chemical pollution, as well as how they promote biological disintegration, which may influence fish capture quality, quantity, the value chain, and human health. To be a part of the solution, they should be trained and educated in their original languages. When appropriate, assistance with selecting community-based fisheries management, changed livelihood development, women and youth motivation, and community mobilization should be offered.

Conservation scientists working jointly with local fisherman to incorporate their experiences into scientific approaches may develop synergies and result in improved management results. Researchers have shown significant results by merging traditional scientific knowledge with local fishing experiences, particularly in poor nations. Furthermore, fishermen who are exposed to diverse habitats and use several gears have a better understanding of connection patterns within seascapes. Incorporating their ecological observations and hands-on experiences would help them achieve their sustainability objectives more quickly. It is critical to understand what makes each town or country's fisheries sector distinctive, and to urge users to look beyond economic concerns alone.

CONCLUSION

Fishing for the future means making sure we don't catch too many fish now so there will be enough for the future. The world still has a lot of progress to make. It is a difficult task for governments, fishing industry, local communities, and other people involved to overcome the obstacles mentioned earlier and restore fish populations and ecosystems. It is important to find a balance that lets people benefit from fish resources without hurting ocean ecosystems and

aquatic biodiversity. Currently, there are some areas that are doing really well in showing that sustainable fisheries can be achieved. It is important to have a good understanding of how different things interact, how they change over time, and how complex the whole system is. This requires involving everyone from different groups and industries in making policies that can change and adapt. This will support a management approach that combines different elements and can change as needed. A fishery and aquaculture sector that is sustainable can provide enough food for the growing global population and ensure a stable economy. This should be possible not only for the next few decades, but for as long as humans exist. If we have enough knowledge, the right tools, a willingness to try new things, and enough help, we can turn our motivation today into our reality tomorrow.

REFERENCES:

[1] C. Piñeiro, B. Cañas, and M. Carrera, "The role of proteomics in the study of the influence of climate change on seafood products," *Food Res. Int.*, 2010, doi: 10.1016/j.foodres.2009.11.012.

[2] I. P. Malyani and I. Cintamulya, "Analisis Berfikir Kritis Siswa yang Bergaya Kognitif Reflektif dan Implusif pada Pembelajaran Biologi melalui Model Think Talk Write (TTW) dengan Media Limbah Pengolahan Hasil Laut," *Proceeding Biol. Educ. Conf.*, 2017.

[3] S. Takahashi, "Hatchery Flounder Going Wild: Authenticity, Aesthetics, and Fetishism of Fish in Japan," *Food Foodways*, 2014, doi: 10.1080/07409710.2014.895566.

[4] J. Quilter, B. Ojeda E., D. M. Pearsall, D. H. Sandweiss, J. G. Jones, and E. S. Wing, "Subsistence economy of El Paraíso, an early Peruvian site," *Science (80-.).*, 1991, doi: 10.1126/science.251.4991.277.

[5] L. Umamaheswari, K. Omar Hattab, P. Nasurudeen, and P. Selvaraj, "Should shrimp farmers pay paddy farmers?: The challenges of examining salinization externalities in South India," in *Environmental Valuation: In South Asia*, 2009. doi: 10.1017/CBO9780511843938.005.

[6] J. Dias, L. Conceic¸ão, and M. T. Dinis, "The increasing role of aquaculture in protein supply: Can we make it environmentally sustainable?," *Ann. Nutr. Metab.*, 2013.

[7] C. Wang, "Connecting Carbon Capture with Oceanic Biomass Production," *Nat. Preced.*, 2010, doi: 10.1038/npre.2010.5161.1.

[8] G. Gerung, "Seaweeds Resources of Indonesia," in *Seaweed Resources of the World*, 1998.

[9] K. Roy, D. K. Pal, S. Basu, D. Nayak, A. De, and S. Lahiri, "Synthesis of a novel ion exchanger, zirconium vanadate, for immobilising 134,137 Cs radionuclides," in *Environmental Radiochemical Analysis II*, 2007. doi: 10.1039/9781847550781-00017.

[10] P. R. Newswire, "World Marine Biotechnology Industry," *LON-Reportbuyer*. 2015.

CHAPTER 9

ADVANCES METHODS IN FISH DISEASE: DIAGNOSIS, AND THERAPEUTICS

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ABSTRACT:
This chapter gives a thorough summary of current improvements in fish health management. It focuses on three essential areas: illness detection, therapeutic measures, and vaccine development to improve the long-term viability and production of aquaculture and wild fish populations. The chapter opens with a discussion of cutting-edge techniques and technology in fish illness diagnosis, such as PCR-based tests and metagenomics. It investigates the function of bioinformatics in detecting new pathogens and highlights the significance of quick, accurate diagnosis in disease outbreak prevention. The therapeutics section digs into the most recent developments in the treatment of fish ailments. It discusses the usage of antibiotics, probiotics, and immunostimulants, highlighting the significance of practising appropriate medicine practices in order to reduce environmental damage. It also covers novel techniques such as phage treatment and nanotechnology-based medication delivery systems. In conclusion, this chapter is a significant resource for academics, veterinarians, and aquaculture professionals since it provides insights into the most recent tactics for fish illness management, therapeutic treatments, and the establishment of sustainable immunization policies. It emphasizes the significance of taking a comprehensive approach to fish health in order to maintain the global aquaculture industry's continuing expansion and resilience.

KEYWORDS:

Chain Reaction, Disease, Enzyme Linker, Fish Farming, Nester PCR.

INTRODUCTION

In the past 50 years, there has been a big increase in the number of fish and fishing around the world. Aquaculture is a rapidly growing industry that produces a lot of fish. Aquaculture is a big industry worldwide because it provides an important source of animal protein for people. Aquaculture has been growing steadily and it is now the fastest-growing sector for producing food worldwide. In 2018, the world caught around 179 million tonnes of fish. These fish were worth about $401 billion. Out of this, 82 million tonnes, valued at $250 billion, were produced through aquaculture. The amount of fish caught worldwide is expected to reach 196 million tons by 2025. Aquaculture, the farming of fish, is predicted to produce more fish than traditional fishing. By the year 2025, it is estimated that aquaculture will provide about 57% of the fish that people eat all over the world. Most of the increase will happen in poorer countries, where the importance of freshwater species is predicted to rise. India is the third biggest fish-making country and the second biggest fish producer in the world. India produces approximately 7% of the world's fish The country also has a lot of different types of fish, more than 10% of all the fish in the world. It is also one of the 17 countries with a lot of different types of plants and animals. Aquaculture is growing quickly, and people are trying different things to make it produce more [1], [2].

The growth of this industry is being slowed down by more and more fish dying, especially because of contagious illnesses spreading. In addition, the trading of live aquatic animals and their products globally has been the main cause of new outbreaks of diseases in animals. The

animals that are being cared for and raised in controlled environments are being exposed to different types of environmental pressures. These pressures can often cause diseases to develop, new pathogens to appear, and for those pathogens to spread to other animals. When fish are in stressful conditions, their immune system becomes weaker, which can lead to them getting sick in fish farms. If we don't have a plan to control diseases that spread easily, the aquaculture industry's demand may keep growing and contribute to too many wild fish being caught. It is very important to have better and faster tests to diagnose problems quickly in order to have a sustainable future for fish farming. We need new and clever ways to create tests that can quickly and correctly detect fish diseases in the field. We also need to find ways to prevent and treat these diseases in aquaculture. We can use molecular techniques to solve those types of problems and make pathogen detection more sensitive and specific. This section investigates how modern biotechnology tools are used to diagnose diseases and promote sustainable aquaculture development [3], [4].

Aquaculture is becoming more focused on raising and selling fish and other aquatic animals in a more controlled and intensified way. Similar to other types of farming, when aquaculture activities become more intense and grow, there is a higher chance of major disease issues occurring. Disease problems are a big worry in fish farming. The cost of production goes up because we lose money on animals that don't survive, we have to spend money on treating sick animals, and the amount and quality of what we produce goes down. So, the fish farming industry has had a lot of diseases and issues caused by viruses, bacteria, fungi, parasites, and other unknown and new pathogens. Many aquatic species are facing a major problem with diseases, which is stopping their growth and causing problems for the economies and societies of many countries. Disease outbreaks are strongly affected by how easily hosts can get sick, how strong the pathogens are, and bad environmental conditions. Intensive and semi-intensive farming methods make it easier for diseases to spread because there are a lot of animals in a small space, which causes stress and not enough water gets replaced.

Many different parasites and germs can infect fish. Most germs are found in small amounts naturally and usually do not cause issues. Fish have ways to protect themselves from getting sick. They have a special layer of mucus that covers their skin and helps keep disease-causing agents away. They also have a strong immune system that fights off any harmful germs that may come their way. These defense mechanisms work together to keep the fish healthy and protected. But when fish that are already crowded in fish farms are put under more stress (like not having enough oxygen, not getting proper food, and being handled too much), their natural defenses against diseases may become weaker and they may not be able to protect themselves against infections as well. Therefore, fish are more likely to get sick from different types of germs like parasites, worms, small creatures with hard shells, bacteria, fungus, and viruses. Severe illness causing a large number of deaths often occurs as a result of, and in reaction to, a very difficult and challenging experience. Most illnesses can be prevented if things are controlled properly. A list of common diseases that spread among fish in India [5], [6].

These tiny organisms are basically harmful germs that enter the body of a fish and make it sick if it is already weak due to stress. Some types of bacteria, like *Aeromonas species, Aeromonas salmonicida, Vibrio spp., Edwardsiella ictaluri, Edwardsiella tarda, Strep tococcus spp.*, and other similar bacteria, can cause illness in fish farming. Gram-negative bacteria cause diseases in fish farming. These diseases are a big problem and limit the production and trade of fish. They also affect the economic development and revenue of the fishing industry in many countries. The current trend is that harmful bacteria like *Aeromonas spp., Flavobacterium spp, Pseudomonas spp,* and *Shewanella putrefaciens* that are usually harmful to fish, are being replaced by other types of bacteria that have not previously been known to harm or cause

diseases in fish. Currently, *Acinetobacter spp., Plesiomonas shigelloides, Sphingomonas paucimobilis,* and *Stenotrophomonas maltophilia* are the most commonly found species in sick fish. Meanwhile, in Central Europe, there are infections caused by a type of bacteria called *Aeromonas spp.* These diseases, called motile *Aeromonas septicemia* (MAS), motile Aeromonas infection (MAI), and furunculosis, are the most common ones that fish can get from bacteria. *Flavobacterium spp*. can cause infections. These events are seen frequently [3], [4].

There have been reports of a widespread infection called Vibrio hemorrhagic septicemia or vibriosis in cultured groupers in several countries including Brunei Darussalam, Malaysia, Taiwan, Indonesia, Kuwait, Thailand, Singapore, and the Philippines. The germs that cause vibriosis are bacteria called Vibrio, which are negative in the Gram staining process. Some examples of these bacteria are *Vibrio parahaemolyticus, Vibrio alginolyticus, Vibrio vulnificus, Vibrio carchariae, Vibrio anguillarum, Vibrio ordalii, Vibrio harveyi, Vibrio mimicus,* and others. These bacteria belong to the Vibrionaceae family. There are more cases of furunculosis infection caused by A. The bacterium *salmonicida* is a type of germ that mainly affects fish like salmon, trout, charr, and grayling. It is a small, non-moving rod-shaped bacterium that is commonly found. But these diseases do not matter much in Indian fish farming.

Unlike bacterial diseases, fish farmers are very worried about parasitic infestations. The growth of fish in the culture system is affected by different parasites that infest them. Fish parasites can quickly increase in number when the conditions are good for them. This can make the fish sick and often leads to many of them dying. Parasites affect how the host's body gets nutrients, mess up how the body uses energy, and harm the digestive system and nervous system. Different types of parasites, such as tiny organisms called protozoan ciliates and monogenetic trematodes, as well as larger crustaceans that live on the outside of fish, can cause diseases in fish. Some examples of these parasites include Ichthyophthirius sp., Trichodina sp, Dactylogyrus sp, and *Gyrodactylus sp. Lernae spp, Argulus spp,* and the freshwater louse (Ergasilus) cause a lot of money loss in fish farms in India. Finding out what germ is causing a disease is an important part of diagnosing and treating it. During disease outbreaks in aquaculture, it is important to quickly identify and remove infected fish. This is necessary to effectively control and manage the health of the fish. Scientists have regularly and thoroughly used classical, immunological, and molecular methods to study this topic.

There are many ways to find harmful germs in fish and the water they live in. This refers to different ways of diagnosing illnesses, which can be either traditional or modern methods. However, sometimes it is necessary to use multiple methods to determine the exact disease a person has. Traditional methods are ways that have been used for a long time. They start by growing and separating the disease-causing organism from infected tissue and then figuring out what type of organism it is. His tology means studying tissues and histopathology means studying changes in tissues caused by disease. Biochemical tests are used to analyze chemicals in the body. Microscopy is the use of a microscope to see things that are too small to see with the naked eye. Electron microscopy uses an electron microscope to see things in even greater detail. These tools are commonly used to diagnose diseases. Two organizations, OIE and FAO/NACA, suggest using three different ways to diagnose emerging and reportable diseases in fish and shellfish. All three levels of diagnostic specialization are needed to confirm newly or rarely seen diseases. There are different ways to diagnose a disease. This is crucial because it is the foundation for activating the other diagnostic levels.

This level includes the study of parasites, diseases in tissues, bacteria, and fungi. It needs a moderate amount of money and training to specialize in these areas. This is the level where experts use specialized technology and need a lot of money and training. Isolation of the

bacteria, fungus, and virus that cause diseases, identifying their characteristics, using laboratory tests like enzyme-linked immunosorbent assays or fluorescent, antibody technique, using advanced microscopes to study them, and using molecular tests like polymerase chain reaction (PCR) and nucleic acid assays with specific probes, all fall into this category. Serological techniques are all about studying how antigens and antibodies react outside of a living organism in a lab. This is called serology and it is a crucial part of diagnosing diseases caused by microorganisms in a clinical setting. Immunodiagnostic tests are used to find and identify a particular substance related to a disease-causing germ by using a reaction between a specific type of molecule. Scientists were able to quickly find and name tiny living things without growing them in the lab thanks to a new method called immunoassays. This method relies on a special connection between a substance in the living thing called an antigen and another substance called an antibody.

The tests can find either the antibodies that are made when the body reacts to a specific substance or the substance itself. Antigens and antibodies react in a very specific way. An antigen will only react with antibodies that were made by its own body or by a similar antigen. Because antibodies and antigens are very precise, they can be used to identify each other. The main thing to know about serologic methods is that they can tell us if animals have had a disease before, but they can't tell us if they are currently infected. The immunoassays can be divided into different groups based on the type of detection systems used. These groups include color detection, radiation detection, chemiluminescence detection, and fluorescence detection. This method is being used in many areas of bacteriology and can be found in various forms, like enzyme immunoassays, immunofluorescence assays, latex agglutination assays, line immunoassays, and lateral-flow immunoassays. Monoclonal antibodies are perfect standardized substances that can be used in these methods, and you can now buy many of these products [7], [8].

