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REVIEW PAPER

Selected topics in sustainable aquaculture research: Current and future focus

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Abstract

Over the last few decades, aquaculture has undergone a dramatic expansion in production, becoming a key source of food for people in many countries. Indeed, aquaculture has become extremely important for food security. However, the rapid expansion has led to many concerns, such as the effects of water shortages, pollution, disease and the depletion of natural fish stocks used as protein and fat sources for aquaculture diets. Against this backdrop, there has been a growing awareness of the need for sustainability to ensure the long-term future of aquaculture. Thus, there have been tremendous efforts made to incorporate the latest procedures to ensure sustainability. For example, the industry has not been slow to address the benefits of polyculture, offshore rather than coastal sites for mariculture, the use of aquaponics and land-based recirculation systems, and improved disease management, including mitigation against the adverse effects of pollution, such as the use of biofloc technology. The therapeutic approach to disease control has moved towards prophylaxis, notably immunoprophylaxis and the use of probiotics and phytobiotics. Unfortunately, there are challenges resulting from the effects of environmental change, i.e. global warming. Some solutions have been found by use of new technologies, including nanotechnology. All these aspects are considered in this review.

Introduction

Sustainability reflects the ability of a society, ecosystem, or any ongoing system of this type to continue to function in the future without declining through depletion or overuse of key resources on which the system depends. The concept of sustainability is simple and important, but it is difficult to translate into specific standards or criteria (Frankic and Hershner, 2003; Troell et al., 2009; Belton, 2020).

The sustainable use of water and natural fishery resources has become an important issue for management and regulators in many countries (e.g. Biodiversity Strategy COM/98/0042, EC, 1998; Water Framework Directive 60/2000/EC (WFD), EC, 2000); Offshore Proposed Fisheries, Law; NOAA, 2006); Maritime Directive 2008/56/EC; EC, 2008). These considerations significantly increase the attractiveness of a simulation modeling alternative (Whitmarsh et al., 2006; Troell et al., 2009; Troell et al., 2013). Lastly, the Food and Agriculture Organization of the United Nations (FAO, 2022) draws the projection how to achieve a sustainable planet in the study “Sustainability in Action.” The importance of the research to be carried out for the transfer of knowledge has become the focus of the scientific world (Kanazawa, 1997; Lawrence and Lee, 1997).

Aquaculture is a sustainable strategic sector that contributes significantly to food security and future supply of nutritious protein required for the rapid increase in human global population, and promoting economic development. Aquaculture provides employment often in rural areas with otherwise limited opportunities, and, when used correctly, contributes to the ecological services offered by the environment (Massa et al., 2021). According to FAO, the contribution of aquaculture to global food security and supply has been broadly demonstrated by industry growth of a staggering 7.5% annually since 1970. In 2018, aquaculture reached an all-time high production of 114.5 million tonnes of live weight with a total farm gate sales value of 263.6 billion USD. This has made aquaculture a key player in the Blue Growth concept, and a strong contributor to some of the Sustainable Development Goals (FAO, 2022)

The most serious concerns in aquaculture are:

(1) destruction of wetlands and other sensitive aquatic habitats by aquaculture projects

(2) conversion of agricultural land into ponds for commercial production

(3) water pollution from effluent discharge of aquaculture waste

(4) overuse of antimicrobial coompounds, including antibiotics, disinfectants and other chemicals for the control of aquatic animal diseases

(5) inefficient use of fishmeal and other natural resources for commercial fish and shrimp production

(6) salinization of soil and natural fresh and marine water from aquaculture discharge effluents, seeps and sediments from brine pools

(7) excessive groundwater use and other freshwater sources to fill and then the required water exchange for aquaculture production

(8) spread of diseases from cultured to native populations

(9) the adverse effects on biodiversity of the escape of non-native species introduced from aquaculture, the extermination of birds and other predators, and the introduction of aquatic organisms into natural waters

(10) conflicts with other resource users and disruption of close communities (Boyd, 2003).

Last year, FAO released the latest edition of its biannual "World Fisheries and Aquaculture" report in its "State of the World" series. While the organization continues to focus on meeting the Sustainable Development Goals set in 2015, the 2020 edition is devoted to "Sustainability in Action."

According to the FAO (2018) fisheries and aquaculture report, it is stated that the total production of fishery products will increase by 28.1% in developed countries, 37.2% in developing countries, and 46.3% in underdeveloped countries in the 2030 projection compared to 2016. In the same report, it is estimated that the production amount from aquaculture will reach 110 million tonnes in 2030 (FAO, 2018). Also, partial to ideally complete fish meal replacement in fish and shrimp food for commercial production is critical (Lawrence et al., 2022).

It is obvious that the world's cultivation must be done with environmentally friendly sustainable methods (Lawrence et al., 2001).

Sustainable Aquaculture Practices may be listed as follows:

a. Polyculture, integrated multi-trophic aquaculture and aquaponics

b. Biofloc: production information for sustainable commercial aquaculture development

c. Energy gain

d. Nanotechnology applications

e. Useful micro-organisms in sustainable aquaculture (including biological control agents, probiotics, prebiotics and phytobiotics)

f. Immunological approach to sustainable aquaculture

g. Fish welfare

h. Site selection and carrying capacity assessment of aquaculture

ı. Recirculation system for sustainability

j. Eco-friendly feeds and sustainable nutrition

k. Offshore mariculture

l. Water quality management in aquaculture research

m. Stock enhancement

n. Spatial planning

o. Climate change

p. Use of ozone in aquaculture

q. Plant based anesthetics

a. Polyculture, Integrated Multi-trophic Aquaculture and Aquaponics

Studies with species combinations are conducted currently to a great extent with the objective of obtaining benefits that will arise from the interaction of aquatic and/or terrestrial organisms with each other. These can be grouped under two main headings; the first is Polyculture and Integrated Multi-trophic Aquaculture, and the second is Aquaponics and their combinations.

Polyculture and Integrated Multi-trophic Aquaculture

In summary, these aquaculture techniques are based on the cultivation of similar and/or different kinds of "aquatic" organisms that have potential to benefit each other in a freshwater or saltwater (e.g. natural and artificial seawater typically from 1-40 mg/L salt) environment. The technique on which research has focused in recent years is integrated multi-trophic aquaculture in earthen or lined ponds and in natural waters. For indoor aquaculture production, the word polyculture should also be considered because either clear water, biofloc or a combination of the two technologies use recirculating aquaculture systems (RAS) and/or filter natural or underground waters.

Integrated multi-trophic aquaculture (IMTA) is an ecosystem approach production technique that has been demonstrated to solve the marine pollution problems that may arise with aquaculture (Troell et al., 2009). The IMTA system is a technique that emerged from the idea that waste from uneaten feed, feces and metabolic excretion of one species is a useful input for the growth of another species, and also has a natural self-cleaning mechanism that is environmentally sustainable (Chopin et al., 2001). There are several options available to reduce the nutrient load from aquaculture, including improving animal feed use and treating wastewater with biological filters. Most of the applications have been made using integrated multiple-trophic systems. Environmental concerns about rapid expansion of intensive aquaculture systems have also led recently to renewed interest in IMTA (FAO, 2006). The cage system benefits from the filtered water and is the focus of studies for IMTA systems. Reid et al. (2010) investigated the absorption efficiency of blue mussel *Mytilus edulis and M. trossulus* on the diet of Atlantic salmon feed and feces particles, and found that the technique of rearing these organisms in close proximity to salmon cages in IMTA systems would be a useful practice for removing solid waste. As a result, it seems reasonable that producers could integrate their mussel production into fish farming to reduce the negative ecological impacts of farming, and it has the potential to become a valuable crop for farmers (MacDonald et al., 2011).