DISCUSSION

Latex agglutination and agglutination tests Agglutination responses are among the simplest immunological assays to conduct. Their use in the presumptive identification of bacteria has long been known, and their applicability to bacterial fish diseases has been proven and reported. It is feasible to validate the identification of the majority of known bacterial fish pathogens with as little as a dozen antisera. Whole-cell agglutination with polyclonal antisera has been widely employed to identify cultures with varying degrees of accuracy depending on the specificity of the antiserum. On a microscope slide, a culture is mixed with antiserum in the presence of saltwater, and clumping of the bacteria indicates a positive result within 2 minutes. The agglutination test is commonly used to identify bacterial fish pathogens from the genera *Vibrio*, *Pasteurella*, *Aeromonas*, *Yersinia*, *Edwardsiella*, and *Pseudomonas*. Antisera/antibodies were adsorbed onto latex particles in latex agglutination, resulting in sensitive latex. On a glass or plastic surface, pure or mixed cultures or pathological material containing pathogens are combined with the reagent. Within minutes, clumping of the latex particles indicates a good response. The latex agglutination test is used to detect specific antibodies or antigens in body fluids such as saliva, urine, cerebrospinal fluid, or blood.

Assays for immunofluorescence: These tests are used to identify antigens of particular disease agents inside infected tissues or cells and to see the antigen in the cell when it binds to a fluorescent-labeled antibody. Finding bacteria in fish tissue is simpler than determining viral infections using a fluorescent antibody test (FAT). The FAT takes use of the fact that some dyes fluorescein isothiocyanate, rhodamine isothiocyanate, and Texas red) glow when exposed to ultraviolet (UV) light. Immunofluorescence may be observed directly or indirectly and occurs when both antigen and antibody are present. An antigen-specific antibody labelled with

a fluoro chrome fluorescein; rhodamine is required for a direct test. A fluorescent labelled antiserum to an agent-specific immunoglobulin is required for an indirect test. The benefit of this technology is that it is particularly sensitive and fast for detecting and identifying disease agents in smears, tissues, or cultures; however, the downside is that it needs the creation of fluorescent-labeled antibody or antiserum. The findings might be subjective, resulting in false-positive or false-negative outcomes. Cross-reaction with another antigen is possible.

Assay for enzyme-linked immunosorbent (ELISA) is one of the most potent immunochemical procedures available. It utilizes a variety of technologies to detect and quantify antigens or antibodies, as well as to examine antigen structure. This method employs antibodies to which enzymes have been covalently linked, preserving both the enzyme's catalytic capabilities and the antibody's specificity. Peroxidase, alkaline phosphatase, and beta-galactosidase are examples of linked enzymes that catalyze reactions with coloured products that can be quantified in extremely low levels using an ELISA reader. There are several methods in which immunoassays may be done. Indirect, direct, and competitive ELISA designs are the most common, however these ELISA designs may be mixed. The antigen of interest is purified in indirect ELISA, and the presence or concentration of antibodies against it is identified or quantified. The presence or concentration of the antigen of interest is identified or assessed in direct or antigen capture ELISA. The presence or quantity of antibody is detected or evaluated in a competitive ELISA, inhibition-type test. Sandwich ELISA detects or measures the presence or concentration of antibodies. Dot-ELISA is a popular immunological technique in both research and analytical/diagnostic labs. The antigen is sandwiched directly between two antibodies that respond with two separate epitopes on the same antigen in sandwich Dot-ELISA [9], [10].

One of the antibodies is immobilized on a solid support, while the other is coupled to an enzyme. The immobilized antibody interacts with the antigen in the test sample first, followed by the second enzyme-linked antibody. In order to determine the quantity of enzyme-linked antibody bound, the strip is incubated with an appropriate chromogenic substrate, which is transformed to a coloured, insoluble product. The latter precipitates onto the strip in the region of enzyme activity, giving rise to the term Dot-ELISA. The intensity of the spot, which is directly proportional to the antigen concentration, indicates enzyme activity. The ELISA test has a high throughput and may be automated. This technique is especially helpful for identifying and quantifying microorganisms during clinical illness, but its sensitivity limitations make it less suitable for subclinical infections. Many bacterial illnesses, such as A. salmonicida in fish, *V. parahaemolyticus* and *V. harveyi* in penaeid shrimp, and viral infections, such as viral hemorrhagic septicemia virus (VHSV) and striped jack neurological necrosis virus in fish, have been detected using the ELISA test. These immunoassays have a wide range of applications for detecting exotic pathogens in cultured finfish, such as channel catfish virus (CCV), infectious hematopoietic necrosis virus (IHNV), infectious pancreatic necrosis virus (IPNV), VHSV, viral nervous necrosis virus (VNN), and bacterial kidney disease (BKD).

Western blotting is a quick and sensitive method for detecting and characterizing proteins. Western blot and dot blot are not often used in fish diagnostics, although they may be beneficial in certain cases. The approach enables the identification of individual proteins by leveraging the specificity inherent in antigeneantibody recognition. Running sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) on a nitrocellulose membrane reveals proteins as separate bands. When serum is administered, the serum's antibodies attach to the proteins on the membrane. Capture antigens, such as proteins, are electrotransferred to a nitrocellulose membrane using this approach. If the material contains target antibodies, they will attach to the antigens on the nitrocellulose strips. A sequence of reactions involving goat

antihuman IgG conjugated with biotin, avidin conjugated with HRP, and the HRP substrate are used to see the antibodies bound to antigen. The antigen-specific bands will be seen on the nitrocellulose strip. These immunodiagnostic techniques are now used to screen for and/or confirm the diagnosis of a variety of fish disorders.

This approach has the benefit of being the most specific viral diagnostic test available, since it offers a complete antibody profile against the illness culprit. The downside is that reagents like monoclonal or polyclonal antibodies are required to execute the experiment. Assay for lateral-flow diffusion This approach is a relatively new serological test, developed more for antigen-specific immunoassay than antibody detection. It creates a visible line in the capture zone when there is a nitrocellulose or nylon membrane by using colloidal gold, carbon, paramagnetic, or coloured latex beads. Lateral-flow tests have been commercially accessible. Typically, the tests are developed using nitrocellulose or nylon membranes housed in a plastic or cardboard container. A capture antigen is linked to the membrane in the antibody detection format, and a second labelled antibody is put on a sample application pad. As the sample migrates down the membrane due to capillary action, the antibody present binds to the labelled antigen and is collected as the complex passes. For a positive result, colloidal gold, carbon, paramagnetic, or coloured latex beads are often employed particles that generate a visible line in the capture zone of the assay membrane. These tests are easy to use, need no training, and have no specific storage requirements.

Once confirmed, this approach has the potential for future usage, notably in detecting pre-exposure of fish to viral infections. Molecular fish disease diagnostics Because of the use of biotechnology in aquaculture, a broad variety of immunodiagnostic and molecular technologies have been developed and improved, and reagents and commercial kits are now more widely accessible. Method development has recently risen significantly as clinical and veterinary care procedures are adopted and enhanced for application in aquaculture. Fish illness diagnosis has developed from old methodologies of pathogen isolation and phenotypic characterisation to current molecular biology methods. Traditional diagnostic approaches are sometimes costly, labor-intensive, and time-consuming, and they may not result in a definitive diagnosis, especially when compared to histology evidence. Immunological procedures are often unable to identify pathogens because the quantity of pathogen present in the sample is less below the assay's sensitivity threshold, or the antigens on the pathogen have been changed and are no longer recognized by the antibody. In regular microbiology labs, nucleic acid-based diagnostics are progressively replacing or supplementing culture-based, biochemical, and immunological methods.

The detection and identification of microorganisms in a range of freshwater, brackish water, and marine samples is the primary duty of all diagnostic bacteriology labs. Again, such molecular approaches make detection and characterisation of unculturable and slow-growing diseases simpler [7,36]. Aside from great sensitivity and the ability to perform quick diagnostics, the major benefit of molecular techniques is the ability to identify uncultivated infectious organisms. Pathogenic bacteria may be identified by deoxyribonucleic acid (DNA) amplification in extremely minute levels, even in the smallest sample volume. Molecular diagnostics are currently the standard regular procedures used in all laboratories. Such procedures are required for any research-based or clinical-diagnostic reasons that include the use of molecular constituents of the cell. Repeated revisions have been necessary in order to enhance with continuing research and assist the future of diagnosis. With the development and availability of improved automated devices, the approach to illness diagnosis has gone another step forward. Molecular diagnostic technologies allow for the detection of infection in its latent or acute stages.

These approaches enable the differentiation of diseases with similar antigenic structures. Recent advancements in detection technologies have paved the way for the development of multiparameter assays based on macroarrays or microarrays, while the introduction of closed-tube real-time PCR systems has resulted in the development of rapid microbial diagnostics with a lower risk of contamination. The most important molecular diagnostic procedures are PCR-based molecular diagnostic methods such as PCR, real-time polymerase chain reaction (RT-PCR), multiplex polymerase chain reaction (multiplex PCR), and so on. These molecular technologies may be utilized for genotyping, determining antibiotic resistance, and performing microbial fingerprinting in addition to detection and identification of microbial pathogens. The polymerase chain reaction (PCR) The development of nucleic acid amplification technologies that allow the multiplication of a few target molecules to detectable levels has resulted in the development of new tools for the rapid, specific, and sensitive detection, identification, and resistance testing of microorganisms starting from sample material without culturing. The PCR method is the most often utilized in research and diagnostic facilities. Kary Mullis created the PCR process in the 1980s, which was a significant and groundbreaking procedure. It is a quick and inexpensive approach to duplicate or amplify tiny regions of DNA. One of the most basic applications of PCR is the identification of infections by amplifying a specific stretch of DNA. A two-step or nested PCR or nested reverse transcriptase PCR (RT-PCR), random amplified polymorphic DNA, reverse cross-blot PCR, real-time PCR, and an RT-enzyme hybridization experiment are all versions of this PCR method [26]. PCR offers a broad range of applications, including ancient DNA research from fossils, DNA fingerprinting, mapping the human genome, and detecting microbes in low quantities in water, food, soil, or other systems.

Kary Mullis discovered the PCR in 1983. PCR is an in vitro and basic type of DNA replication, which is a physiological process utilized by all live cells to copy their genetic material prior to cell division. In theory, the rise in product quantity after each cycle will be geometric. Every PCR cycle, the quantity of DNA doubles and is repeated in the next cycle, and a fresh strand of DNA functions as a template for replication. This causes an exponential rise in the number of targeted DNA segments during PCR. Depending on the predicted yield of the PCR products, 25e40 PCR cycles are performed. It has a shorter cultivation time and produces results more faster than culture. The test has also been used to identify *Vibrio penaeicida* and *A. salmonicida* subspecies salmonicida in shrimp. The fundamental necessity of PCR is to construct particular primer sequences for important target genes that may describe and distinguish distinct strains/species of organisms or animals. To fully harness the potential use of PCR technology in diagnostics, work on detecting particular target genes for distinct fish infections is required. Nested and heminested PCRs are intended to improve PCR sensitivity by reamplifying the result of a first PCR with a second PCR. Internal to the first primer pair are nested PCR primers.

The bigger fragment from the first round of PCR serves as the template for the second round of PCR. Nested PCR may also be conducted with a single nested primer and one of the initial primer pairs. The second round of PCR in heminested PCR employs one of the first-round primers and one new, internal primer. The second round of PCR produced a shorter amplicon than the first. The sensitivity and specificity of DNA amplification might be considerably increased using this method. The benefit of nested PCR is that it substantially increases the sensitivity and specificity of both DNA and RNA amplification. Because this approach virtually always removes any erroneous nonspecific amplification products, specificity is greatly improved. The disadvantages of nested PCR include the additional time and expense involved with two rounds of PCR, as well as the increased potential of contamination during the transfer of first-round amplification products to a second tube. PCR multiplexing Multiplex PCR (mPCR) refers to the use of polymerase chain reaction to enhance a number of different DNA configurations at the same time. Multiplex PCR incorporates two or more primer sets

designed for the expansion of several targets into the same PCR process. Using several primers and a temperature-mediated DNA polymerase, this approach amplifies DNA in assays. All primer sets' preparatory plans should be tuned so that they can all function at the same annealing temperature during PCR.

Because DNA polymerase needs a double-stranded DNA template, RNA must be transcribed into complementary cDNA by the enzyme reverse transcriptase prior to PCR because Taq has low reverse transcriptase activity. This technique of RNA amplification is known as reverse transcriptase polymerase chain reaction (RT-PCR). Moloney murine leukemia virus reverse transcriptase and Avian myeloblastosis virus reverse transcriptase are two reverse transcriptase enzymes extensively utilized in RT PCR. Both enzymes perform the same basic functions but vary in other aspects, such as optimal temperature and pH. These enzymes are available in RT-PCR kits that have been pre-optimized. The reverse transcriptase enzyme and a thermostable DNA polymerase are combined in a single tube for synthesis and amplification of the target RNA sequence in one-tube RT-PCR. For routine analysis, this is the recommended method. Commercial RT-PCR kits are available, and reagent mixtures may also be produced from different component component. There are several types of primers that can be used to make cDNAs: oligo-dT will prime cDNA synthesis on all polyadenylated RNAs, random primed cDNA synthesis gives a wide range of cDNAs and is not limited to polyadenylated RNAs, and oligonucleotide primers complementary to the RNA of interest can be used to make highly specific cDNAs. RT-PCR is often used to identify RNA shrimp viruses that have wreaked havoc in aquaculture. RT-PCR was performed by many studies to identify Tilapia Lake Virus in fish, and the assay was shown to be highly sensitive and specific.

CONCLUSION

This text summarizes the main points and emphasizes the importance of the progress talked about in the previous chapters. In this part, the chapter gives a brief overview of the most significant progress made in diagnosing and treating diseases in fish, which are discussed in the book. This text looks again at the important ways of doing things, technologies, and approaches that have become very important in the field. The chapter tells us that it's really important to combine research from different people and chapters in the book. It talks about how these shared ideas help us better understand how to take care of fish. It is important to solve the problems and drawbacks of the methods used currently. This chapter recognizes that there are still things we don't know and ways that technology can be improved. It shows where more research needs to be done. It also shows possible future ideas and advancements. The last chapter highlights the real-life effects of the progress talked about in the book. This text shows how these ways can be used in real-life situations to make fish populations healthier and more sustainable. This includes both fish farms and natural habitats. The chapter might have final thoughts from the people who wrote or edited the book. They will share their ideas about what might happen in the future with diagnosing and treating fish diseases. It can also recognize the teamwork that went into making the book. At the end, the chapter might ask researchers, veterinarians, policymakers, and industry professionals to accept and improve the discussed methods to handle the changing problems in fish health management. In simple terms, the last chapter of the book gives a complete summary of what the book talks about, emphasizes its importance, and encourages people to keep working to improve the way we diagnose and treat fish diseases.

REFERENCES:

[1] T. Haferlach, A. Kohlmann, W. Kern, W. Hiddemann, S. Schnittger, and C. Schoch, "Gene Expression Profiling as a Tool for the Diagnosis of Acute Leukemias," *Semin. Hematol.*, 2003, doi: 10.1016/S0037-1963(03)00193-8.

[2] H. Pretorius, H. Schumacher, T. Quistorf, N. Richards, and E. Budke, "P4–270: Omega-3 fish oil with purified eicosapentanenoic acid stimulates cerebral perfusion," *Alzheimer's Dement.*, 2013, doi: 10.1016/j.jalz.2013.05.1663.

[3] S. Naseem, "Newer flow cytometric markers in CLL: Utility in diagnosis and prognosis," *Indian J. Hematol. Blood Transfus.*, 2013.

[4] H. R. F. COLBACK, "Veterinary Report for 1939.," 1940.

[5] H. Ferguson, "Program and abstracts of the sixteenth Annual Meeting of the American Society of Parasitologists, Philadelphia, Pennsylvania, December 30 and 31, 1940, and January 1, 1941.," in *Journal of Parasitology*, 1940.

[6] P. E. Sax *et al.*, "Demographic properties of an outlier population of," *J. Infect. Dis.*, 1998.

[7] N. N. Khatib, M. Raheesa., Konanavar, "Modern Identification Methods of Bacteria," *Research & Reviews: Journal of Agriculture and Allied Sciences*. 2014.

[8] N. Halaihel *et al.*, "A new real time PCR-based assay for diagnosing Renibacterium salmoninarum in rainbow trout (Oncorhynchus mykiss) and comparison with other techniques," *J. Microbiol. Methods*, 2009, doi: 10.1016/j.mimet.2008.09.014.

[9] P. Ciaurriz, F. Fernández, E. Tellechea, J. F. Moran, and A. C. Asensio, "Comparison of four functionalization methods of gold nanoparticles for enhancing the enzyme-linked immunosorbent assay (ELISA)," *Beilstein J. Nanotechnol.*, 2017, doi: 10.3762/bjnano.8.27.

[10] E. Engvall and P. Perlmann, "Enzyme-linked immunosorbent assay (ELISA) quantitative assay of immunoglobulin G," *Immunochemistry*, 1971, doi: 10.1016/0019-2791(71)90454-X.

CHAPTER 10

EXPLORING THE FISH VACCINE TYPES: DISEASE TREATMENT AND AQUACULTURE SUSTAINABILITY

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ABSTRACT:
This chapter talks about different types of vaccines used to treat and prevent diseases in fish populations. The main goal is to make aquaculture practices more sustainable. Inactivated vaccines don't make you sick but still help your body's defenses. This text is about studying how vaccines are made for fish, when they should be given, and how well they work to protect fish health. Subunit vaccines are vaccines that use specific parts of the virus or bacteria to create an immune response. They focus on using antigens, which are proteins or peptides, to develop the vaccine. This chapter looks at how accurate and safe subunit vaccines are in targeting specific diseases in fish. DNA vaccines are a new way to protect fish from diseases. They work by giving fish genetic material that encodes antigens, which help their bodies fight off infections. This chapter explores how DNA vaccination works, its benefits, and the difficulties it poses. Live attenuated vaccines are studied because they can simulate natural infections but are not as harmful. This section talks about choosing weaker strains and the dangers that come with it. Recombinant vector vaccines are discussed in the chapter. These vaccines use viral carriers to send substances called antigens into fish cells. It studies how well this method can make someone immune to something. The importance of using adjuvants and advanced delivery systems to make vaccines work better is emphasized. The chapter talks about different things that help fish vaccines work better, like substances called adjuvants and special ways to deliver the vaccines. To sum up, this chapter gives a detailed explanation of the different types of vaccines used to treat and prevent diseases in fish farming. This text highlights the importance of vaccines in supporting fish farming, preventing losses due to diseases, and maintaining the overall health and productivity of aquatic ecosystems.

KEYWORDS:

DNA Vaccine, Fish, Gene Expression, Immune Respondse, Vaccine.

INTRODUCTION

Different types of modern vaccines include vaccines made from whole cells that have been killed or inactivated, vaccines made from live but weakened cells, vaccines made from DNA, vaccines made from synthetic peptides, vaccines made from genetically engineered substances, and vaccines made from specific subunits. Vaccines that are given to the whole organism worked better than other types of immunizations. But most immunizations don't completely prevent sickness. An inactivated or killed vaccine can be made from completely inactivated cells or certain parts taken from harmful bacteria using physical, chemical, or biological methods. There are three types of vaccines: ones made from whole cells that have been destroyed, ones made from parts of the virus that have been inactivated, and ones that protect against multiple viruses. The harmful germ is killed and used in dead bacterial vaccines to help the immune system. These vaccines are created by growing the harmful bacterium in ideal conditions and then destroying it using a chemical called formalin. For vaccinating fish, either the entire bacterial cell or a specific amount of inactive cells will be used. In simple words, because inactivated harmful bacteria have not been changed, they still have their original features that can trigger an immune response [1], [2].

Most bacterial vaccines used in aquaculture are made from specific strains of bacteria that have been grown in a liquid and then treated with a chemical called formalin to make them inactive. Many studies have shown that these vaccines are effective. The way in which vaccines work and how long they provide protection can be influenced by different substances used in their creation. Most of the vaccines used in fish farming today can kill the fish. Creating vaccines to kill viruses has been too expensive and takes too much time to be considered good enough. A type of vaccine made from proteins or sugars could be used in the fractional inactivated vaccine. The harmful bacteria are usually killed using a chemical called formalin in some vaccines. Only the harmless parts of the bacteria are taken out and used to make the vaccine. To fight against a serious illness called red sea bream iridoviral disease, which causes a lot of death in farmed marine fish, there is a vaccine that can be injected to help prevent it. In Japan, this vaccine is called the "Formalin-inactivated Iridovirus *streptococcosis-vibriosis* combined vaccine" and is used to protect marine fish [3], [4].

However, inactivated vaccines may only give long-term protection when adjuvants are added to make the vaccine stronger and more effective. Oil-based substances were added to salmonid vaccines in the 1990s to help prevent infectious diseases, which led to a significant reduction in disease outbreaks. The great success and long-lasting protection provided by water-in-oil emulsion adjuvants have been very important for the growth of salmon farming. In Japan, animals raised in the ocean are commonly given vaccines through injections. They have been effective against bacteria, like L. Yellowtail can get sick from garvieae infection and red sea bream can get sick from iridoviral infection. These infections can change how often the diseases happen. Fish can be protected from important bacterial infections by giving them vaccinations that can be bought. A type of vaccination called A is available for these infections. The bacteria called salmonicida, anguillarum, ruckeri, and salmoninarum can affect fish. *Flavobacterium columnare* and *Piscirickettsia salmonis* are types of bacteria. L is an unknown abbreviation that needs more information. Psychrophilum is a type of bacteria. Streptococcus iniae is a type of bacteria called *P. Damselae* subspecies Garvieae, Pis a type of bacteria. *Damselae* subspecies Piscicida is a substance that can harm or kill fish [5], [6].

These subunit vaccines are beneficial because they only contain parts of the virus that cause an immune response. They do not cause illness because they cannot grow or reproduce in the body. However, due to the limited number of recognizable parts of the virus, subunit vaccines are not as effective at triggering a strong immune response compared to vaccines made from dead or live whole cells. A polyvalent vaccination is a vaccine that protects against many illnesses that a certain kind of fish is prone to getting. This kind of vaccine needs to include all of the main types of each germ that can cause diseases in a particular area. Polyvalent salmon vaccines have multiple strains of *Vibrio species* and *A. Salmonicida* antigens can also be easily obtained. There are different types of commercial *V. Anguillarum* vaccines have been made to be used by bathing or injecting. Most of these vaccines contain only O1, or a mix of O1 and O2a serotypes. Different types of oil-based vaccines include different combinations of V. The text can be rewritten as: Another illness called V can be found along with *anguillarum. Ordalii*, V Rewrite this text in simple words: Ordalii, V. Translate the text to simple language: Ordalii, V. Salmonicida and A are two things. Baumannii is a type of bacteria. Salmonicida, Moritella viscosa, and IPNV can also be used in salmon by injecting them into their abdomen [7], [8].

When making polyvalent vaccines, either bulk numbers of monovalent antigens are manufactured and then combined as a phase in the manufacturing process, or the antigens are synthesized separately, maintained separate, and then blended before injection. ICTHIOVAC VR/PD is a Pasteurella/Vibriosis vaccine with four antigens P. damselae subsp. piscicida, *Listonella anguillarum* serotype O1, *Listonella anguillarum* serotype O2a, *Listonella*

anguillarum serotype O2b). AQUAVAC VIBRIO, a fish vaccine that covers both biotype I and biotype II strains of the bacteria *V. anguillarum*, is also available for purchase. These immunizations have been shown to be especially effective when delivered orally or intravenously. In terms of effectiveness, polyvalent vaccinations beat monovalent vaccines; nevertheless, caution must be used in the formulation of polyvalent vaccines because antigen competition may occur, especially when these vaccines are administered through injection. A whole-cell culture of Yersinia enterica vaccine that has been formalin-inactivated is commercially available. Ruckeri serovar I, Biotype 1 Hagerman strain) was shown to be quite effective.

One aqueous live immunization against R. Novartis' salmoninarum has been licensed for BKD prevention once again under the trade name Renogen. The first commercial E. coli bacterins were created to treat catfish intestinal septicemia. Although ictaluri has been licensed for use by immersion or oral routes, killed vaccines have been shown to be ineffective. An E. coli strain that has been attenuated and lacks the O antigen. An ictaluri strain was created after a single bath immersion in 9e14 days old channel catfish without booster vaccination [89]. It was safe and provided high long-lasting acquired immunity. This had an effect on live E. Since 2000, Intervet Inc. has been manufacturing ictaluri vaccine under the trade name AQUAVAC-ESCO, which is the first licensed bacterial live vaccination in aquaculture manufactured with an attenuated pathogenic strain. Inactivated vaccines usually need many doses. In general, the first dose does not generate protective immunity, but rather 'primes' the immune system. The immune response in this context is primarily humoral, albeit the antibody titer declines with time.

A second booster dose must be provided 3e4 weeks after the original dose to establish a protective immune response in fish that will be cultured for a prolonged length of time. Adjuvants are typically used in conjunction with inactivated immunization to produce a stronger and longer-lasting immunological response, which boosts antigen presentation to fish immune cells and extends the period of protection. Although several furunculosis bacterins have been used in salmonids by injection, immersion, or oral administration, their efficacy has been questioned owing to a lack of reproducible results and/or a short protective duration. The best protection results in salmonids were obtained with mineral oil adjuvanted vaccines, however weight gain was hampered. To avoid these concerns, nonmineral oil-adjuvanted vaccines have recently been developed and commercialized. These vaccines offer the following benefits they are easy to make, they are stable in storage, they are less expensive, and there are no virulence issues. These vaccines are designed in such a manner that they primarily target germs' exterior surfaces or inner sections while keeping the ability to grow when administered to the host. IHNV, *A. V. salmonicida* and *V. salmonicida* are two infections that may be prevented by using killed immunizations. These vaccines are popular in salmon and trout, with a growing market in sea bass, sea bream, tilapia, turbot, halibut, yellowtail, cod, and other species. The vast majority of commercially available vaccines are whole-cell killed. Despite the prevalence of numerous diseases in aquaculture and the ease of delivery, no efforts are being made to develop multivalent inactivated vaccines for fish infections.

Live-attenuated immunizations are made from live bacteria that have lost their virulence after being treated and no longer have the ability to cause significant fish sickness. These are created by putting organisms to a variety of laboratory passes as well as physical and chemical attenuation. Chemical therapy, molecular change of the virulence gene, such as gene depletion or point mutations on many genes, and serial passage culturing utilizing tissue culture conditions might all be utilized to attenuate harmful bacteria without killing them. Although live attenuated germs do not generally cause sickness, they must proliferate in the vaccinated

fish. A live-attenuated vaccination should elicit the same immune response as a natural disease. Vaccination with an attenuated vaccine is a simulation model of a disease, and antigen dissemination in the population would occur over time if vaccinated fish could distribute the vaccine strain. These vaccinations have the added advantage of significantly increasing cellular immunity. They are also capable of stimulating humoral and mucosal immunity.

Hydrophila isolates were obtained by continuous passage on braineheart infusion agar over an 8-year period. They discovered that the immunogenic capacity of these variations was unaffected and that they might be employed as vaccine candidates for fish vaccination to manage MAS illness. Agrinnovate India Ltd., New Delhi, is already commercializing the vaccine. In the United States, a live-attenuated vaccine against BKD, intestinal septicemia of catfish illness, and columnaris disease has been approved for commercialization, and one viral vaccine for Koi herpesvirus for carp has been approved in Israel. Similarly, an attenuated viral vaccination against carp virus spring viremia is utilized in China by immersion. Live vaccinations have been demonstrated in laboratory trials to be efficacious in fish. They stimulate mucosal, cellular, and humoral immune responses. Without causing any clinical symptoms, the attenuated bacterium multiplies in the intended host. The primary worry with utilizing modified live vaccinations is safety. Several research groups have argued that administering attenuated or avirulent versions of the virus may be undesirable because residual virulence in targeted species may disseminate virulence to nontarget species.

In terms of management convenience, live-attenuated vaccines must be kept at low temperatures to be alive. As a result, appropriate storage should be seriously addressed when transporting and supplying vaccinations across long distances. Recent breakthroughs in vaccine creation in recent years, gene sequences from bacterial, viral, and metazoan genomes, together with knowledge of gene functions and derived proteins, have led to the development of innovative approaches for fish immunization. With the advent of biotechnology, recombinant vaccines were developed, in which just the immunogenic portions of a pathogen were produced in heterologous hosts and employed as vaccination. Recombinant proteins have been shown to provide effective protection against a variety of human and animal infections as vaccine antigens. The following are recent advances in vaccination technology. Vaccines based on DNA. Vaccines that are recombinant. Technology based on vectors. Pathogens that have been genetically modified. Vaccine based on synthetic peptides. Deoxyribonucleic acid vaccines DNA vaccines are new forms of vaccinations that have emerged as a consequence of advances in molecular biology. Because conventional oil-based vaccinations have comparable efficacies to bacterial DNA vaccines and there are no viable oil-based vaccines for viral infections, attempts are currently being made to produce DNA vaccines against fish viral pathogens. Despite significant investment in the research of DNA vaccines to protect veterinary animal species and people against viruses, only a handful have made it to the market.

To create a DNA vaccine, instead of using the antigen as a vaccine, the gene that codes for the antigen is extracted using molecular procedures and administered as a vaccine. DNA vaccines are made up of a specific section of genetic material that, once integrated into the animal, may constantly create a specific immune stimulating component of a pathogen, so providing a "internal" supply of vaccine material. The DNA vaccination principle is to transfer to the host fish a DNA or RNA sequence coding for an antigen. The DNA is taken up by host cells and transcribed to mRNA, which is then translated into vaccination proteins. These proteins will be identified as a foreigner or antigen by the fish immune system, resulting in an immunological response. The fish immune system response, once activated, includes both cell-mediated and humoral components. Similarly, more than 20 distinct viral DNA vaccines targeting viruses such as rhabdoviridae, orthomyxoviridae, togaviridae, and nodaviridae have

been produced experimentally for preventive use in fish. The rhabdoviridae DNA vaccines have shown significant levels of efficacy, whilst others have demonstrated moderate to poor efficacy. Apex-IHN is a recombinant vaccine that has recently been developed and marketed for the protection of salmonids against IHNV.