Siccardi et al. (2006) reported increased growth by shrimp eating feces from polycultured sea urchins using clear filtered seawater in indoor tanks. Jensen (1991) reported increased growth of shrimp ontside cages containing sea urchins when polycultured using clear artificial seawater in indoor tanks. Moreover, Rubino et al. (1983) obtained lower growth and production in indoor tanks using clear filtered natural seawater when polyculturing *Litopenaeus stylirostris* (Pacific blue shrimp) with *Farfantepanaeus aztecus* (Gulf of Mexico brown shrinp). However, Luszcynski et al. (1988) obtained greater growth for *L. vannamei* (Pacific white legged shrimp) and

L. stylirostriis polycultured in cages in earthen ponds using natural seawater. Furthermore, Hutchins et al. (1981) showed that a ratio of 4:1 to 5:1 *L. vannamei* to *L. stylirostris* gave the greatest production in earthen ponds using natural sea water.

In southeast Asia two to three species of freshwater carp could be cultured together with greater production using earthen and lined ponds and outoor tanks. However, crustaceans are known to bioaccumulate human pathogenic organisms, including *Vibrio* spp., hepatitis A virus, human sapovirus, and adenovirus. Several investigations point to the potential for shellfish to serve as reservoirs for fish pathogens. Therefore, the integration of shellfish into fish farms, as in IMTA, is thought to potentially change the infection dynamics for fish pathogens (Pietrak et al., 2012).

Sea cucumbers and abalone are valuable species that are candidates to be cultured with appropriate species that can consume fish, other aquatic animals (e.g. polychaetes and copepods), bacteria, protozoa, microalgae, and macroalgae in IMTA systems. However, many investigations have focused on land-based systems, and, to date only a few have explored possibilities of open water IMTA farming. Over the last 15 years, the integration of seaweeds with marine fish farming has been researched in Canada, Japan, Chile, New Zealand, Scotland and the USA. Also, the integration of mussels and oysters as biofilters in aquaculture has been studied in numerous countries, including Australia, USA, Canada, France, Chile and Spain. Recent reviews of IMTA research include focusing on seaweeds, bivalves, crustaceans, and integrated cultures from a coastal zone management perspective. Some integrated agricultural environmental systems by creating natural fishery products are very important in terms of the protection of ecosystems as well as economic development. Studies on this subject have also shed light on future ecological and sustainable fisheries research.

Eating habits (including carnivorous versus herbivorous, time of day the species is active, species role in the ecosystem, harvesting and processing methods, and marketing information) must be considered in selecting the species for poluculture and IMTA. By not fully considering the preceding aspects will lead to a greater probability that a negative return on investment will be obtained.

Aquaponic combination

An example is the use of waste water from aquaculture (e.g. shrimp) which is used to supplement the nutrient requirement of water used to raise vegetables, such as lettuce, by hydroponics. This was developed to the level that lettuce produced with this system was sold commercially.

In summary, these aquaculture techniques are based on raising similar or different species of "terrestrial and aquatic" living things together, which have the potential to benefit each other in freshwater or with water typically having <2 mg/L salinity. The technical focus of research in recent years is aquaponic aquaculture. Hydroponics means agriculture with water without using soil. In this production technique, production is made using only water containing nutrients required for plant production. Aquaponics uses solids in waste water from aquaaculture production systems as nutrient supplements for the production of agriculture crops. If discharge waste water is used as irrigation water for the production of agricultural crops it will supplement the agricultural crop nutrient requirement required from the added commercial fertilizer. This would reduce the fertilizer cost for irrigated agricultural crops. At the same time, the use of groundwater having a salinity <2 mg/L is reduced further lessening cost, and is environmentally friendly. In this regard, aquaponics is an innovative, environmentally friendly and sustainable agricultural production system created by the integration of land based agriculture and aquaculture, which is made by using underground water in regions where access to water is difficult or unfavorable, allowing soil-less agricultural production as well as fish farming (Love et al., 2015; Shete et al., 2016). However, there are areas, such as the desert in Arizona, USA, having <2 mg/L natural underground water which may be used to commercially produce shrimp in raceways and earthen ponds (Samocha et al., 1999; 2001; 2004). The low salinity underground discharge water from the production shrimp in raceways and ponds contain waste which supplements the nutrients from the commercial fertilizer added to the irrigation underground water and at the same time reducing the amount of irrigation low salinity underground water required for production of hard red wheat. This may be done commercially in Arizona because the soil characteristcs prevent continued salt accumulation in the irrigated soil.

b. Biofloc: Production Information for Sustainable Commercial Aquaculture Development

Biofloc technology was developed as a consequence of restriction of water exchange because of costs and environmental regulations, and as a means to provide bio-secure systems to minimize disease (Avnimelech, 2009). Bioflocs have a diameter ranging from 0.1 mm to several mm (Avnimelech, 2009), and comprise a consortium of microorganisms consisting of primarily bacteria, single cell protein (SCP), micro/macro invertebrates, filamentous organisms, exocellular polymers (e.g., nucleotides, vitamins, amino acids, and oligopeptides), microminerals, uneaten feed and is free of deleterious levels of anti-nutritional factors (Kuhn et al., 2011; 2012, Logan et al., 2010).

Micro-organisms play four major roles in biofloc systems: (i) maintenance of water quality, by the uptake of nitrogen compounds generating *in-situ* microbial protein; (ii) reduction of water usage and reuse of water over multiple culture cycles; (iii) nutrition, increasing culture feasibility by reducing protein, vitamin, phosphorus and micromineral levels in the diet and the feed conversion ratio (FCR) thus significantly reducing feed costs; (iv) competition with pathogens (biosecurity); (v) boosting the health status of cultivated species by improving the immune system and resistance against infections and stress and (vi) sequestration of greenhouse gases (GHG) (Velasco et al., 2000, 2001; Velasco and Lawrence, 2001; Wasielesky et al, 2006; Hargreaves, 2013; Esparza-Lel et al., 2015; Manan et al., 2016; Wang et al., 2016a; 2016b; Liu et al. 2017; Emerenciano et al., 2017; Ferreira et al, 2020; Ogello et al., 2021). The basic principle of biofloc systems is removal of nitrogenous compounds by heterotrophic and chemoautotrophic bacteria, and the process depends on the Carbon and Nitrogen ratio present in the system (Avnimelech, 1999). The required C:N ratio is ~15-20 for ammonia assimilation by heterotrophic bacteria (Avnimelech, 1999; Asaduzzaman et al., 2008). The ratio is realized by the addition of a carbon source being one of the crucial processes for the success of the technology (Ebeling et al., 2006; Browdy et al., 2014). Different types of organic carbon sources applied in biofloc systems are often by-products derived from human and/or animal food industry, preferentially cheap and locally available (e.g., glucose, acetate, starch, wheat, glycerol and molasses) (Deng et al., 2018).

Bioflocs in culture water provide a medium upon which both chemoautotrophic and heterotrophic bacteria colonize (Avnimelech, 2009). Autotrophic nitrification is a two-step process in which ammonia is biologically oxidized into nitrate and then to nitrate (Rittmann and McCarty, 2001). Nitrifying bacteria within the biofloc perform the function of a biological filter in a recirculating system (Avnimelech, 2009). An adequate amount of biofloc is essential to maintain good water quality in reduced to zero water exchange aquaculture production systems.