The Apex-IHN DNA vaccine has shown to be quite effective, and additional DNA vaccines against various piscine viruses are in the advanced stages of development. The European Medicines Agency (EMA) proposed in 2016 that Clynav, a DNA vaccine against salmon pancreatic disease be approved for sale in the EU. Advances in pathogen genome sequencing may hasten the emergence of possibilities to research next-generation vaccinations. To face the difficulty of developing effective vaccines, a systems vaccinology strategy combining transcriptomics, epigenetics, proteomics, and metabolomics platforms, as well as bioinformatics, may be required. In order to be effective, a vaccine must be able to produce innate mechanisms, a sufficient antibody response, T-cell responses, and specific immunological memory in the host fish species. In this regard, the Apex-IHN DNA vaccine has shown to be quite effective, and additional DNA vaccines against various piscine viruses are in the advanced stages of development.

Intramuscular (IM) injection of these vaccines provides rapid and long-lasting protection against economically significant illnesses like as IHNV and VHSV, which were previously controlled by DNA vaccines. It has been demonstrated that DNA vaccination induces strong and protective immunity to some viral infections in fish, particularly Rhabdoviruses that infect rainbow trout and Atlantic salmon, as well as channel catfish herpesvirus infection . A DNA vaccine is first designed by identifying and cloning a protective antigen from the pathogen. For example, protective antibodies against the surface glycoprotein of various pathogenic fish viruses, such as VHSV and IHNV, are known. As a result, the glycoprotein gene and the regulatory sequences that enable eukaryotic cell expression were considered for the creation of DNA vaccines. The plasmid is synthesized in bacterial culture, purified, and quality-assured before being used as a vaccine. Following this, a DNA vaccine will be delivered and taken up by host cells to generate the glycoprotein. This circumstance causes the antigen to be detected by the fish's immune system. In theory, DNA vaccines provide benefits over conventional immunizations. A DNA vaccine has the benefit of being noninfectious since it is based on pure plasmid DNA bearing just a single gene from the disease.

Because it is unable to multiply inside the host, there is no chance of the vaccination transmitting the real illness. This vaccine type in fish or other animals is essentially the vaccine's long-term immunogenicity, safety, and stability for storage and shipment. As a result, DNA vaccinations are thought to be safer than traditional immunizations. Because these vaccinations do not contain adjuvants like traditional vaccines, they are devoid of post-vaccination adverse effects. The primary disadvantages of DNA vaccines include the ability to produce antibodies against DNA as well as immunologic tolerance by antigens expressed inside the host body. Because the DNA sequence encodes just one microbial gene, there should be no return to virulence, which is a significant feature in aquaculture environmental safety.

DISCUSSION

Real-time PCR has the benefit of being unaffected by nonspecific amplification. This assay requires a less quantity of template material. The main benefit of this PCR method over others is that it measures the template DNA or RNA contained in the sample. Amplification may be checked in real time using this approach. This assay requires a less quantity of template material. It does not need any post-PCR processing. The downside of real-time PCR is that it only detects the presence of antigenic material during infection and does not show whether or

not a host was infected. It requires specialized biocontainment facilities staffed by highly skilled workers, making it a costly and difficult to scale test. Real-time polymerase chain reaction (RT-PCR) kits are not accessible for all genes and diseases, and technical and standardized techniques are restricted. Furthermore, designing an RT-PCR test requires a greater level of experience and technical abilities.

Under isothermal circumstances, this LAMP amplifies DNA with great specificity, efficiency, and speed. A DNA polymerase with strong strand displacement activity and pairs of specifically designed inner and outer primers are used in the LAMP reaction. This method is based on autocycling strand displacement in isothermal DNA synthesis in the presence of Bst DNA polymerase. Six separate primers are carefully engineered to detect eight unique locations on a target gene in this approach, with amplification happening only if all of the primers bind and generate a product. LAMP, unlike PCR, is performed at a constant temperature range of 60e65 C, removing the requirement for a thermal cycler and making it a portable instrument. Each inner primer at the three prime terminal has a complementary sequence to one chain of the amplification area and is identical to the inner region of the same chain at the five prime terminal. Using the previously mentioned stem-loop sections as a stage, DNA polymerase-mediated strand displacement synthesis sequentially repeats the elongation processes.

This method generates a significant number of DNA amplification products with a mutually compatible sequence and an alternating, repetitive structure. The process is carried out at isothermal conditions, since strand displacement causes strand denaturation. All four primers are engaged in the earliest phases of the LAMP reaction; however, only the inner primers are employed for strand displacement DNA synthesis in the later cycling reaction. An inner primer comprising sense and antisense strands of the target DNA initiates the LAMP reaction. Following this, a single-stranded DNA is released by priming using an outside primer. This single-stranded DNA will act as a template for DNA synthesis, which will be aided by the second inner and outer primers, which may hybridize at the target's other end. A stem-loop DNA structure will be formed as a consequence of this process. Following the LAMP cycling stage, one inner primer will hybridize to the product's loop and induce strand displacement DNA synthesis, resulting in the original stem-loop DNA and a new stem-loop. Cycling lasts roughly 1 hour and resulting in the accumulation of 109 copies of the target.

To view the reaction on a membrane, several commercial LAMP kits use an enzyme substrate system. The amplification of the target gene takes 1 hour at temperatures ranging from 60 to 65 degrees Celsius. Because of the strand displacement activity of the Bst polymerase enzyme, a high quantity of product is generated. Because of this feature, positive reaction identification does not need any extra processing or electrophoresis. Proofreading LAMP (PR-LAMP), reverse transcription LAMP (RT-LAMP), in situ LAMP, allele-specific LAMP (AS-LAMP), real-time LAMP, and peptide nucleic acid LAMP (PNA-LAMP) are all examples of LAMP. For VHSV detection, Soliman and El-Matbouli employed a one-step RT-LAMP assay. Based on the G-protein sequences of the VHSV serotypes, a set of six primers was constructed. The whole LAMP technique may be completed in 90 minutes, and since it is done under isothermal conditions, it can be done without a thermocycler, making it appropriate for a field test if a small oven is available. LAMP has been employed in both animals and plants to identify bacterial, viral, fungal, and parasite illnesses. In aquaculture, LAMP-based detection of pathogens such as E. coli in fish and shellfish is used. E. tarda. There have been reports of ictaluri, Nocardia seriolae, white spot syndrome virus, and IHNV.

A variety of LAMP kits for aquaculture are currently available on the market. LAMP has the benefit of being a relatively straightforward and simple approach to perform after the appropriate primers are generated, needing just a DNA polymerase, four primers, and a

standard laboratory water bath or heat block for reaction. Furthermore, it is exceedingly exact for the target sequence and efficiently amplifies DNA, generating 109 copies of the target sequence in less than an hour. Another advantage of LAMP is that when used with reverse transcription, it may effectively amplify RNA sequences. Restriction fragment length polymorphism/DNA fingerprinting Restriction fragment length polymorphism (RFLP) is a widely used approach for genotyping that may be used to practically all species, including plants, animals, and humans. In genetic and genomic research, such as genome mapping and gene identification, RFLP is commonly employed. The existence of polymorphism areas in isolated fragments has been identified thanks to the availability of a number of restriction endonuclease enzymes that cleave DNA at specified places. The variation in the number of tandem repeats (VNTR) of a short DNA region causes such RFLP findings. These VNTR sequences may be utilized to uniquely identify a person and are therefore employed in DNA fingerprinting and paternity testing. As a molecular marker, RFLP is restricted to a single clone/restriction enzyme combination. To find DNA sequence polymorphisms in genes or DNA areas of interest, restriction endonuclease digestion is used. Aliquots of genomic DNA from each family members are digested to completion with the restriction enzyme known to create the polymorphism of interest when researching families for RFLP inheritance. Following agarose gel size fractionation,

In a high-salt buffer, capillary action transports DNA to a membrane. The gel is first denatured with NaOH, then neutralized before being put between buffer-soaked filter paper and a sheet of membrane. The labelled probe is hybridized to the Southern blot overnight. The blot is rinsed and subjected to X-ray film under circumstances intended to eliminate any nonspecifically adherent probe. The sizes of identified fragments vary across people and may be tracked from generation to generation. An RFLP probe is a labelled DNA sequence that hybridizes with one or more pieces of the digested DNA sample after gel electrophoresis, showing a distinct blotting pattern indicative of a certain genotype at a specific locus. RFLP probes are generally short, single- or low-copy genomic DNA or cDNA clones. RFLP probes are widely employed in genome mapping and variation analysis (genotyping, forensics, paternity testing, hereditary illness diagnosis, and so on). Certain requirements, such as proximity to the disease gene, must be met for RFLP to be helpful as a genetic disease marker. Materials and methods for detecting RFLP are reviewed, with a current focus on PCR-based amplification processes.

The benefit of RFLP is that it is a very specific, reliable, and repeatable approach, although it takes longer to get results. A microarray is a technique for measuring gene expression. It measures the quantity of mRNA produced by a certain gene in an organism to determine its expression or activity. Microarray analysis is a test for detecting gene expression that is seldom used to identify infectious pathogens. Microarray analysis is a variation on traditional RT-PCR in which numerous RT-PCR reactions for various target sequences may be conducted concurrently. As a result, the concepts of microarrays are the same as those of RT-PCR. A labelled probe molecule is hybridized to target DNA or RNA bound to a membrane in this approach. The probe is bonded to the support substrate, which is typically a nonporous solid surface, and then the labelled DNA or RNA hybridizes to the probe. A microarray is often used to assess gene response to a stimuli. An agent's influence on the activity of several hundred genes in an organism may be assessed. The main benefit of this method is that the material may be tested with thousands of probes at the same time. This method enables for the simultaneous measurement of the expression rates of thousands of genes in a single sample in a single test. The use of DNA microarrays for gene expression profiling offers considerable potential for the future of molecular diagnostics. Microarrays have been proven in diagnostics to be an effective approach for identifying and diagnosing fish illnesses caused by viruses, bacteria, fungus, and protozoa in a single step .

This approach allows for the simultaneous measurement of the activity of several hundred genes in an organism; nevertheless, the function of the majority of these genes, particularly in aquatic species, remains unclear. Microarrays may be a superior solution for wide-scale diagnostic testing since they can concurrently scan a sample for a large number of sequences. Because the majority of pathogens' genetic sequences are accessible in GenBank, oligonucleotide probes complementary to all pathogens may be designed and placed into microarrays, allowing a wide range of germs to be identified by a single microarray chip. As a consequence, microarray-based technology might be useful for detecting fish diseases in fish populations. Microarrays are already useful for measuring gene expression [9], [10]. An array with a high number of probes may indicate which genes are expressed or present in the sample. This sort of array would be very beneficial in pathogen investigations, where the presence of certain genes or gene products signals whether or not the organism is harmful. The expense of setting up DNA microarrays is significant. The use of microarrays in research projects for gene expression studies is widely known, but their usage in diagnostic applications for microbiology is still in its infancy. DNA chips are also being developed by diagnostic firms, as seen by the recent development of an Affymetrix array Food-Expert-ID.

The prefix nano, which comes from the Greek word meaning dwarf, is commonly coupled with a noun to make phrases like nanometer, nanorobot, and nanotechnology. Nanotechnology is typically characterized as systems or devices with nanoscale size characteristics. Nanoscience and nanotechnology have witnessed a wealth of new advances in practically every aspect of science and technology, particularly biology and medicine, during the previous two decades. Because of the tiny size of this technology, nanoarrays and nanochips have been used as test platforms. Recent advances in nanotechnology have resulted in the creation of nanoparticle-based simple assays for the selective detection of clinically relevant bioanalytes. Nanotechnology provides an excellent chance to produce rapid, accurate, and cost-effective diagnostics for pathogenic infectious pathogens. Because of their small size and large surface area, the properties observed in nanomaterials differ from those observed in bulk materials, resulting in enhanced surface reactivity, quantum confinement effects, enhanced electrical conductivity, and enhanced magnetic properties, among other things. Most notably, changes on the surface of the nanostructures may substantially modify certain of their characteristics. As a result, a single binding event may be recorded. As a result of these processes, a variety of nanostructures have been designed to detect specific molecular targets in biodiagnostic applications, including pathogen detection.

Surface biomolecule functionalization of nanomaterials has resulted in the creation of new multidisciplinary research fields such as biomedical nanotechnology, nanomedicine, diagnostic devices, theranostics, contrast agents, nanobiosensors, and targeted drug delivery vehicles. Nanomaterials may gain functionality by interacting with biological molecules or structures. The ability to evaluate a sample for an array of infectious agents on a single chip is one benefit of this technology. Applications include identifying individual strains or serotypes of disease agents, as well as distinguishing infections caused by distinct viruses but with similar clinical symptoms. The use of nanoparticles to mark antibodies is another aspect of nanotechnology. The labelled antibodies may subsequently be used to identify particular diseases, chemicals, or structures in different experiments. Gold nanoparticles (GNPs), nanobarcodes, quantum dots, and nanoparticle probes are all examples of nanoparticle technology. GNPs with distinct optical characteristics and a large surface area are widely employed for the rapid detection of bioanalytes of interest in samples.

CONCLUSION

Quickly determining if a fish is infected and promptly getting rid of the infected fish are important steps to take in order to effectively manage and control the spread of disease during outbreaks. Molecular tools are becoming more and more important in understanding and treating diseases in fish. The process of analyzing the entire genetic makeup of disease-causing organisms is helping scientists to better understand their behavior and find ways to diagnose and manage these harmful organisms. By using nucleic acid as targets and new methods to analyze differences in this nucleic acid, we can improve the accuracy, ability to detect, and speed of diagnosing diseases. This can also help us understand the connections between a pathogen's genetic makeup and how it presents itself in individuals. Using different methods to study various aspects of fish, scientists have been able to gain a better understanding of how fish develop resistance to infections from pathogens. These are important for keeping track of disease rates, finding new diseases early, and improving how we manage aquaculture operations. Advancements in methods help scientists study diseases and figure out what causes them or when harmful germs are present. So, molecular biology can help find better ways to diagnose and control fish diseases. It can also help study how these diseases spread among fish. To protect fish and aquaculture animals from diseases and keep the environment safe, it is important to use immunoprophylaxis or vaccination. This method is effective and cost-friendly in preventing the growth of harmful microbes and keeping the food safe for consumption. Different vaccines are now being sold and can be used to improve the protection of fish against different diseases. This would be very helpful in preventing diseases in fish farming. Nanomaterial-based vaccines have several benefits. They can help make antigens more stable, target specific areas for delivery, and increase the effectiveness of the immune response and protection. There are some problems when it comes to making vaccines for fish. First, we don't know enough about how fish immune systems work. Second, making the vaccines can be too expensive. Lastly, giving the vaccines to fish can be a stressful process. We hope that future vaccines will use several killed antigens along with a boost to make the vaccine work better.

REFERENCES:

[1] I. Sommerset, B. Krossøy, E. Biering, and P. Frost, “Vaccines for fish in aquaculture,” *Expert Review of Vaccines*. 2005. doi: 10.1586/14760584.4.1.89.

[2] M. Dadar *et al.*, “Advances in Aquaculture Vaccines Against Fish Pathogens: Global Status and Current Trends,” *Reviews in Fisheries Science and Aquaculture*. 2017. doi: 10.1080/23308249.2016.1261277.

[3] B. E. Brudeseth *et al.*, “Status and future perspectives of vaccines for industrialised fin-fish farming,” *Fish and Shellfish Immunology*. 2013. doi: 10.1016/j.fsi.2013.05.029.

[4] N. Lorenzen and S. E. LaPatra, “DNA vaccines for aquacultured fish,” *OIE Revue Scientifique et Technique*. 2005. doi: 10.20506/rst.24.1.1565.

[5] C. A. Shoemaker, P. H. Klesius, J. D. Drennan, and J. J. Evans, “Efficacy of a modified live Flavobacterium columnare vaccine in fish,” *Fish Shellfish Immunol.*, 2011, doi: 10.1016/j.fsi.2010.11.001.

[6] S. Poobalane *et al.*, “Production and efficacy of an Aeromonas hydrophila recombinant S-layer protein vaccine for fish,” *Vaccine*, 2010, doi: 10.1016/j.vaccine.2010.03.011.

[7] C. Tafalla, J. Bøgwald, and R. A. Dalmo, “Adjuvants and immunostimulants in fish vaccines: Current knowledge and future perspectives,” *Fish and Shellfish Immunology*. 2013. doi: 10.1016/j.fsi.2013.02.029.

[8] C. W. E. Embregts and M. Forlenza, "Oral vaccination of fish: Lessons from humans and veterinary species," *Dev. Comp. Immunol.*, 2016, doi: 10.1016/j.dci.2016.03.024.

[9] D. J. Pasnik and S. A. Smith, "Immunogenic and protective effects of a DNA vaccine for Mycobacterium marinum in fish," *Vet. Immunol. Immunopathol.*, 2005, doi: 10.1016/j.vetimm.2004.08.017.

[10] D. Zhang *et al.*, "More than just antibodies: Protective mechanisms of a mucosal vaccine against fish pathogen Flavobacterium columnare," *Fish Shellfish Immunol.*, 2017, doi: 10.1016/j.fsi.2017.10.001.

CHAPTER 11

GENE EDITING PRINCIPLES: TOOLS FOR PRECISION FISH GENE MODIFICATION

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ABSTRACT:

Aquaculture is becoming one of the biggest ways we produce seafood for people and animals to eat. It is growing quickly and becoming the main source of seafood. Selective breeding methods can be used to make animals or plants better in certain ways, like being able to resist diseases. However, there are limits to how much improvement can be made because of how genes are inherited and the time it takes for new generations to be born. CRISPR/Cas9 genome editing is a useful tool to help improve genetic traits in aquaculture quickly. GE can change the genetic makeup faster and create new versions of genes, combine genes from different species or types, or fix genes in charge of certain traits. Most fish species can reproduce a lot, and external fertilization can make it easier to study them and use their genes in ways that would be difficult to do with mammals that live on land. Having enough food that is safe to eat is a big problem, and more and more people want good meat to eat. Crop and animal production will not be good because people will need to use the land for other things. Aquaculture is expected to be a big help in meeting the growing demand for food and nutrition. To increase production and reliability, we will need to use new and innovative technologies in areas such as engineering, health, nutrition, and genetic improvement. We need to make better use of the genes of fish that we already have in order to improve fish farming. This also involves important genetic information in methods of breeding that use markers to help select the desired traits. Genome modification, such as adding, removing, or changing one base, can be done using GE tools. GE technologies have a lot of potential to make fish farming better in the long run and more efficient. Zinc finger nucleases (ZFNs) and transcription activator-like endonucleases (TALENs) were some of the first genetic engineering tools used. The newest genetic engineering technique, called CRISPR-Cas technology, is now the most dominant. This option allows changes to be made in the genes and traits through engineering. The CRISPR technology is now known as a very powerful and accurate genetic engineering technology that is also easy to use and affordable. In this chapter, we will briefly talk about the different CRISPR-Casemediated technologies that can be used to make changes in genes. We will also look at how they are currently being used in aquaculture and the difficulties in using these technologies.

KEYWORDS:

CRISPR CAS9, Fish, Genetic, Prime Editing, Speices.

INTRODUCTION

Scientists are working hard to be able to modify genetic information ever since they discovered the structure and function of DNA. Restriction endonucleases are enzymes that can break DNA. Ligases are enzymes that can stick DNA pieces together. Polymerases are enzymes that can make new DNA. Cells have ways to repair DNA strand breaks that occur naturally within the cell. The main thing for GE is the skill to create breaks in a specific order. There have been different ways to change DNA that have developed in the past 20 years, such as using nuclease to target specific mutations or using oligonucleotides to direct mutations. There are four different types of site-directed nucleases called meganucleases, TALEN, ZFN, and CRISPR-

Cas. The text is not provided ZFN is made of pieces of DNA that can recognize and attach to specific target DNA sequences. It also includes a protein called FokI nuclease. The complex can be made to find specific parts of DNA that are 9e18 nucleotides long, and the FokI enzyme can cause breaks in both strands of DNA when it pairs up with another molecule. Using ZFN was made difficult because it was hard to create, build, and confirm. Because of these problems, a new tool called TALEN was created in 2010-11.

TALEN is simpler to confirm and create compared to ZFN, and it only identifies a smaller number of nucleotides, which makes it superior to ZFN. However, the process of testing, creating, and making proteins is still complicated and difficult, which means that there are restrictions on how much this technology can be used. CRISPR-Cas GE technology is a new and important discovery. Scientists have used a prokaryotic immune system to develop this technology. This mechanism uses RNA to recognize and turn off foreign nucleic acids depending on their specific order of building blocks. Bacteria have CRISPR arrays that are made up of sequences that target specific parts of their genome. These sequences are separated by repeating sections and are accompanied by Cas enzymes that are encoded in operons. The repurposed CRISPR-Cas9 is a two-part system used for genetic engineering. The first one is a tiny guide RNA, and the second one is an enzyme called Cas9 that can cut DNA directed by the guide RNA. The gRNA tells the Cas9 where to go on the DNA and make a cut. ZFN, TALEN, and CRISPR-Cas systems can all create a specific type of damage to a specific site in DNA. When DSB forms, it triggers the cell's own repair systems. Nonhomologous end joining (NHEJ) and homology-directed repair (HDR) are two different ways that cells can fix damaged DNA. The text talks about someone's response to a specific question. NHEJ is a process that tries to fix mistakes in DNA, but it often messes up and changes the DNA in different ways [1], [2].

It can delete, add, or swap nucleotides. When a person gives their DNA that matches the surrounding DNA near a specific site called DBS, HDR is turned on. The way a sequence is added, changed, or fixed in DBS depends on the type of donor. These changes in DNA can either put in a new gene or deactivate an existing gene. To improve editing with great accuracy, scientists have created and experimented with base editing and prime editing technologies in many different living things. These technologies use a method called Cas9 nickases to make exact changes to the target genes at a very precise level, down to a single letter change in the genetic code. There are three different tools for editing DNA bases. They are called cytosine base editor (CBE), adenine base editor (ABE), and C-to-G base editor (CGBE). CBEs were created to change C-G to T-A in DNA, while ABEs were created to change A-T to G-C. New tools called CGBE have been created by different teams to edit the C to G bases. These three types of base editors are really helpful for accurately making changes to the genetic code. A new genetic engineering technology called prime editing has been created to make precise changes in the genome by adding or removing specific parts. Prime editing uses a special type of RNA called prime editing guide RNA. It can make specific changes to a single unit in our genetic material and can also add or remove small pieces. It can do all of these changes accurately and in a specific way [3], [4].

GE technology in aquaculture currently has many opportunities available. 113 out of 10. Recently, certain kinds of fish like salmon, catfish, and carp, as well as sea bream, Nile tilapia, and Pacific oyster, have been modified using a gene editing technique called CRISPR-Cas9. This has been done both in cell cultures and in living organisms. We have not yet seen successful CRISPR-Cas9 based genome engineering in shrimp, which are important aquatic organisms. This could be because of practical challenges, which will be briefly explained. Most of the research used prototypes and tested CRISPR Cas9 in model organisms such as zebrafish.

Most of the studies typically focused on genes that show obvious traits to see how well editing works for example, changing color. Lately, scientists used a tool called CRISPR-Cas9 to change the genes of red tilapia fish. In aquaculture, when we want to change the genes of a species, we usually inject special materials called CRISPR-Cas9 into very young eggs. Usually, a combination of mRNA and gRNA is used to edit genes. This has been shown to work well in many different species. Using Cas9 protein instead of mRNA has also been found to be effective. In most experiments, NHEJ is used to make mutations. However, in rohu carp, the HDR method has been successfully used by inserting a template DNA. Furthermore, various studies have mentioned the transmission of genes from one generation to another in organisms [5], [6].

Due to the high occurrence of variation in edited animals, it has been proposed that cutting with Cas9 and genetic editing may go beyond the initial stage of development. This is seen as a significant concern and should be investigated further in future studies. In the farming of aquatic species, genetic engineering has been used in the past to change traits such as not being able to have babies, getting bigger faster, and being able to fight diseases better. Scientists have used CRISPR/Cas9 technology to make catfish and Atlantic salmon infertile. This is done to prevent the genes from spreading to wild populations and to avoid negative effects on the fish's growth and development. Different groups have changed the myostatin gene in fish to make them grow better and have improved traits related to growth. Scientists have already studied how Grass carp and Rohu fish defend against diseases using genetic engineering. They think that more research in this area will help improve and understand disease resistance even further. For instance, changing the TLR22 gene in carp on purpose shows how GE can be used. These simulations can help us understand how fish react to diseases, which could help us find better ways to treat them. similarly, GE technologies can be used to make fish cell lines better by removing important,

The parts of the interferon pathway mechanisms, for example, that help make viruses so they can be used to make vaccines. There are two practical reasons why genetic modification is useful for studying and using farmed fish. First, their embryos are big enough to be injected with small tools. Second, there are many thousands of externally fertilized fish available for research. Being part of a big family helps to control our genetic background and allows us to compare successfully transformed individuals with their siblings. It is common to do many tests and evaluations on babies to understand how their bodies fight off germs when they get sick. In simpler words: If we find good traits in plants, we can spread these traits quickly through the breeding programs. Good-quality, well-understood reference genomes are available for many important species [7], [8].

To successfully design gRNA targets with accuracy and minimal unintended changes, it is necessary to have a high-quality reference genome specific to the species, considering the frequent occurrence of whole-genome duplication events in various fish species, like salmon. Among the many challenges faced by aquaculture, the biggest threat is infectious diseases. It is estimated that these diseases cause a loss of around 40% of the total potential productivity every year. Because many aquaculture species are still being bred, there is a chance that new options and disease constraints could lead to the need for certain genes in populations that are highly influential. This could make it easier to use genetic engineering to increase the rate of desirable traits. GE is creating new opportunities for making fish farming more sustainable and productive. GE technologies can be used to improve genetics in three major ways. The first is by changing, finding, strengthening, or resolving specific groups of genes in a breeding program's current population. The second is by transferring positive traits from different species, strains, or populations to enhance or introduce new characteristics in a population. The

third is by creating new useful traits that have not been seen before and using them to generate energy or enhance new characteristics.

DISCUSSION

Most animal breeding and genetics research focuses on finding and using certain genes that affect the characteristics important for production. However, there haven't been many successful discoveries in this area. Simulations show that using editing to enhance certain beneficial genes at multiple genetic points can speed up genetic progress compared to relying solely on genomic selection or pedigree. The strategy's success is hindered by the difficulty in accurately identifying differences in function below QTLs, particularly those that are not very significant. To simplify this text: Researchers can use different tools to narrow down a large number of potential changes found in extensive studies of genes throughout the whole genome. GE has the potential to be very useful in the field of functional genomics. Big differences and harmful effects caused by many genes, which are always present in groups of people, can be gotten rid of using the same method. In addition, in order to make a big impact using this method, many genes must be modified all at once in the same group of animals used for breeding. This requires enhancing and creating new strategies to modify multiple genes at a time.

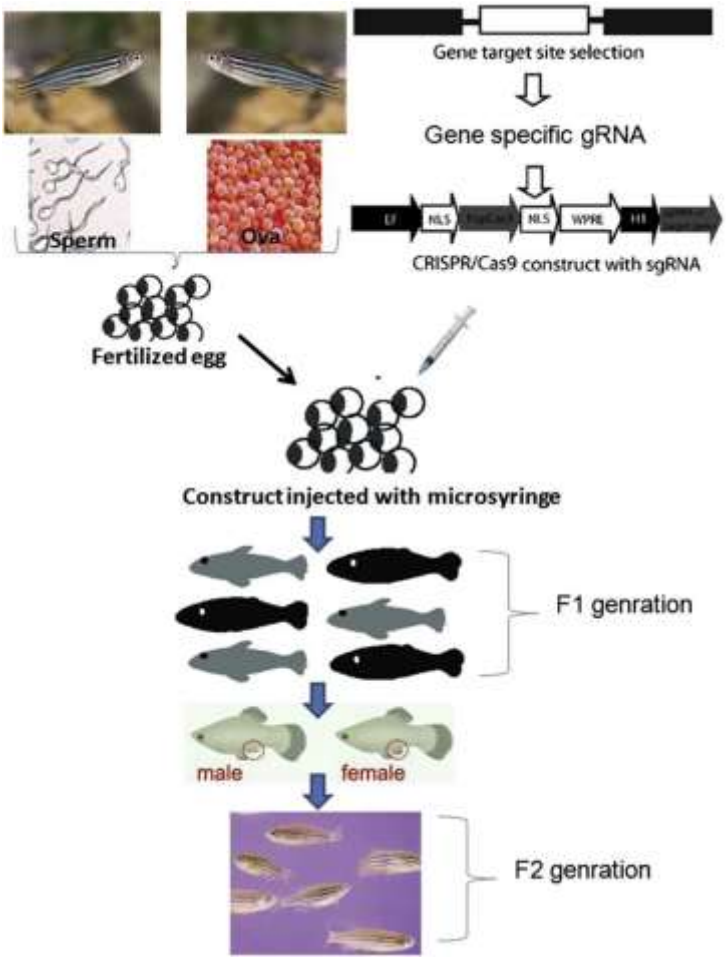

Figure 1: Representing the genetically modified fish using CRISPR/Cas9 technology [Research Gate. Net].

One of the most fascinating uses of GE is the ability to get different genes from unrelated groups of animals without having to spend a lot of time and money on traditional breeding

methods, or in situations where traditional breeding is not possible. CRISPR technology can be used to change harmful or deadly genes in a specific species or strain of living things. This can be done by adding in the beneficial or non-deadly gene from a similar species or strain (Figure 1). GE allows for new ways to avoid traditional methods of combining genes, while also preventing the negative effects of introducing genes from wild strains on growth rate. It also allows for using genetic diversity in other species and strains that would not be possible with traditional methods. Sea lice, which are a type of parasite called copepods, harm Atlantic salmon farming. They cause a loss of USD 880 million every year. Aquaculture means growing fish or other sea animals near natural populations that have good qualities. Coho salmon and pink salmon are able to handle sea lice attacks without much trouble. They have a strong immune response to the parasite. This raises the exciting idea of trying to give Atlantic salmon the same ability to fight off diseases as their hosts. The scientific community has put in a lot of effort to understand why different types of fish have different ways of resisting diseases. It's possible that the important genes that control how Atlantic salmon resist diseases could be changed using genetic engineering to make them react like Coho salmon when attacked by sea lice.