Bioflocs may supplement the nutritional requirement of shrimp. Retention of nitrogen in shrimp contributed by biofloc can be significant (Burford et al., 2004). Biofloc recycles un-utilized feed primarily by heterotrophic microbial processes forming microbial protein. Nitrifying bacteria, through the chemo-oxidation process, convert only about 10-14% resulting in production of material, as compared with 50% in heterotrophs (Avnimelech, 2009). Biofloc or bacterial based single cell proteins (SCP) can be produced by either *in-situ* or *ex-situ* technology (Kuhn and Lawrence, 2012; Kuhn et al., 2012). The production of *in-situ* biofloc in ponds (Logan et al., 2010) or tanks (Crockett et al., 2013; Crockett and Lawrence, 2017) by manipulating carbon to nitrogen ratio (C:N) in culture water have been reported. Under high C:N ratios (up to 23:1) heterotrophic bacteria are primary components of SCP or bioflocs in contrast to autotrophic bacteria using lower C:N ratios (down to 5:1). Aquatic animals such as shrimp can either graze on these bioflocs (SCP) for nutrition or bioflocs can be harvested from culture water and put into diets for aquatic animals. The production of *ex-situ* biofloc can be produced in suspended growth biological reactors (SGBRs) of which there are two types, sequencing batch reactors (SBRs) and membrane batch reactors (MBRs). SGBRs producing SCP (biofloc) have been used to treat aquaculture production waste (Kuhn et al., 2010) and shown either no effect or increased growth and production with partial to complete fish meal replacement (FMR) (Kuhn et al., 2008; 2009; 2010; 2016; Bru et al., 2019, Lawrence et al., 2022), and increased attractability in shrimp (Lawrence et al., 2022; Yuan et al., 2021). Also, Lawrence et al. (2022) reported that complete FMR with a bacterial based SCP significantly increased the body protein and decreased the body fat levels in shrimp. These results will significantly reduce fish meal usage by aquaculture resulting in making the global natural fisheries healthy and sustainable, and will increase the amount of edible healthy protein food to satisfy the nutritional requirements of the rapidly increasing human global population in the future.

In-situ bioreactors representing shallow water biofloc nurseries in tanks with 20 to 30 cm water depth were used successfully to produce juvenile *Litopenaeus vannamei*, Pacific white-legged shrimp, at the Texas AgriLife Research Mariculture laboratory in Port Aransas, Texas. The focus of simulated production trials was to develop methods for the commercial production of juvenile shrimp at inland sites where seawater is unavailable.

The reduction of salinity levels in water used within recirculating aquaculture systems may lead to the production of shrimp at lower costs because of the need for less sodium choride (Schuler et al., 2010). However, ammonia and nitrite toxicity increase as salinity decreases (Lin and Chen, 2001; Lin and Chen, 2003). High nitrate levels are detrimental to shrimp, especially at low salinity, as they may reduce growth, decrease survival, and cause negative effects on product marketability (Kuhn et al., 2010). Initial simulated production trials were carried out in full strength seawater (28 mg/L salt) with the objective to develop an acceptable minimal water exchange process, before testing it in low salinity water where the inorganic nitrogen level is more critical.

During initial biofloc trials, ammonia and nitrite levels were controlled throughout the entire production trial primarily through oxidation by nitrifying bacteria in autotrophic dominant biofloc (Crockett et al., 2012). A commercially available product (Fritz Turbostart, Fritz Aquatics, 500 North Sam Houston Rd. Bldg. B, Mesquite, TX 75149) was used to inoculate the system with nitrifying bacteria, and both ammonia and nitrite were kept at levels acceptable for shrimp culture in low salinity water. However, this technique resulted in nitrate nitrogen levels higher than optimal (90 mg/L) for low salinity shrimp culture. The end product of nitrification is nitrate nitrogen (Rittmann and McCarty, 2001). Attempts were made to dilute nitrates; however, the result was a spike in nitrite nitrogen. Water exchange rates above 30% per day wash more nitrifying bacteria out of a system than the amount required to maintain steady nitrification (Avnimelech, 2009). When nitrate nitrogen was diluted from 38 mg/L to 12 mg/L by addition of fresh water, nitrite nitrogen surged from < 1 mg/L to 10 mg/L (Crockett et al., 2012).

An option was to promote heterotrophic bacteria, so that ammonia and nitrites would be assimilated or reduced, rather than allowing nitrifying bacteria to oxidize these types of inorganic nitrogen to nitrates. A methodology was developed to control inorganic nitrogen by sequencing autotrophic and heterotrophic bacterial dominance (Crockett et al., 2013). In this process ammonia and nitrite nitrogen were initially controlled through oxidation, followed by inorganic nitrogen bacterial assimilation. The system was inoculated with nitrifying bacteria on day zero. On day 3 or 4, when enough suspended particulate matter within the water column had been established by unconsumed feed and shrimp feces to serve as media on which heterotrophic bacteria colonies could develop, heterotrophic dominance was promoted. A commercially available heterotrophic bacterial product (BiOWiSH AquaFarm, BiOWiSH Technologies, 2717 Eire Avenue, Cincinnati, Ohio 45208) was used to inoculate the system.

Organic carbon is necessary for heterotrophic bacteria to assimilate inorganic nitrogen for cell synthesis (Rittmann and McCarty, 2001). Nitrogen input was reduced at the same time organic carbon input was increased by decreasing the feed protein level. Organic carbon input was also increased by applying a carbon source to reduce the concentration of inorganic nitrogen in the production system.

Initially, the methodology was developed to keep inorganic nitrogen levels close to zero (Crockett, et al., 2013). However, it is desirable to have a residual level of nitrate nitrogen for proactive prevention of sulfates being reduced to hydrogen sulfide, should anaerobic pockets develop (Churchill and Elmer, 1999, US Peroxide, 2014). Low levels of nitrates (up to 35 mg/L) are not detrimental to shrimp, even at greatly reduced salinities (Kuhn et al., 2010). Less organic carbon application is required if low levels of nitrate nitrogen remain in the system. If less organic carbon is applied, there is less organic loading and production costs. A mathematical procedure was developed to quantify the amount of organic carbon required to leave a nitrate nitrogen residual of approximately 11 mg/L (Crockett et al., 2014; Crockett and Lawrence, 2017). Using stoichiometric analysis, it has been reported that heterotrophic bacteria require 6.07 g of organic carbon for each gram of total ammonia nitrogen (TAN) assimilated (Ebeling et al. 2006). `Also, it has been reported that there are no totally autotrophic and no totally heterotrophic systems, and there is always a mix between the two types of bacteria (Avnimelech, 2009).

When sequencing autotrophic and heterotrophic dominance, autotrophic bacteria were initially inoculated followed by promotion of heterotrophic bacterial dominance. It was assumed both autotrophic and heterotrophic bacterial populations were present, and that both oxidation and assimilation of inorganic nitrogen was occurring simultaneously (Crockett et al., 2014).

Ammonia nitrogen may be assimilated relatively easily by heterotrophic bacteria because it is more reduced than other forms of inorganic nitrogen. Nitrate and nitrite must be reduced by enzymes to ammonia before assimilation occurs, but all types of inorganic nitrogen can be incorporated into organic material by heterotrophic bacteria if organic carbon is available (Prescott et al., 1993).

The amount of organic carbon required for assimilation of TAN, NO_2-N, and NO_3-N was taken into consideration to control inorganic nitrogen. Organic carbon required to assimilate inorganic nitrogen is proportional to the ratio of carbon and nitrogen in microbial cells. It was assumed that the C:N ratio of bacterial biomass is

5.17:1. Potential microbial biomass that can be generated from inorganic nitrogen was projected using 9.46% nitrogen biomass content. Required amount of carbon to become bacterial tissue was estimated using 48.9% carbon biomass content (Crockett and Lawrence, 2017).

During metabolism some organic carbon is lost as carbon dioxide due to cellular respiration through catabolism and some carbon becomes microbial biomass through anabolism (Rittman and McCarty, 2001). Percentage of assimilated carbon with respect to metabolized feed carbon is defined as microbial conversion efficiency (MCE) and is in the range of 40-60% (Avnielech, 2009). It was determined that assuming a 60% MCE resulted in acceptable inorganic nitrogen removal and less organic loading (Crockett et al., 2014; Crockett and Lawrence, 2017).

What makes bioflocs composed of primarily bacteria-based single cell protein for sustainable commercial aquaculture development and the increase in growth and aquaculture production with no effect on survival above 90%? Kuhn et al. (2011) proposed that it was not due to soluble carbohydrate, mineral and fiber but is probably due to soluble protein, oligopeptides, dipeptides, amino acids, biogenic amines, prebiotics and probiotics, nucleotides, chemoattractants, stress and growth promoters, and/or stress resistant components of biofloc.

Of significance: (1) *in-situ* technology in tanks and ponds for bacteria based single cell protein (SCP) (biofloc) has been developed for water quality control or to provide nutrients for aquatic animal consumption of biofloc from culture water or including harvested biofloc from culture water into aquatic animal diets for consumption; and (2) more recently *ex-situ* echnology using suspended growth biological reactors has been developed for use both for cleaning up aquaculture production system effluents and for partial and complete fish meal replacement with increased growth and production and no decrease in survival. It is predicted that aquaculture production systems using bacteria based SCP technology will result in reducing fish meal usage by aquaculture resulting in making the global natural fisheries healthy and sustainable leading to significant production of badly needed quality protein food to satisfy the growing demands due to significantly increasing global human populations.

c. Energy Gain

Fossil fuels are the most important source of energy used today. These fuels, such as oil, coal, and natural gas, were formed with the decay of animals and plants that died millions of years ago under high heat and pressure. Research indicates that fossil fuel reserves, which meet a significant portion of the world's energy needs, will be depleted in the second half of this century. The energy demand is constantly increasing, and resources are decreasing, so it is vital to ensure that energy is used efficiently (Hall et al., 2014). The possibility that the world's fuel may run out in a short time increases the oil demand and the price of oil. Annual demand is more than four times greater than that of existing fuel, including new reserves emerging. According to the latest data obtained, the daily fuel oil consumption is 81 million barrels. Predictions show that this amount may increase to 121 million barrels per day in 2025. With the increasing demand, the largest fuel reserves will decrease by 4%-5% annually, and the oil need will not be met (Conti et al., 2009). Experts agree that only the visible part remains of the reserves, which were initially as large as an iceberg (Pahl and McKibben, 2005).

In addition to the depletion of oil reserves, another significant issue that we should not neglect is the rapidly spreading environmental pollution. Studies have estimated that the average temperature of the earth's surface has increased by 0.6 °C in the last century due to greenhouse gases, and the average temperature will increase by 1.4–5.8 °C by 2100. Greenhouse gases cause not only global warming but also affect the environment and human life. The oceans absorb only about a third of the CO_2 released by human activities each year, with the rest being released into the atmosphere. As the level of CO_2 in the atmosphere increases, the amount of solute in the oceans also increases, and the pH of the water becomes more acidic. This pH drop can result in a rapid loss of coral reefs and marine ecosystem biodiversity, significant impacts on ocean life and, consequently, on earth life (Ormerod et al., 2002). The information obtained from all these studies has prompted many scientists to prepare a climate agreement to make the world still livable in the future. In December 1988, at Malta's endeavor, the resolution on "Preserving the Global Climate for Present and Future Generations of Humankind" was adopted by the UN General Assembly. In the resolution, while the global climate is the common heritage of humanity, climate change is a common problem that was defined. Next, The United Nations Conference on Environment and Development was held in Rio in 1992. Then, the Kyoto Protocol, which was signed in 1997 and entered into application in 2005 and was valid until 2020, and the Paris Agreement, which entered into force in 2020 and limited global warming to 1.5°C, was signed (Erdoğan, 2018). One of the most important articles of the agreements signed to prevent climate change is related to renewable energy sources. It is an accepted idea that fossil fuels, which are non-renewable fuels because their formation takes many years, should now be replaced by renewable energy sources. One of the most desirable features of renewable energy sources is their sustainability. Renewable energy systems are known as clean energy because they stand out, particularly with their environmental friendliness and neutral effects in terms of ecological balance. Solar, wind, geothermal, hydraulic, wave, hydrogen, and biodiesel can be listed as clean and renewable energy sources. In particular, biodiesel obtained from plant sources is twice as beneficial to the environment. Because the plant sources used in biodiesel production convert the carbon dioxide from the atmosphere through photosynthesis and provide the carbon cycle, they do not increase the greenhouse effect. (Kann et al., 2002; Yüceer, 2003).

Biodiesel can be produced from oils extracted from various terrestrial plants such as soybean, canola, palm, corn, and coconut in many countries (Antolin et al., 2002; Felizardo et al., 2006). It is known that the danger of hunger is a threat to people in our rapidly growing world. While producing terrestrial plants, hectares of land are needed, and the danger of starvation will increase if the soil is used in the production of plants for biodiesel. The renewable and sustainable energy production is essential for a renewable and sustainable life. For this purpose, it will be ecologically and economically efficient to produce biodiesel from algae, where hectares of land are not needed, and soil is not used during its cultivation. Many small-scale studies exist on the production of biodiesel from algae. Many companies claim that they will economically produce biodiesel from algae within the next few years. However, there has not been enough production to replace fossil fuels yet. Therefore, more work is needed on this subject.

d. Nanotechnology Applications

The US National Nanotechnology Initiative (NNI) defined nanotechnology as the "understanding and control of matter at the nanoscale, at dimensions between approximately 1 and 100 nm, where unique phenomena enable novel applications" (Fajardo et al., 2022). Nanotechnology has great potential with applications encompassing many areas of science (Aibinu et al., 2022). The approach could deliver innovation to aquaculture systems leading to reduced costs, increased efficiency and a reduction in the impact to the environment. The outcomes could lead to improvements in the ability to provide nutrition to the growing human population of Planet Earth (Fajardo et al., 2022). The essential tools include nanomaterials, nanosensors, nanovaccines, gene delivery and smart drug delivery systems, which have the potential to resolve many issues related to health including disease control, production and reproduction (Elhamed et al., 2021). Nanotechnology has multiple applications in aquaculture, and could revolutionize the industry. Certainly, the applications could include the recognition and control of pathogens, water treatment, sterilization of ponds, and improved delivery of nutrients and medicinal compounds (Fajardo et al., 2022).

Nanominerals have higher surface area affinity, higher solubility, thermal resistance, low toxicity, slow excretion rate, and sustained release. These minerals may be beneficial for metabolic, physiological, and biological functions. For example, iron and zinc are important trace minerals in fish nutrition as they play a key role in various metabolic pathways such as prostaglandin metabolism and a structural role in nucleoproteins (Elhamed et al., 2021).

It can be argued that disease causes major obstacles for the sustainability and development of aquaculture (Fajardo et al., 2022). Aquaculture suffers substantial losses annually because of the presence of infectious diseases. The effective detection and control of disease is crucial for maximizing productivity, and ensuring the satisfactory quality of the final product for human consumption (Luis et al., 2019). Certainly, there is great potential for nanotechnology to provide novelty for disease diagnosis and health management. Some approaches involve solid core drug delivery systems, which coat solid nanoparticles with a fatty acid shell. This protects the medicinal substance. To date, nanoparticles have been used successfully with labile and thermo-sensitive inhibitory compounds (Fajardo et al., 2022).