We can modify the coding area and/or the regulatory sequence in a specific way. Six Creating new variations using known traits: Genetic engineering uses existing genetic variations to solve issues like quality, production, and animal welfare. It also has the potential to create new beneficial traits that don't naturally exist. We can create new genetic variations using a system called CRISPR/Cas9. We use what we already know about certain genes to make these new variations. Or, we can discover new genes related to certain characteristics by studying the entire genome. Changing the dnd allele to make Atlantic salmon unable to reproduce, and targeting the mstn1 gene in different types of fish to make them grow faster, have both been used in aquaculture CRISPR screens is a powerful way to find changes in our genes that make us more likely to get sick or more likely to stay healthy. It looks at all of our genes to see which ones might cause or prevent diseases. But, in order to use CRISPR screens, we need to have well-known cell lines. One of the main obstacles in aquaculture research is the absence of reliable and well-studied cell lines for certain species that are important in this field. Researchers recently used a gene-editing tool called CRISPR/Cas9 to change the genetic material in cell lines from chinook salmon, medaka, and carp. They saw good results from this experiment. Researchers are focusing on studying virus-related infections using CRISPR/Cas9 in laboratories. This is because of natural ways our body reacts to things and can be easily studied because they are inside our cells. Using these technologies, it would be easier to bring together big genetic tests to understand more about the genetics of being able to resist diseases. This would help us find genes or alleles that could be used in profitable and commercial fish breeding [9], [10].

To improve the chances of using GE systems in aquaculture species, there are several important technological difficulties that need to be solved. First, in species where CRISPR/Cas9 has been used, scientists want to improve the technique to make it better at making changes and preventing mistakes in the first generation. Off-target editing may lead to unintended changes in the organism's genes, which could have unforeseen effects. In addition to studying specific sites in the DNA that may be edited by mistake, we can use a cheaper method called whole-genome resequencing to regularly check for these errors. Getting better information about the order of aquaculture species will help create specific gRNAs that target a particular area accurately. To fight against mosaicism, scientists have created Cas9 proteins that don't last long. These proteins only cause DNA breaks during the very early stages of a fertilized embryo's development. It is possible to test for the most common changes in genes in the

offspring of mutant animals. This is done to find animals that have two copies of a single changed gene, rather than having changes in multiple genes.

Base editing, prime editing, and HDR can be used to make precise changes in the F0 generation. This includes swapping one nucleotide for another, causing a coding sequence to stop prematurely or altering the building blocks of proteins. These techniques can also add or remove small pieces of DNA as desired. The effectiveness of gRNAs can be tested in a lab or cell culture before using them in live editing. This helps choose the most effective gRNAs that were designed using computer simulations. Other ways to use CRISPR/Cas9, like editing cells before they turn into eggs or injecting cells into unfertilized eggs, could be tested. This is especially important for species where it's difficult to get newly fertilized embryos, like certain shrimp species. A different way to make changes to an animal's genes is by replacing germ cells with edited ones. This can be done by using many sterile surrogates. It is a way to make improvements to specific gene variations that we are worried about. In Japan, they have allowed the sale of two fish that have been edited using CRISPR technology. The Regional Fish Institute, along with Kyoto University and Kindai University, worked together to change the genetic makeup of a tiger puffer and a red sea bream. The genetically modified fish grow bigger than the normal fish. When scientists remove the gene that controls hunger from tiger puffer fish, they eat more and get fatter. In simpler terms, the red sea bream had its myostatin gene turned off so that its muscles could grow bigger. The research found that the tiger puffer fish and red sea bream fish grow almost twice and slightly more than once as big as their normal counterparts when given the same amount of food.

CONCLUSION

Aquaculture species are able to produce a large number of offspring, and when they reproduce, the fertilization happens outside of their bodies. This allows scientists to study their genes in great detail, which can help us learn more about and enhance their complex traits. CRISPR/Cas9 and other genetic engineering technologies can help increase the genetic traits that are important for producing what we want. Infectious disease is a big problem that affects aquaculture and reduces production and productivity. Finding ways to prevent and control these diseases is a main focus for genetic engineering and selective breeding techniques. There are different ways to use genetic engineering (GE), such as finding different variations that affect important traits and adding them to plants or animals through editing. It's also possible to take beneficial genes from nearby populations or species and add them to breeding systems through editing. Another way is to create new genes with useful traits and use them. In addition to the usual CRISPR-Cas9 method, other advanced tools such as base editing and prime editing can help us make specific changes to genes more easily. These tools are very helpful in understanding gene functions and creating specific traits in fish. However, if those tools become accessible, it will open up new research opportunities that would eventually be beneficial for the sector in the long run.

REFERENCES:

[1] D. Zhang, Z. Li, and J. F. Li, “Targeted Gene Manipulation in Plants Using the CRISPR/Cas Technology,” *Journal of Genetics and Genomics*. 2016. doi: 10.1016/j.jgg.2016.03.001.

[2] J. Lin *et al.*, “Progress and Application of CRISPR/Cas Technology in Biological and Biomedical Investigation,” *J. Cell. Biochem.*, 2017, doi: 10.1002/jcb.26198.

[3] P. M. Rogowsky, “CRISPR-Cas Technology in Plant Science,” *Potato Res.*, 2017, doi: 10.1007/s11540-018-9387-y.

[4] E. Stout, T. Klaenhammer, and R. Barrangou, "CRISPR-Cas Technologies and Applications in Food Bacteria," *Annual Review of Food Science and Technology*. 2017. doi: 10.1146/annurev-food-072816-024723.

[5] D. Burstein *et al.*, "New CRISPR-Cas systems from uncultivated microbes," *Nature*, 2017, doi: 10.1038/nature21059.

[6] X. Liu, S. Wu, J. Xu, C. Sui, and J. Wei, "Application of CRISPR/Cas9 in plant biology," *Acta Pharmaceutica Sinica B*. 2017. doi: 10.1016/j.apsb.2017.01.002.

[7] F. Port, N. Muschalik, and S. L. Bullock, "Systematic evaluation of Drosophila CRISPR tools reveals safe and robust alternatives to autonomous gene drives in basic research," *G3 Genes, Genomes, Genet.*, 2015, doi: 10.1534/g3.115.019083.

[8] D. Seruggia and L. Montoliu, "The new CRISPR–Cas system: RNA-guided genome engineering to efficiently produce any desired genetic alteration in animals," *Transgenic Research*. 2014. doi: 10.1007/s11248-014-9823-y.

[9] D. Ma, S. Peng, and Z. Xie, "Integration and exchange of split dCas9 domains for transcriptional controls in mammalian cells," *Nat. Commun.*, 2016, doi: 10.1038/ncomms13056.

[10] L. Qi and A. Lo, "Genetic and epigenetic control of gene expression by CRISPR-Cas systems," *F1000Research*. 2017. doi: 10.12688/f1000research.11113.1.

CHAPTER 12

ULTRAVIOLET-B RADIATION: THREATS TO AQUATIC ORGANISMS AND MITIGATION STRATEGIES

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ABSTRACT:
Ultraviolet-B (UV-B) radiation is a type of sunlight that has a big effect on water environments. This chapter explains how UV-B radiation can harm water-dwelling organisms and provides ways to protect them from its effects. UV-B radiation can harm different types of plants and animals that live in water, like tiny plants, tiny animals, fish, and amphibians. Too much UV-B exposure can cause harm to DNA, make plants not able to do photosynthesis as well, affect their ability to reproduce successfully, and change how groups of plants and animals interact with each other. The outcomes of these effects can spread through the food chains in water and have a domino effect on the overall health of ecosystems. To lessen the harmful effects of UV-B radiation on water creatures, different methods and techniques have been created and put into action. These strategies include actions done by nature and actions done by people. Plants and animals have ways to protect themselves against UV-B rays, like making special compounds and moving to lower depths of water. Moreover, by restoring plants along the riverside and reducing the amount of nutrients in the water, we can indirectly help protect the ecosystem from the harmful effects of UV-B. Moreover, technology has helped create special shields and screens that can be placed in water to stop harmful UV-B rays. These engineering solutions can protect vulnerable water organisms when used carefully. This chapter discusses how UV-B radiation affects aquatic ecosystems. It also gives information on different ways to reduce its impact. By learning about the different ways UV-B radiation affects our environment and taking steps to reduce its harmful effects, we can do a better job of keeping our water ecosystems and the living things in them safe, even as the environment continues to change.

KEYWORDS:

Fish, Immune System, Protection, Ultraviolet radiation, Water.

INTRODUCTION

Ultraviolet radiation (UVR) is a type of light from the sun that has a lot of energy. It has more energy than any other type of light that reaches the Earth's surface. The UVR is split into three groups depending on how long the wavelengths are: UV-A, which has wavelengths between 315 and 400 nanometers; UV-B, which has wavelengths between 280 and 320 nanometers; and UV-C, which has wavelengths between 200 and 280 nanometers. Out of these three types of UV light, UV-C gets absorbed by oxygen, ozone, and other gases in the stratosphere and doesn't make it to the surface of the Earth. UV-B is a small part of sunlight, but it has a lot of energy. The amount of ultraviolet radiation (UVR) on the Earth's surface changes a lot based on certain factors. Latitude refers to how far north or south a place is from the Equator. Time of day refers to what time it is during daylight hours. Time of year refers to what season it is in a particular location. Presence of cloud and aerosols refers to whether there are clouds or particles in the air like pollution or dust. The position of the sun in the sky affects the amount of UV radiation throughout the year. This powerful light particle can harm the land and water environments. The strength of ultraviolet radiation (UVR) in water depends on two important things: how deep the water is and how much organic matter is in the water. UV-B rays can go deep into the water because water can absorb some UV-B rays [1], [2].

In clear ocean water, 10% of the sun's ultraviolet rays (UVR) can go as deep as 25 meters. In lakes with little dissolved organic carbon (DOC), the UVR can go over 10 meters deep. UV-B has been seen as a big reason why amphibians are decreasing in number. Studying how UV-B rays affect aquatic animals is important for both the environment and aquaculture. When natural waters are exposed to UV-B light with DOC (dissolved organic carbon), it creates hydrogen peroxide (H_2O_2) and other harmful substances called reactive oxygen species (ROS). These substances are believed to contribute to the harm caused by UV radiation in fish. When UV-B radiation causes the production of ROS in surface water, hydrogen peroxide is formed and builds up because of the activation of DOC by light. ROS come from when oxygen is partly reduced during how our body uses oxygen for energy. ROS can also be produced from chemicals that are harmful to light-sensitive cells. When there is no charge on H_2O_2, it creates a substance called hydroxyl radicals (OH). These radicals can cause harm to cells and tissues by causing oxidative damage. Iron-catalyzed reduction of water using superoxide anions can create the very reactive hydroxyl radical (OH) [3], [4].

The sunlight's UV-B rays can seriously affect the water-based organisms in tropical countries like India. There is a decrease of 5% to 15% in the amount of ozone over Delhi, Pune, and Trivandrum every ten years. The strength of UV-B rays was measured at the surface of Naini Lake in Delhi, India. The lowest amount of UV-B radiation, measured in units of power, was 24e27 mW/cm2 during the months of January and February. In the month of June/July, the highest value is 180. In India, lots of fish are mating and growing in small ponds during May to July. The lakes and rivers are getting shallower because the land around them is getting higher. There are a lot of fishes that live on top of the water. Some fishes show changes in their behavior caused by UV-Bein. The amount of exposure can greatly impact the chance of survival, the body functions, and the growth of young fish and adult fish. In lakes and oceans, baby fish and shellfish float near the top of the water and are exposed to the sun's rays. Fish babies are very sensitive to UV-B because their bodies have less color and no scales in their skin. The types of species with color are better at handling UV-B rays than the ones that are see-through. Exposing young aquatic organisms to certain conditions can impact how likely they are to survive. The sun's UV-B radiation has harmed the survival of young fish in their early stages. These are the names of different kinds of fish: plaice, Atlantic cod, yellow perch, northern anchovy, steelhead trout, coho salmon, and Atlantic salmon. In yellow perch eggs that were exposed to UVR, all of them died [5], [6].

The Kaplan and Meier product-limit model demonstrates that the more time catla catla larvae are exposed to UV-B, the lower their survival rate. A lot of baby fish dying in the early stages has a bad effect on the growing and making of fish farms. Some fish, like young coho salmon and red seabream, have ways to protect themselves from the sun's harmful rays. The cones in the larvae's eyes can detect how strong the UV light is. The ability of fish to reorganize themselves when exposed to ultraviolet radiation (UVR) changes as they grow. When fish are exposed to UV-B radiation, it harms their eye lens by causing oxidative stress. It makes it harder for the lens to get rid of hydrogen peroxide because it slows down the cells in the lens that do this. The UV-B radiation hurts the microridges in the eyes of ayu fish, which makes it harder for them to see. Scientists have observed that young cod fish can develop a condition called cataract. The morhua fish were placed in a recirculating system and exposed to ultraviolet radiation (254 nm). Poor vision in fish has a negative impact on their ability to find food and escape from predators.

UV-B radiation hurts the gills in fish, which are important for breathing and getting rid of waste. Exposure to UV-B radiation can harm three different types of cells found in the gill filaments and lamellae of fish: pavement cells, mitochondrion-rich cells, and accessory cells.

UV-B light breaks down the PVCs and makes the MRCs visible in catla larvae. PVCs are the most common cells found in the surface layer of the gill, making up over 90% of the gill surface. These cells help in the transfer of gases in the gills. Fish have very sensitive skin and are more likely to be damaged by ultraviolet radiation (UVR) than humans. The pigment layer that protects the skin is found in the deeper layer of the skin in teleosts. Many rainbow trout fish have been observed to have sunburn. Mykiss, young plaice, and young paddlefish. The skin cells of the fish called ayu have less of the tiny ridges that cover them when they are exposed to UV-B light. The small ridges on the skin are also broken into pieces and found in certain parts. This causes the neuromast and goblet cells to be exposed. The neuromast cells have specific roles, meaning they have particular jobs. Assist the fish in dealing with the pressure and forces of the water and help them breathe through their skin. Goblet and mucus cells are important parts of a fish's skin. The damage from UV rays hurts the fish's skin, which is their first way of protecting themselves, and this could start outbreaks of diseases. Flexibacteriosis has been found in Atlantic salmon, also known as Salmo salar. When fish are exposed to UV-B, it harms the protective layer of their skin and makes it easier for harmful germs to get inside [7], [8].