There is extensive literature that supports nanotechnology in the effective delivery of dietary supplements and nutraceuticals. These systems enhance the bioavailability, bio-accessibility and efficacy of nutrients by enhancing their solubility and protection from the harsh conditions in the digestive tract. For example, it was determined that feeding common carp (*Cyprinus carpio*) with 1 mg of nano-selenium/kg of diet led to significant improvement in growth and the antioxidant defence system as compared to the controls (Shah and Mraz, 2020). Moreover, DNA nanovaccines, i.e. short strands of DNA within nanocapsules, have been used to induce immunity in fish. Moreover, iron nanoparticles accelerate development, which is linked to a programmed release of antimicobial compounds. For example, alginate, which is a naturally occurring polymer of β-D-mannuronic acid and α-L-guluronic acid in some brown algae and bacteria, may be used to produce nanoparticles by emulsification. A combination of chitosan-alginate has been used effectively for oral vaccination of rainbow trout (*Oncorhynchus mykiss*) against lactococcosis and streptococcosis, which are caused by *Lactococcus garvieae* and *Streptococcus iniae*, respectively, leading to improved survival after challenge and enhanced immunomodulation compared to the non-coated vaccine and the controls (Fajardo et al., 2022).

Aquaculture has faced increasing challenges with infectious diseases, and the presence of antimicrobial resistance (AMR) in micro-organisms. Thus, research on non-chemical approaches to controlling disease, such as the use of nanobubbles (NBs), which are <100 nm in diameter, has increased recently to reduce the risk of AMR and to address production losses caused by the emergence of pathogenic AMR bacterial strains. Recently, ozone nanobubbles (NB-O_3) have been demonstrated to reduce populations of pathogenic bacteria, improve dissolved oxygen (DO) in water, and modulate the immune systems of fish against (bacterial) infections (Dien et al., 2022). In addition, anesthetics have been utilized to reduce stress in fish. However, many anesthetics have been banned for use in aquaculture because of undesirable side effects and toxicity. As an alternative, some essential oils (EO) have shown anesthetic abilities in fish while having reduced toxicity and greater biodegradability compared to synthetic compounds. Yet, despite their useful properties, EOs have some important limitations for use in aquaculture. Therefore, new approaches are needed, of which nanoencapsulation has possibilities (Luis et al., 2019). The efficient detection and control of diseases is, therefore, of importance for maximizing productivity and ensuring the satisfactory quality of the final product. Use of nanobiosensors offers an innovative way to resolve some of the existing problems. These sensors, which may be based on different nanomaterials, e.g. carbon nanotubes, permit detection of low concentrations of bacterial and viral pathogens and parasites, and pollutants (Luis et al., 2019). Many metal nanoparticles (NP), such as silver, titanium and copper, have been considered for use in disease prevention and treatment. These compounds have various modes of action against bacteria, of which one of the strongest is against the cell membrane and cell wall as a result of electrostatic interaction leading to disruption of the microbial cells. Colloidal silver NPs comprise one of the principal nanotechnology products used against a wide spectrum of microbial pathogens and parasites. The inhibitory activity reflects the highly damaging oxidation capacity to DNA and proteins. For example, silver NPs are capable of inactivating the human superbug, methicillin-resistant *Staphylococcus aureus* (Fajardo et al., 2022).

The principal attributes that confer advantages to the nanomaterials include high absorption and bioavailability, better dispersion and solubility, improving stability against environmental degradation during food processing, and controlled release kinetics. Additionally, the use of nanomaterials for delivery systems may improve the nutritional profiles of feed and the food conversion rate. These advantages improve efficiency, reduce waste and financial burden, and improve yield and quality of the farmed product. The delivery of molecules via nanotechnology may be more effective in controlling diseases through the precision of delivery and the controlled release of therapeutants and prophylactants thereby decreasing the risks associated with health and environmental factors, and reducing the need for chemicals. Nanoparticles for targeted delivery may facilitate new methods of administering inhibitory compounds. These methods could be faster, non-intrusive and more cost effective than conventional approaches. Furthermore, disease control methods that combine diagnostics and therapy in a single step (= theragnostics) would improve the effectiveness of treatments and substsantially lower the costs (Fajardo et al., 2022).

e. Useful Microorganisms in Sustainable Aquaculture (including Biological Control Agents, Probiotics, Prebiotics and Phytobiotics)

From therapy with antimicrobial compounds particularly antibiotics and chemicals/ disinfectants to prophylaxis with vaccines, disease control strategies have evolved to include use of probiotics, prebiotics, postbiotics, symbiotics, nonspecific immunostimulants and phytobiotics/herbal medicine and their by-products (e.g., Butt et al., 2021; Mugwanya et al., 2021; Pereira et al., 2020; 2021; Silva et al., 2021). The beneficial effects centre on improved growth, immunostimulation and protection against bacterial and parasitic diseases.

Development of probiotics started with the work of Elie Metchnikoff, who while working in Bulgaria during 1907 noted the longevity of impoverished individuals. These people were observed to consume diets rich in fermented milk products. From this work, *Lactobacillus bulgaricus* was recovered, and recognized as the first probiotic (see Ozen and Dinleyici, 2015; Gasbarini et al., 2016). A current definition of a probiotic centers on live micro-organisms, which are administered in food and exert a beneficial effect to the host particularly the intestinal microflora (Fuller, 1989; 1992). Use of probiotics has extended from human to agricultural (Hossain et al., 2017) and aquacultural contexts (e.g. Austin and Sharifuzzaman, 2022).

Human and agricultural use of probiotics has involved the use of predominantly Gram-positive lactic acid producing bacteria, i.e., tentatively associated with lactobacilli which include representatives involved in the production of yogurt (Hossain et al., 2017; Hungin et al., 2018; Sanders et al., 2018). However, aquaculture has considered use of a far greater range of organisms, encompassing Gram-positive and Gram-negative bacteria, micro-algae, yeasts and bacteriophages. Certainly, there is concern over the use of Gram-negative bacteria, e.g., *Aeromonas hydrophila,* from taxa associated with diseases of aquatic animals (Austin and Sharifuzzaman, 2022). The issue surrounds the theoretical possibility of some Gram-negative bacteria resorting to pathogenicity, namely by the acquisition of virulence (Austin and Sharifuzzaman, 2022) and/or antibiotic resistance genes (Patel et al., 2012). Yet, this has not ever occurred in practice.

The first use of a probiotic in aquaculture involved endospores of *Bacillus toyii*, which improved growth of yellowtail (*Seriola quinqueradiata)* and conferred resistance of Japanese eel (*Seriola quinqueradiata)* against edwardsiellosis (Kozasa, 1986). Since then, many Gram-positive taxa including the lactic-acid bacteria, i.e., *Lactobacillus,* endospore-forming *Bacillus* and Gram-negative representatives, notably *Aeromonas* and *Vibrio* have been evaluated singly or in combinations of two or more organisms orally or via water in a multitude of aquatic animal species (Hoseinifar et al., 2018; James et al., 2021; Austin and Sharifuzzaman, 2022). Preparations may be viable (e.g., Mani et al., 2021) or deliberately or accidentally nonviable (paraprobiotics; Luo et al., 2021; Tran et al., 2022); viability is not always determined by researchers. Dosages range typically from 10^6-10^9 colony forming units/g of feed with application typically in the range of 14-84 days (Cerezo et al., 2022). The recipient farmed animals include finfish, crustacea and mollusks from larval stages, through juveniles to adults (Austin and Sharifuzzaman, 2022).

The effectiveness of probiotics may be related to the recipient species. Thus, lactic acid bacteria used for *Litopenaeus vannamei*, *Astyanax bimaculatus* and *Oreochromis niloticus* had different effective lives (= action times) (Vieira et al., 2008; Jatobá et al., 2018a; 2018b). However, the action time of probiotics is rarely considered. Other highlights influencing the effectiveness of probiotics include frequency of their supply, as offering them at low frequency (25% or less) may change the intestinal microbiota without improving animal health. Often, autochthonous bacteria demonstrate more positive/beneficial effects than allochthonous organisms, as the former already coexist in the environment (water and host), however this does not preclude the use of allochthonous bacteria (Jatobá and Jesus, 2022; Silva et al., 2022).

Traditionally, the mode of action is considered to involve competitive exclusion in which harmful organisms in the digestive tract are inhibited by the action of the probiotic cells (Plaza-Diaz et al., 2019; Knipe et al., 2021). Also, probiotics may influence nutrition via enzymic activity (Francavilla et al., 2017) and be important in immunostimulation. The latter involves stimulation principally of innate and cellular immunity, including increased erythrocyte and leucocyte populations, enhanced macrophage phagocytic and lysozyme activities, (Nguyen et al., 2022). Similar benefit has been reported with paraprobiotic preparations (Li and Tran, 2022).

Prebiotics, which are non-digestible [= roughage] feed ingredients that aid growth, development and efficacy of probiotics in the digestive tract, include beans and plantains, which contain fibres, e.g., of fructo-oligosaccharides (Buttriss and Stokes, 2008). Prebiotics have been combined with probiotics [= synbiotics] and used successfully in aquaculture. For example, use of *Bacillus* and mannan-oligosacccharide led to improved growth and survival of

European lobsters (*Homarus gammarus*) (Daniels et al., 2013). Furthermore, an extract from king oyster mushroom (*Pleurotus eryngii*) with *Lactobacillus plantarum* improved the growth and health of white shrimp *Litopenaeus vannamei* (Prabawati et al., 2022).

Attention has been given to determining active components of probiotics with outcomes pointing to the value of a wide range of subcellular compounds including cell surface proteins, enzymes, polysaccharides and short chain fatty acids (Ang et al., 2020). The soluble compounds resulting from probiotic metabolism are referred to as postbiotics and have comparative value to the intact host microbial cells. The possible use of postbiotics in aquaculture has been largely ignored although there is increasing awareness of potential value for improving health (Ang et al., 2020). For example, Abbass et al. (2010) protected rainbow trout (*Oncorhynchus mykiss*) against challenge with *Yersinia ruckeri* following administration intraperitoneally of lipopolysaccharide and various proteins derived from probiotic *Aeromonas sobria* and *Bacillus subtilis.*

In parallel with the development and evaluation of probiotics, workers examined a possible role of plant products (= phytobiotics) in aquaculture. The outcome is that a diverse range of plants, including garlic (*Allium sativum*), basil (*Ocimum basilicum*), cinnamon (*Cinnamomum zeylanicum*), peppermint (*Mentha piperita*) and turmeric (*Curcuma longa*) used singly or in combination (Ghafarifarsani et al., 2021; Pereira et al., 2020; 2021, Raissy et al., 2022), have exhibited possible positive effects when applied orally in terms of improved growth, immunostimulation and protection against challenge with some microbial pathogens (Awad and Awaad, 2017; Kuebutornye et al,. 2020; Bilen et al. 2021; Tadese et al., 2022). The beneficial effects have been linked to plant components, for example alkaloids, glycosides, steroids and terpenoids (Mendam et al., 2015). Doses range from 0.01 – 25% of diets although typically the supplements account for 0.01-5% (Bulfon et al., 2015) with administration for 1 – 16 weeks (Awad and Awaad, 2017). However, there may be variation in effect of the plant material because of geographical conditions including temperature and climate (Wang et al., 2014).

Plant material has been combined with probiotics. For example, peppermint has been combined with *Bacillus coagulans* leading to improved growth and resistance of Indian carp (*Catla catla*) to *Aeromonas hydrophila* (Bhatnagar and Saluja, 2019). Yet, it is often unclear whether or not combinations of probiotics and plant material are better than the components used separately.

There remain many unanswered questions, for example how long and to which age groups should the supplements be administered to farmed animals? Does the beneficial effect continue after the cessation of administration, and if so for how long?

f. Immunological Approach to Sustainable Aquaculture

Disease is a major concern for the sustainability of global aquaculture and one of the main reasons for fish, crustacean and mollusc losses during production. It has been estimated that 10 % of aquatic animals are lost to disease annually, equating to an annual loss of >10 billion USD for the global aquaculture industry (Evensen, 2016). Effective disease management is needed to help prevent disease outbreaks and reduce aquatic animal commercial production losses. Routine husbandry and changing environmental conditions can lead to stress-related immunosuppression in fish culture (Makrinos and Bowden, 2016). Understanding how these factors influence the fish's ability to resist disease and their impact on immune function will help farmers predict immunosuppressive events, enabling them to take appropriate action to reduce their impact (Thompson, 2017).

Fish and other aquatic animals have a very effective immune system to protect them from invading pathogens. Skin, scales, gills and the gastrointestinal tract, and the mucus layer covering these surfaces, act as physical barriers to invading pathogens. If the pathogen breaches these defences, humoral and cellular components of the innate immune system respond to prevent the infection from progressing. If the innate immune response is unable to control the infection, cell-mediated (B and T lymphocytes cells) and humoral components (antibodies) of the adaptive immune system will respond (Uribe et al., 2011). The adaptive immune response targets specific pathogens and produces a memory response to the pathogen so the host can respond against it should it re-encounter the pathogen (Smith et al., 2019). Antigen-presenting cells, i.e. dendritic cells, monocyte/macrophages and B cells, from the innate immune system present processed phagocytosed materials to the T cells of the adaptive immune system, demonstrating the co-operation between the innate and adaptive immune systems (Smith et al., 2019).

A deeper insight into the fish's immune response is not only necessary to deal with farming-related immunosuppression, but also for developing vaccines, functional feeds (with immunostimulants and probiotics), breeding programs for disease resistance, to understand the fish's response to emerging pathogens, and to examine the effects of climate change, pollution and alterative feed ingredients on fish health.

Vaccines are non-pathogenic preparations of the pathogen that induce protection against subsequent infections through adaptive immunity (Adams, 2019). The use of vaccines in aquaculture has increased significantly since commercial vaccines were first introduced over four decades ago, with regard to the number of microbial diseases and fish species targeted (Håstein et al., 2005, Evensen, 2009). Vaccination is now a routine part of fish husbandry, particularly for higher value species like Atlantic salmon. However, there is still a lack of commercial vaccines for other fish species (Adams, 2019). Some farmers are unwilling to use vaccines because they are concerned about the cost of vaccinating lower value fish species such as tilapia. However, the success of fish vaccination has led to both a decrease in disease outbreaks and a reduction in the amount of antibiotics used by the aquaculture industry, particularly by Atlantic salmon farmers in Norway and the UK (O'Neill, 2015, Norwegian Ministries, 2020). The types of vaccines licensed for use in aquaculture include whole, killed pathogens (usually using formalin inactivation), recombinant proteins, subunit, DNA and live attenuated vaccines.