DISCUSSION

Exposing fish to UV-B radiation has harmful effects on their bodies. UV-B radiation primarily affects proteins, nucleic acids, and lipids. The harmful sun rays can hurt the body, tissues, and cells. This can make living things not grow well and can even make them die. It changes how fish's body processes food and grows when they are still young. The catla larvae that were exposed to UV light for 10-15 minutes had much lower amounts of amylase, total protease, and trypsin activities compared to the fish that were not exposed to UV light. When the digestive enzymes in fish are not working well, it leads to poor growth and production. Slowly, the fish will become weak and more likely to get sick. The impact of UV-B radiation on the immune system has been examined in numerous fish species. When fish are exposed to UV-B light, their immune systems, both the nonspecific and specific ones, become less active. UV-B rays from the sun can make fish more vulnerable to getting sick from germs. The immune system of fish can be measured using certain tests like lysozyme, myeloperoxidase, hemagglutination titer, and nitric oxide synthase (NOS).

These tests can tell us if the fish is healthy or not. Lysozyme helps protect fish from germs. It breaks the walls of certain types of bacteria called gram-positive bacteria. In addition to killing bacteria, it helps the body's white blood cells called polymorphonuclear leukocytes and macrophages to attack them. It can do this by directly activating the cells or by making the bacteria easier for the cells to grab onto. In fish, lysozyme is mostly found in certain types of white blood cells called neutrophils and monocytes, and there is also a small amount in a type of immune cell called macrophages. Myelo peroxidase is a protein that is found a lot in the body. This means that fish have the ability to eat and kill bacteria. NOS is a group of enzymes that helps make a chemical called nitric oxide (NO) which sends signals within cells. Nitric oxide is a chemical messenger inside cells that activates guanylate cyclase. The low levels of these important measures indicate that the fish have a weak immune system. The fish that are exposed to UV-B light have more white blood cells, which helps them fight off infections.

The UVR causes the body to produce harmful molecules called free radicals. When fish are exposed to UV-B, certain enzymes in their bodies become more active. These enzymes, such as superoxide dismutase (SOD), work to protect the fish from oxidative stress. The blood tests of the fish, like serum protein, albumin, globulin, SGOT, and SGPT, show how the fish is doing physically. When fish are exposed to UV-B, they have higher amounts of protein, albumin, and globulin in their bodies. This suggests that the fish are experiencing stress. High levels of

SGOT and SGPT can be seen in damaged tissue. The test for lipid peroxidation products, malondialdehyde/thiobarbituric acid reactive substance (TBARS), detects the harmful effects of UV light on cell membranes. In samples from living things, the most common way to study the final result of protein damage caused by reactive oxygen species is by measuring protein carbonyls. Carbonyl groups (aldehydes and ketones) are formed on protein side chains through oxidation. We can create protein carbonyl derivatives by breaking down proteins through oxidation. Proteins can quickly cause damage to cells because they often act as catalysts rather than simply carrying out reactions in a specific ratio. Stress proteins are proteins that can show if cells are damaged. These proteins are linked to the immune response and autoimmune diseases. Heat shock protein 70 (Hsp70) is a type of protein that is found all over the body. It is made more when the body is exposed to difficult conditions like high temperatures, UV radiation, and chemicals. It is also known to help [9], [10].

Even though organisms have ways to protect and repair cells from UV-B, too much exposure can still harm the organism's abilities. When fish are exposed to UV-B for a long time, it can cause DNA damage that cannot be fixed. Then cells go through complicated chemical reactions and use up a lot of energy, which can cause the cells to die in different ways like apoptosis, necrosis, or other forms of cell death. When our skin is exposed to UV-B rays, it can cause damage to the DNA in our cells. This damage happens because the UV-B rays directly affect the building blocks of DNA, which can result in changes to our genes and cause parts of our chromosomes to break apart. The changes that happen most often are cyclobutane pyrimidine dimers and pyrimidineepyrimidone (6-4) photoproducts. UV-B radiation can change DNA by making reactions happen in a roundabout way. This causes the production of harmful molecules called free radicals and ROS. This ROS causes damage to DNA by breaking its building blocks. Fishes have been found to experience DNA damage caused by UV light from the sun. The main ways to deal with the harmful effects of UV-B rays are to: avoid being in the sun too much, use sunscreen, and fix any damage caused by UV rays.

Proteins and DNA are important for our body, and toxic substances can harm us. Out of these, avoiding and protecting against UVR are really important for staying safe. Many living things in water have substances that can protect them from the sun's harmful rays. These substances can either soak up the rays themselves or help in some other way. Many aquatic organisms have sunscreening compounds like mycosporine-like amino acids (MAAs) and carotenoids. They help protect them from the sun. Scientists have discovered that many water-dwelling organisms can handle more sunlight when they have more MAAs in their bodies. Only microorganisms can make MAAs. Fish can get MAAs by eating other organisms in the food chain. Living organisms have special substances that help protect them from harmful effects caused by certain molecules called free radicals. These substances, like glutathione, and enzymes such as SOD, CAT, glutathione S-transferase, and glutathione peroxidase, are able to neutralize these harmful molecules. Substances like flavonoids, carotenoids, vitamin C, and vitamin E help protect fish from the harmful effects of UV-B radiation.

Vitamin C is a strong substance that helps cells by reducing other substances. It helps put oxygen into different materials. Researchers have studied how vitamin C can help fish fight off diseases. Fish and crustaceans cannot make vitamin C because they do not have the right enzyme needed to finish making it. As a result, they need to always have enough vitamin C in their food. Feeding catla larvae with diets containing added vitamin C (0. 5%) helps protect the fish from UV-B exposure. Vitamin C, an antioxidant, helps regulate the immune system and protects against stress. It reduces the levels of certain substances that are higher in the control group of catla fish on a regular diet compared to those on an enriched diet. Adding natural plant ingredients to fish food can help protect them from the harmful effects of UV-B radiation. The

leaves and seeds of a plant called Achyranthes aspera L, which is native to a certain place, have medicinal properties. Different species of plants from the family Amaranthaceae have been added to fish food in different amounts. 01%, 025%, and 05% can be rewritten as one-tenth of one percent, one-quarter of one percent, and one-half of one percent. Studies have shown that adding leaves and seeds at a 0. 5% concentration provides the most effective protection against UV-B radiation for the fish species rohu, also known as Labeo rohita. The scientific study found that the seeds and leaves of plant A have certain chemical properties. Asparagus are a type of food that contain a lot of protein, lipid, and ash. In seeds, there are 18 important amino acids and in leaves, there are 17 important and non-important amino acids. Seeds and leaves have a lot of n-6 polyunsaturated fatty acids and n-3 polyunsaturated fatty acids. Certain vitamins can be found in seeds and certain vitamins can be found in leaves of A. Aspera is a type of software or technology. So, adding these plant ingredients to fish's diets makes their immune system stronger and helps protect them from harmful UV-B radiation.

CONCLUSION

Basically, UV-B radiation is becoming a bigger problem for animals and plants that live in water. This chapter has explained how UV-B radiation can harm aquatic life, including small plants and animals to larger ones. These outcomes, such as harm to DNA, decreased ability of plants to make food through photosynthesis, and changes in how living things work together, show that we must take action right away to find ways to lessen the problems. Reducing the harm of UV-B radiation on water-dwelling creatures needs a variety of methods. Natural processes, like the creation of substances that protect the body and changes in behavior, help to make living things more resilient. However, people must get involved. Restoring the natural vegetation and reducing harmful substances in water can indirectly help protect water organisms by making the environment more stable. Moreover, new technology like UV-B shields and screens provide direct protection. When used in a smart way, these actions can protect species that are at risk and keep the balance of underwater environments. As the earth's climate changes, the amount of UV-B radiation also changes. It is important to take action and prevent the negative impacts of this radiation. Working together to study the issue, create plans, and inform the public is very important in dealing with this new environmental problem. In simple words: By understanding and taking action against the dangers of UV-B radiation, we can protect the variety of living things in our water environments and make sure they can continue to exist for the next generations.

REFERENCES:

[1] E. Sucré, F. Vidussi, B. Mostajir, G. Charmantier, and C. Lorin-Nebel, “Impact of ultraviolet-B radiation on planktonic fish larvae: Alteration of the osmoregulatory function,” *Aquat. Toxicol.*, 2012, doi: 10.1016/j.aquatox.2011.09.020.

[2] V. S. Blazer, D. L. Fabacher, E. E. Little, M. S. Ewing, and K. M. Kocan, “Effects of ultraviolet-b radiation on fish: Histologic comparison of a uvb-sensitive and a uvb-tolerant species,” *J. Aquat. Anim. Health*, 1997, doi: 10.1577/1548-8667(1997)009<0132:EOUBRO>2.3.CO;2.

[3] D. L. Fabacher, E. E. Little, and G. K. Ostrander, “Tolerance of an albino fish to ultraviolet-B radiation,” *Environ. Sci. Pollut. Res.*, 1999, doi: 10.1007/BF02987550.

[4] S. E. Markkula, H. M. Salo, A. K. Rikalainen, and E. I. Jokinen, “Different sensitivity of carp (Cyprinus carpio) and rainbow trout (Oncorhynchus mykiss) to the immunomodulatory effects of UVB irradiation,” *Fish Shellfish Immunol.*, 2006, doi: 10.1016/j.fsi.2005.10.007.

[5] C. G. Otoni, R. J. Avena-Bustillos, B. Sen Chiou, C. Bilbao-Sainz, P. J. Bechtel, and T. H. McHugh, "Ultraviolet-B Radiation Induced Cross-linking Improves Physical Properties of Cold- and Warm-Water Fish Gelatin Gels and Films," *J. Food Sci.*, 2012, doi: 10.1111/j.1750-3841.2012.02839.x.

[6] D. L. Mitchell *et al.*, "Ultraviolet A does not induce melanomas in a Xiphophorus hybrid fish model," *Proc. Natl. Acad. Sci. U. S. A.*, 2010, doi: 10.1073/pnas.1000324107.

[7] M. K. Singh, J. Sharma, and R. Chakrabarti, "Effect of UV-B radiation on the defence system of Labeo rohita (Actinopterygii: Cypriniformes: Cyprinidae) larvae and its modulation by seed of devil's horsewhip, Achyranthes aspera," *Acta Ichthyol. Piscat.*, 2013, doi: 10.3750/AIP2013.43.2.04.

[8] Y. Fukunishi *et al.*, "Sub-lethal exposure to ultraviolet radiation reduces prey consumption by Atlantic cod larvae (Gadus morhua)," *Mar. Biol.*, 2013, doi: 10.1007/s00227-013-2253-1.

[9] D. L. Fabacher, E. E. Little, and G. K. O, "Research Articles: Biological Effects Tolerance of an Albino Fish to Ultraviolet-B Radiation," *Tetrahedron*, 1999.

[10] M. Llabrés *et al.*, "Impact of elevated UVB radiation on marine biota: A meta-analysis," *Glob. Ecol. Biogeogr.*, 2013, doi: 10.1111/j.1466-8238.2012.00784.x.

CHAPTER 13

AQUACULTURE NUTRITION AND FISHERIES: BIOTECHNOLOGY'S ROLE AND APPLICATIONS

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ABSTRACT:
This chapter talks about how biotechnology is changing the way we feed fish and manage fisheries. As more and more people want to eat seafood, we need to find ways to produce it that are good for the environment and don't harm wild fish populations. Biotechnology progress has become helpful in reaching these goals. The chapter starts by talking about how important it is to have good food for fish and other water animals that are being farmed. It says that balanced meals are needed to help them grow, stay healthy, and produce good quality products. Biotechnology is important in creating special types of animal food, making sure animals use the nutrients efficiently, and improving how well they convert food into energy. Additionally, this text talks about using biotechnology in fisheries management. It explains how genetic markers can be used to identify different fish stocks, assess the population, and create breeding programs to selectively breed fish with desirable traits. DNA fingerprinting and genomics can greatly change and improve sustainable fishing methods. The chapter also talks about how biotechnology in aquaculture helps the environment. It reduces the amount of waste produced and prevents diseases from spreading by making farmed species stronger against diseases. Furthermore, there are promising solutions to help reduce the strain on wild fish stocks. These solutions involve using biotechnology to create new protein sources, like microalgae and insect-based feeds. In short, this chapter talks about how biotechnology is changing the way we feed and manage fish, which helps make seafood production more sustainable, efficient, and environmentally friendly. This text highlights how biotechnological advancements can help us feed more people while also protecting our water ecosystems.

KEYWORDS:

Aquaculture, Biotechnology, Disease, Fish, Farming.

INTRODUCTION

The number of people on Earth is increasing quickly, and the amount of money people earn is also increasing rapidly in Asia. Most of the people who want to buy more fish in the future will be from the lower- and middle-income groups in these areas. Fisheries and aquaculture are important industries that produce food. They help ensure that people have enough to eat, contribute to the export of fish and seafood, and provide jobs for many people. Over 40% of fish, shellfish, plants, and algae that live in water are grown in many different fresh water systems around the world. The fishing industry has been steadily growing in the past 50 years, mainly through farming fish instead of catching them in the wild. This is because the amount of fish caught in the wild has stayed at around 90 million tons in the past 25 years. In 2018, fish farms produced 51. 3 million tonnes of fish, which was 62. 5% of all the fish produced for food around the world. The total amount of money is predicted to be $401 billion. Out of this, $250 billion is from aquaculture production, which is 82 million tonnes [1], [2].

This was a 5. 4% increase compared to the average of the previous three years. Using biotechnology to increase the production of aquatic species has the potential to help meet demand for seafood and improve aquaculture. Biotechnology is a type of science that uses

living things or organisms to make things. It is useful in fishing and fish farming because it helps scientists improve the quality and productivity of fish and shellfish. In simpler terms, biotechnology helps make farmed species grow faster, makes the food for aquatic animals more nutritious, makes fish healthier, helps save and protect the environment, and improves the preservation of wild animals. So, in order to develop and use biotechnology, we need to have a good understanding of biology, how organisms work, disease, how molecules interact, and genetics. So, this chapter explains how biotechnology is used in different areas of fish farming and how it will affect the future of fish farming in the present era.

India is one of the biggest producers of fish and seafood in the world. In 1980, it produced 0. 37 million tonnes of fish and seafood, but by 2010, it had increased to 4. This means that the production grew by 10 times. The industry has been growing at an average rate of 6% each year. The amount produced in 2017 was about 11. In 2018, people around the world caught a total of 96. 4 million tonnes of fish. This was mainly done in the sea and in rivers and lakes. In 2018 and 2018, the country produced a record-breaking amount of 14. 16 million metric tonnes of fish. The fishing industry adds 1. 24% to the overall economy and 7. 28% to the agricultural economy. In 2018, the Food and Agriculture Organization shared that Asia produces the most fish worldwide, accounting for 89% of the total production over the past 20 years. This rapid increase in aquaculture has greatly helped both traditional methods and new technologies. It is thought that new technologies in biology will continue to help the industry meet the worldwide need for seafood in the next few decades. Aquaculture is a new way to raise fish and other aquatic animals in many countries, which is becoming more popular than traditional livestock or crop farming [3], [4].

The methods used here provide effective tools for the long-term growth of fish farming, fishing, and the food industry as a whole. Biotechnologies are not used as much in fisheries management as they are in aquaculture. For many years now, biotechnology has made progress in giving us the means to change genes and chromosomes in living things. When biotechnology came about, people started using aquaculture's knowledge more to make it more successful and sustainable. The fish farming industry needs fish that grow quickly and are resistant to diseases. They also need affordable and effective vaccines, methods to diagnose diseases, cells that can be grown outside of a living organism, and good bacteria to promote fish health. Biotechnology can make aquaculture better by creating better types of fish, keeping them healthy, improving their genetic diversity, and using markers to study them. More and more people want to have aquaculture, and biotechnology can assist with meeting that demand. In simple words, the food fish that people eat all around the world is expected to increase to 165 million tonnes by the year 2030.