Inactivated, whole-cell vaccine preparations are most often used in commercial vaccines, frequently containing multivalent pathogen components and an adjuvant (Adams and Subasinghe, 2019), whereas few recombinant protein, subunit and DNA vaccines are licensed for use (Adams, 2019). Live attenuated vaccines have the advantage that they survive and replicate within their host, eliciting both strong cellular and humoral immune responses with a long duration of immunity. Attenuation is based on repeatedly sub-culturing of the pathogen *in vitro*, producing random mutations that result in the attenuation. Defined genetic modifications by targeting specific genes would allow better control over the mutation process and help to overcome concerns that the attenuated organism might revert to virulence (Frey, 2007). These pathogens would be classified as genetically modified organisms making their licensing more complex (Brudeseth et al., 2013). Intraperitoneal injection is the most commonly used method of vaccine delivery, with automated vaccination machines used to mass vaccinate high-value fish species. This route of vaccine delivery produces high levels of long-lasting protection, especially if adjuvants are added to improve the immunogenicity of the pathogen. DNA vaccines are administered by intramuscular injection, whereas immersion tends to be used to vaccinate smaller fish. Oral vaccination is an ideal route of vaccine delivery insofar as there is no animal handling during vaccination. This reflects in improved animal welfare during vaccination and the stimulation of mucosal immunity. However, poor vaccine efficacy is currently limiting the use of oral vaccination. Research is focusing on adjuvants that can stimulate cellular immunity against intracellular bacteria and viruses for immersion and oral delivery (Taffala et al., 2013). There is interest in using nanoparticle technologies for fish vaccines, as vaccine delivery systems or adjuvants (Vinay et al., 2019). These technologies could enhance the immunogenicity of weakly immunogenic antigens. The surface area of the nanoparticle allows higher antigenic loads to be incorporated into the vaccine compared to traditional vaccines or antigens that could be encapsulated within the particles. The types of nanoparticles currently being assessed for aquaculture are comprised of poly-lactide-co-glycolide, alginate, immune-stimulating complexes (ISCOMs) chitosan, various metals, carbon nanotubes, liposomes or virosomes (Taffala et al., 2013, Vinay et al., 2019).

Functional feeds, containing immuno-stimulatory products, such as glucans, probiotics, prebiotics and medicinal herbs, enhance the fish's immune system during immunosuppression events or before juvenile fish are immunocompetent (Newaj-Fyzul and Austin 2015; Dawood et al., 2018). The use of functional feeds is of interest because of their ability to increase resistance to disease (Sakai, 1999; Bairwa et al., 2012; Meena, et al., 2013), improve growth and enhance the immune response particularly during periods of stress (Ringø et al., 2012; Dong et al., 2015). β-glucans are commonly used immunostimulants in aquaculture, particularly β-glucan (β-1,3 and 1,6 glucans) obtained from the cell wall of *Saccharomyces cerevisiae* (= baker's yeast). However, other sources of β-glucan have been researched (Sirimanapong et al., 2015). β-glucans act by enhancing innate defence mechanisms such as macrophage activity (Ranjan et al., 2012), complement and lysozyme activity, and enhance antibody responses (Sakai, 1999; Dong, et al., 2015). There are many reports of immunostimulants enhancing resistance to disease (see the review by Newaj-Fyzul and Austin, 2015).

More sophisticated analytical techniques, including next-generation sequencing, single-cell sequencing, proteomics, and epigenetic studies, are being used to assess the complexity of the fish's immune system at a molecular level. Also, tools are becoming available to characterize populations of immune cells (e.g., cell markers) and their products (e.g., cytokines) at a protein level. This information will inform how the fish's immune system responds to pathogens and environmental changes in more detail. Because mucosal surfaces are important routes for pathogen entry, this information will help develop the next-generation vaccines that stimulate mucosal immunity, and will be based on novel adjuvants and delivery systems for immersion or oral vaccine delivery. An immunological approach to controlling disease in fish-farming systems, using vaccines and functional feeds, will ultimately contribute to the overall sustainability of the aquaculture industry.

g. Fish Welfare

"Fish welfare" is defined as providing minimum standards by taking into account the general biological characteristics of fish (Broom, 1986). This definition is generally limited to only cultivated species. However, in every aquatic system, fish and other aquatic animals must be able to sustain their existence in prosperity. Therefore, it is necessary to understand the definition of fish welfare by considering all aquatic systems. Starting from global warming, human-induced pollution, structural changes on aquatic systems and pressure on stocks should be considered as factors that threaten fish welfare.

Specifically, when aquaculture systems are discussed, clear and resolvable items can be mentioned about fish welfare, taking into account different species. First, the water quality required by fish should be at optimum on the basis of the species. According to the

European Union directives (98/58/EC, 2021), it is stated in the animal welfare section that species that are grown should be kept in suitable conditions. In this context, effects of classical raceway systems, recirculated systems, and even aquaponics applications on fish welfare are examined in terms of water quality values (D'orbcastela et al., 2009; Yavuzcan Yıldız et al., 2017). Optimum water quality criteria alone are not sufficient for fish welfare. Appropriate aquaculture facilities and equipment within the facility must be suitable for the species and aquatic systems. According to EC directives, it is necessary to design the correct equipment in terms of animal welfare. It is requested that the animals be treated in such a way as to cause minimal damage. Another important component of fish welfare is the provision of food specific to each species. Fish need to be fed with the correct ration and feeding regimen. The positive effects of feeding regime and ration content on fish welfare are reported in different studies (Attia et al., 2012). Within the scope of the European Union directives, it is stated in directive 98/58/EC that the fish should be fed with the required rations. Another important issue is that all kinds of practices are carried out in accordance with ethical rules during the breeding process. From the breeding to the harvesting process, all kinds of applications are important for fish welfare. The subjects of this stage are the improvement of the transport conditions of the fish, stocking density, being careful in vaccination against diseases, and appropriate treatment with appropriate chemicals. In recent years, issues such as the use of ceramic balls to ensure fish welfare in live fish transfer (Akdemir et al., 2022), and the organization of studies that will allow the reduction of side effects of existing fish vaccines (Midtlyng, 1997) could be exemplified under this heading. Also, the presence of experienced and trained personnel in all these applications is an important issue (Kayış, 2019; FAO, 2019).

Some genetic manipulations have started to be included among important topics of fish welfare in recent years. In the production process, it is necessary to breed the fish, provided that the health and welfare of the fish are not harmed due to the genotype and phenotype (FAO, 2019).

h. Site Selection and Carrying Capacity Assessment of Aquaculture

The definition of carrying capacity has developed into a comprehensive four-pronged approach that has centred on physical, production, ecological and social carrying capacities to reflect management objectives (McKindsey et al., 2006), and finally, governance factors were adopted with the addition of the regulatory carrying capacity definition (Ferreira et al., 2013, Weitzman and Filgueira, 2020).

Site selection is one of the most important decisions as it provides the foundation not only for economic benefit, but also the sustainability, reputation and longevity of an individual farm and the industry as a whole (Falconer et al., 2016). Site selection for sustainable aquaculture is divided into the four modules:

(1) site classification

(2) location selection

(3) holding density

(4) economic evaluation.

Each module is described with the overall process including welfare issues and staff employment (Massa et al., 2021).

Determination of the carrying capacity for aquaculture involves multiple management objectives, and offers methodologies to evaluate ecological, social, governance and economic interactions (Weitzman and Filgueira, 2020), In the sustainable management of carrying capacity, the synchronization of researchers and decision makers and raising the awareness of producers on this issue will be among the important issues that should be emphasized now and in the future.

i. Recirculation Systems for Sustainability

Recirculating aquaculture systems (RAS) offer sustainable methods for producing marine and freshwater fish (Tal et al., 2009). A key factor is the ability of RAS to manage, collect and process nutrient wastes, which have been accumulated during fish growth. This has importance in future developments of environmentally sound fish production systems.

Tal et al. (2009) reported the development of a completely enclosed, land-based, marine recirculating aquaculture system with minimal environmental impact resulting from adoption of high-efficiency biological waste treatment and water recycling. Thus, >99% of the water by volume was recycled daily involving aerobic nitrification, which removes toxic ammonia, and, for the first time, anaerobic denitrification and anaerobic ammonium oxidation. This converts ammonia and nitrate into nitrogen gas. The system is site independent, biosafe, free of environmental pollutants and not limited to a single species.

Treatment methods applied for the treatment of aquaculture wastewater can be broadly classified as physical, chemical and biological processes. Physical processes apply [physical] forces to remove contaminants from the system. The removal of solids is accomplished by sedimentation or mechanical filtration (van Rijn et al., 1996). Often, chemical unit processes used for wastewater treatment are used together with physical and biological processes. In particular, the biological approaches, notably nitrification, are most important for wastewater treatment (Crab et al., 2007).