DISCUSSION

As fishing in the wild is almost fully used worldwide, a lot of the future increase in fish supply will come from fish farming. The main aim of fish nutrition is to make food that helps fish grow well, stay healthy, and be of good quality. This way, people can have safe and healthy fish to eat without spending too much money. It is important to find different types of fish and good food options to feed them. Researchers are studying how to safely use nutrients when we replace expensive fish meals and oil with other plant-based and marine substances. Biotechnology helps address technical and environmental issues related to fish farming, specifically focusing on the food that fish eat. It is expected that aquaculture farming will keep growing, and because food and feeding are very important for sustainable aquaculture, the cost of feed makes up about 40% to 50% of the total cost of aquaculture farming. The well-rounded and complete meal that provides good nutrition may continue to be important for raising fish. So, we should look for better feed ingredients and ways to manage ponds and make them more

productive using biotechnology. Biotechnology is being used to make fish food. Fish meal is a good source of protein from leftover fish after processing. This protein is commonly used in fish farming. However, using fish meals as a source of protein for fish also has some disadvantages. Firstly, it is expensive, so we need a less expensive choice for fish meals. Secondly, sometimes there is not enough fish meal available. Additionally, using fish meal for aquaculture can harm the environment and cause various environmental issues. This is mainly because it has a lot of phosphorus [5], [6].

When there is too much phosphorus in water, it can cause algae to grow, which is called eutrophication. So, replacing fish meals with any other type of protein from plants could be the best way to fix this problem. Currently, around 20 million tons of specially made fish food are being used in fish farming, with most of it being eaten by the fish being farmed. If aquaculture continues to grow quickly, the amount of food needed will increase by a lot. So, we need to know more about science and do more things to make sure we can keep doing aquaculture in a way that lasts a long time in the country. Biotechnology is used to address problems related to fish food, specifically technical and environmental issues. Scientists are using biotechnology to create different plant-based proteins and insect larva as alternative sources. The oil from black soldier fly larvae can be a replacement for fish or soy oil in the diet of young rainbow trout. Similarly, Hossain and their team. Researchers found that adding insect meal made from black soldier fly larvae (BSFL) to soy-based diets helps rainbow trout grow better. Biotechnology is used a lot in taking care of fish health [7], [8].

Monoclonal antibodies are useful to find pathogens quickly. Antibodies that target different fish species can be used to track the response to vaccines. Monoclonal antibodies can also be used to check if brood stock have been exposed to pathogens before. New scientific methods like PCR, real-time PCR, and nucleic acid sequence-based amplification can now be used to find, recognize, and measure small amounts of aquatic diseases. These techniques are very helpful in studying issues related to waterborne diseases. Microarray technologies enhance the ability to simultaneously screen for multiple pathogens and responses from hosts. Recombinant DNA technology helps make vaccines in large amounts and at a low cost. In the future, DNA vaccination, proteomics, adjuvant design, and oral vaccine delivery will also help improve fish vaccines. One of the biggest advancements in biotechnology is vaccination. Vaccination is a process where a specific vaccine is made to protect against a certain disease. It includes how the vaccine is given, when it is given, and if it needs to be given again later. People in the aquafarming industry have started using the information and methods of vaccination.

The use of vaccines in aquafarming has been very important in keeping fish healthy and preventing diseases. This has helped increase the amount of fish being produced worldwide to meet the growing need for food for humans. In aquaculture, scientists have made vaccines to fight against bacteria. Some vaccines are made by killing tiny organisms, while others are made using proteins, genetically modified organisms, and DNA. These new vaccines are still being worked on. New research has found that using certain substances like lipopolysaccharides, peptidoglycans, or glucans can activate our body's natural defense system. Some substances, like glucan and levamisole, can make fish's immune system work better. They help the fish's cells eat harmful things and make antibodies to fight off infections. The newest technology is also very important in making vaccines and substances that strengthen the immune system for fish and other aquatic animals. These helps prevent diseases by using vaccines. Fish vaccines are a good and cheap way to control illnesses in farm-raised fish [9], [10].

This method has been used for about 20 to 30 years and has been proven to work well. The vaccines can be given to animals by adding them to their food, letting them soak in it, or by injecting them directly. Scientists are making vaccines for fish by changing their genes to

protect them from diseases. The main problem that is stopping the growth of aquaculture is diseases. Biotechnological tools like gene probes and PCR have the potential to help fish stay healthy by preventing infections. Researchers have created tools called gene probes and PCR-based diagnostic methods to help identify diseases that harm fish and shrimp. In finfish farming, scientists have made various vaccines to protect against bacteria and viruses. However, a new type of vaccine is being worked on right now. It is made up of protein subunit vaccines, genetically modified organisms, and DNA vaccines. Biotechnology can help with keeping diseases under control in aquaculture. When diseases break out, they can cause a lot of problems for fish farming, like fish dying and growing slower. It has been noticed that disease is the biggest problem for producing a lot of shrimp, salmon, carp, and tilapia. Sometimes, up to 90% of the fish can die because of it.

Aquatic animal diseases are easier to spread through water than diseases in land animals. Controlling these diseases is also challenging because aquatic animals are kept in crowded conditions in water. The disease happens in all types of systems, from big to small, although there may be significant losses in small-scale production systems. Intensive and semi-intensive fish farming can greatly impact the water quality where the animals are being raised. Low quality water can lead to more diseases and environmental issues, like too many nutrients in the water, not enough oxygen, and too much algae. This can cause many deaths in the aquatic animals. So, we need a different and better way to take care of animals and help them grow and stay healthy. This will also help keep diseases from spreading too much. There is a bigger need for managers to get involved in intensive systems. Biotech tools can be useful in managing things. They have many uses like working as sensors in factories, managing waste, and detecting and controlling diseases. Usually, diseases are not controlled until after many deaths have been observed. In the past, fish diseases were mainly diagnosed by looking at the tissues under a microscope and studying parasites, bacteria, and viruses found during autopsies and in the laboratory. These techniques have been proven to be effective. However, we need people who are very skilled and it takes a lot of time to do this work well. This work cannot be done automatically. Because of these reasons, even though it requires special training, PCR technology has become an important tool for checking pathogens in developing countries. For instance, it is used in the shrimp industries of Asia and Latin America.

In the 1980s, because of major advances in biology and techniques for manipulating DNA, scientists were able to isolate individual genes, combine them into small circular DNA molecules called plasmids, make many copies of them in the lab, and then transfer them into tiny organisms for studying how they work. By using this new technology, scientists believed that when they put a foreign gene into an animal cell or fertilized egg, it would become part of the cell or egg's genome and do its job as the cell grows and changes. Some people believed that, in certain situations, the joined genes could stay in the reproductive cells of the grown animals and then get passed on to future generations. They also think that because we know what genes do, the genes that are transferred into animal eggs must have a specific purpose. So, when these foreign genes are turned on and cause changes in the animal's appearance, they can predict how those changes will affect the animal as it grows. The animal created through this process is called a 'transgenic animal. " In theory, this method appears to be a good and perfect way to produce new breeds of animals. Transgenesis or transgenics means putting genes/DNA from outside into the genes of an organism. This makes the new genes become a permanent part of the organism's genes, and they can be passed on to the next generations and used by the organism.

This method is a great chance to change or make better the genetic traits of fish, shellfish, and seafood that are important for farming. Gene transfer is important for improving fish

populations used in fish farming and is also useful for studying how genes are controlled in living cells or organisms. Palmiter and others Scientists first discovered a special kind of mouse, called a "super mouse," by adding a growth hormone gene to the eggs of regular mice. Afterwards, other writers mentioned that some genetically modified chickens, cows, pigs, rabbits, and sheep were also created. Some writers want to use gene transfer in fish eggs to study the effects of changing genes and controlling gene expression while the fish is growing. In these situations, fish that have a known genetic background, a short life cycle, and eggs that work well for experiments in controlled conditions have been chosen. Biotechnology can be used to help manage the environment and clean up pollution. People have criticized aquaculture for not being sustainable and harming the environment. While there have been some cases where aquaculture has not met global sustainable development expectations, these claims are not entirely baseless. Most aquaculture practices are done sustainably and with a focus on protecting the environment. It is important to think about reducing the negative effects of waste water discharge, making water cleaner, and using water responsibly when developing aquaculture projects.

Scientists are using various biotechnologies to solve different problems. For example, they use a process called bioremediation to break down dangerous waste. They also use vaccinations and probiotics to reduce the need for antibiotics. Additionally, they use DNA-based methods to quickly detect harmful algae that produce toxins. The ways diseases spread in fish farming are closely related to the environment they are in. Additionally, a method called bioremediation has been used in aquaculture due to the unique way it works. This means that using probiotics can help prevent harmful bacteria from growing and causing sickness. Getting vaccinated against disease is something that many people do. But fish have a weak immune system. Biotechnology can help create molecules that can make the immune system of shrimp stronger. New research has found that the body's defense system can be improved by using substances from microbes like lipo polysaccharides, peptidoglycans, or glucans.

Some substances called glucans and levamisole can help fish's immune system work better by making certain cells in their body do a better job of eating harmful things and making special proteins called antibodies. Water-dwelling creatures are more easily affected by their surroundings than those that live on land. They need water to get oxygen and essential chemicals and the water also carries their waste products and pollution from the nearby environment. To effectively control and treat diseases in aquatic animals, it is important to have testing facilities that are fast, reliable, and sensitive. DNA-based technologies are now being used more often to identify different types and variations of diseases. These are used to help keep fish healthy by selecting fish that are naturally more resistant to diseases and by using special methods to study and identify disease-causing agents at a molecular level. In many cases, pathogens are grown directly in a laboratory. But these methods take a lot of time and money, and there hasn't been a practical way to grow viruses available. These problems have led to the development of immunoassay and DNA-based diagnostic methods and PCR amplification techniques.

Biotechnology uses living things to create various items. It has grown quickly because we can now change an organism's DNA. Modern biotechnology affects every part of our daily life. It impacts the food we eat, the safety of our drinking water, the clothes we wear and clean, the medications we use, and the fuel we put in our cars. In aquaculture, biotechnology refers to the use of man-made hormones to help fish reproduce, producing populations of fish that have only one sex, come from only one parent, or have more sets of genetic material than normal. It also involves using molecular biology to study fish genetics and create fish with altered genes. Biotechnology in aquaculture also includes storing fish genes, creating better fish food and

ways to keep the fish healthy, and developing useful products from underwater organisms. New discoveries in feed for aquaculture are looking good and will help make the system more diverse. Aquaculture biotechnology is getting better, allowing us to produce strong, fast-growing, and high-quality water creatures in a way that is good for the environment. Biotechnology is being used in aquaculture and it is making a big impact. But, it is important to use it along with existing technologies instead of replacing them with this one. Although this place has the potential to increase fish production, it should be used based on the need for fish rather than just using modern techniques. In summary, the use of biotechnology in aquaculture has many benefits and is the most important factor in the industry's growth. In simple words, developing countries can use ways that are based on what people want, instead of just using technology, when they start using biotechnology in aquaculture. This will help them make more money and become self-sufficient. Furthermore, to fulfill their social responsibility, companies should clearly label DNA-vaccinated or hybridized products so that consumers can easily know what they are buying.

CONCLUSION

In summary, using biotechnology in aquaculture and fisheries can greatly change how we produce seafood and protect the environment in the future. Biotechnology has the potential to greatly improve the way fish are fed in aquaculture. It can help create diets that are specific to each fish's needs, make better use of nutrients, and increase the efficiency of how feed is converted into fish growth. These improvements make fish farming better for the fish and help make it more efficient and sustainable. In fisheries management, biotechnology provides helpful tools for identifying fish populations, assessing their numbers, and creating breeding programs that focus on specific traits. Genetic markers, DNA fingerprinting, and genomics help gather important information to make smart choices that protect wild fish populations and make sure they survive for a long time. Furthermore, biotechnology helps protect the environment by decreasing the amount of waste produced and preventing the spread of diseases in farm animals by making them more resistant to illnesses. New and creative ways of using biotechnology, like making protein from plants and bugs, can help us rely less on fish that are becoming fewer in number. As more and more people want to eat seafood, scientists are using biotechnology to help make enough food without harming the environment. Collaborating on research, implementing it responsibly, and having rules in place will be important in fully using biotechnology to create a more sustainable and strong future for aquaculture and fisheries. This chapter emphasizes how important it is to constantly come up with new ideas and adjust to changes in order to meet the needs of a changing world.

REFERENCES:

[1] S. H. Thilsted *et al.*, “Sustaining healthy diets: The role of capture fisheries and aquaculture for improving nutrition in the post-2015 era,” *Food Policy*, 2016, doi: 10.1016/j.foodpol.2016.02.005.

[2] B. Belton, I. J. M. van Asseldonk, and S. H. Thilsted, “Faltering fisheries and ascendant aquaculture: Implications for food and nutrition security in Bangladesh,” *Food Policy*, 2014, doi: 10.1016/j.foodpol.2013.11.003.

[3] C. Béné *et al.*, “Feeding 9 billion by 2050 – Putting fish back on the menu,” *Food Secur.*, 2015, doi: 10.1007/s12571-015-0427-z.

[4] B. Belton and S. H. Thilsted, “Fisheries in transition: Food and nutrition security implications for the global South,” *Global Food Security*. 2014. doi: 10.1016/j.gfs.2013.10.001.

[5] L. Bernatchez *et al.*, "Harnessing the Power of Genomics to Secure the Future of Seafood," *Trends in Ecology and Evolution*. 2017. doi: 10.1016/j.tree.2017.06.010.

[6] N. Kawarazuka, "The contribution of fish intake, aquaculture, and small-scale fisheries to improving food and nutrition security: A literature review ," *WorldFish Work. Pap. No.2106.*, 2010.

[7] M. C. M. Beveridge, S. H. Thilsted, M. J. Phillips, M. Metian, M. Troell, and S. J. Hall, "Meeting the food and nutrition needs of the poor: The role of fish and the opportunities and challenges emerging from the rise of aquaculturea," *J. Fish Biol.*, 2013, doi: 10.1111/jfb.12187.

[8] J. A. Gephart, L. Deutsch, M. L. Pace, M. Troell, and D. A. Seekell, "Shocks to fish production: Identification, trends, and consequences," *Glob. Environ. Chang.*, 2017, doi: 10.1016/j.gloenvcha.2016.11.003.

[9] R. G. Richards *et al.*, "Effects and mitigations of ocean acidification on wild and aquaculture scallop and prawn fisheries in Queensland, Australia," *Fisheries Research*. 2015. doi: 10.1016/j.fishres.2014.06.013.

[10] U. R. Sumaila, C. Bellmann, and A. Tipping, "Fishing for the future: An overview of challenges and opportunities," *Mar. Policy*, 2016, doi: 10.1016/j.marpol.2016.01.003.