Although the initial investment cost is high, the system can pay off its costs in a short time thanks to its advantages. Similarly, it is clear that other disadvantages are also compensatable. Besides, the application of renewable energy has potential in reducing environmental impacts. Certainly, technological innovations are needed to improve energy efficiency in RAS, and may be coupled with an increased utilization of renewable energy. This would be more cost-effective than reliance on fossil based fuels (Badiola et al., 2018). In the future, the dissemination of RAS, especially with the use of renewable energy, will play an important role in reducing the environmental impact of aquaculture, as it is today.

Many comparisons have been made, especially on trout farming, in closed or semi-closed circuit units, and it has been shown that these systems are more advantageous than continuous flow systems in every way. In terms of water usage, it is reported that more than 50 m^3 of water is required for the production of 1 kg of trout per year in continuously flowing systems, whereas 0.1-1 m^3 of water is required in semi-closed systems and less than 0.1 m^3 in fully controlled systems (Martins et al., 2010).

Currently and for the future, it is clear that RAS comprise eco-friendly, water efficient, highly productive intensive aquaculture systems, which are not associated with adverse environmental impacts. These would include habitat destruction, water pollution and eutrophication, biotic depletion, ecological effects on biodiversity due to the escape of farmed fish particularly if exotic for the local environment, disease and parasitism. It is especially significant that RAS operate in indoor controlled environments, and thus, are only minimally affected by climatic factors, namely rainfall variation, flood, drought, global warming, cyclone, salinity fluctuation, ocean acidification, and sea level rise (Ahmed and Turchini, 2021).

j. Eco-Friendly Feeds and Sustainable Nutrition

Feed comprises ~70% of the total operating costs in aquaculture (Dossou et al., 2018; Kop et al., 2019; Dawood and Koshio, 2020) with upward prices of aquatic diets causing an increase in the total cost of production (El Basuini et al., 2017; Hossain et al., 2016; Dawood and Koshio, 2020). Arguably, the future of aquaculture depends on the supply of sustainable high quality feed ingredients

(Arru et al., 2019; Kop et al., 2019). Over the last few decades, there has been a significant increase in aquaculture production. However, due to the limited sources of fish meal (FM), fish oil, and other marine protein sources needed for carnivorous fish diets, alternative feed additives have come to the fore (Tacon, 2004; Arru et al., 2019). As an alternative to fish protein in feeds, consideration has been given to utilizing a variety of products derived from terrestrial animal and plant protein sources, agricultural by-products, synthetic amino acids, fungi, single-celled organisms, namely bacteria, algae, and aquacultural waste. There are ongoing studies to research raw materials that may be used as an alternative to fish meal in aquaculture (e.g. Hardy and Tacon, 2002).

Aquaculturists must use feeds effectively as they contribute an increasingly higher share of the total production cost. Thus, the producers need to ensure that there is a reduction in the potential environmental effects of uneaten feeds. Feeding on well-managed farms is carefully regulated to ensure maximum food intake by fish and shrimp, and producers aim for not more than 5% wastage. To improve the feed intake of fish and shrimp, the pellets are produced as floating or slowly sinking, taking into account the nutritional habits of the farmed species. Overfeeding or underfeeding will increase the feed conversion rate (FCR). Also, improvement in feed quality and feeding techniques reduces nitrogen pollution from aquaculture (Jensen, 1991). Similarly, depending on the water temperature, high-energy feeds increase the use of nutrients and consequently reduce the solid waste and nutrient load in the receiving waters. Although necessary as components of fish feeds, it is critically important to reduce the amount of nitrogen and phosphorus (in the feed). This process ensures a careful selection of ingredients when formulating fish feeds (Akinrotimi et al., 2007). In addition, controlled and restricted feeding reduces the nutrient load as it provides the fish with higher nutrient absorption efficiency (Usher et al., 1990). In short, better feed conversion is essential to reduce the nutrient load in aquaculture (Kibria et al., 1998). For this reason, many farms have underwater surveillance-monitoring systems as well as devices that control the supply and distribution of feed. For example, ocean sensor technologies help fish farmers reduce feed costs and impacts. Feeding cameras are located deep below the feeder areas and face the surface. These may be connected to remote videos to monitor the feeding efficiency in real-time. In addition, a detector suspended in the cage records uneaten bait that falls to the bottom of the fish cage. This detector reduces inedible feeds, and increases the feed conversion rate. Thus, the producer saves on feed costs, while at the same time reducing the environmental impact of uneaten feed on the seafloor below the production cages.

Lawrence et al. (2001) in their review of environmentally friendly feed and feed management for aquaculture research stated that the three major classes of pollutants from feeds are nitrogenous compounds, phosphate and dissolved and particulate matter, and that feeds are the major cause for pollution of natural fresh and marine waters. Also, they pointed out that the following factors affected pollutant discharge:

(1) feed physical properties,

(2) feed protein density and composition,

(3) feed phosphorus density and composition,

(4) feed energy density and composition,

(5) biotic factors.

(6) abiotic factors,

(7) feed attractability,

(8) feed digestibility, and

(9) feed management

According to Huntington (2009), aquaculture industries should consider two points;

1. Improve feed conversion and reduce waste. The industry must continue to develop efficient and cost-effective ways to reduce pollutant emissions per unit of production.

Investing in new technological systems is necessary to improve feed conversion and reduce waste.

2. Use of sustainable fish feeds. The industry should use fish feeds produced from sustainably sourced raw materials, including natural fishery products, that are sustainable by national or international regulatory authorities.

Simard et al. (2008) made recommendations for environmentally friendly aquatic feeds, as follows:

1. Regarding feeds and technology, the use of formulated feeds should be recommended, and feed production technologies and feed quality and management need improvement.

2. The use of alternative sources for feed ingredients and other available sources of marine proteins and fats should be encouraged.

3. Regarding the optimization of nutrients:

(a) cultivation of low trophic species is needed

(b) the integration of aquaculture with other agricultural farming activities should be encouraged.

k. Offshore Mariculture

Offshore mariculture, also known as aquaculture in the open ocean, is a comparatively new approach in which production sites move away from shore to the open seas. Farms are located in deeper, less sheltered sites where oceanic currents are inevitably much stronger than onshore (Naylor and Burke, 2005). There is great interest in offshore aquaculture (Morro et al., 2022) in response to the lack of suitable, sheltered coastal areas and potential livestock advantages, such as increased water quality and oxygen supply of oceanic sites, increasing production efficiency and fish quality. However, there are challenges, including problems of extreme weather conditions offshore leading to a focus on new building concepts, remote monitoring and greater automation to keep the cost of constructions within an economically viable range (Jensen et al., 2007). The issue of distance from shore or a safe harbor is often, but not always, a factor (Drumm, 2010). Thus, there is potential for expansion particularly in regard to the context of competing use of the coastal zones, and the global requirement for an additional thirty million tonnes of aquaculture produce by 2050 (Ferreira et al., 2014). Conversely, the multifunctional use of offshore waters may lead to more sustainable aquaculture "in areas that can be used simultaneously for other activities, such as energy production" (Lado-Insua et al., 2009). Certainly, sites for fin- and shellfish are being constructed. Globally, aquaculture and energy production could be combined with similar approaches. However, some negative environmental conditions may be turned into positivity by good management of materials.

The interaction and compatibility of aquaculture with the environment and *vice versa* is one of the main controversial issues associated with the sustainability of aquaculture. The projected future development and intensification of mariculture is linked to a diverse range of environmental concerns reflecting long-term issues with sustainability (Massa et al., 2017). These environmental concerns are likely to increase as offshore fish farming increases. Research has been conducted to assess potential environmental problems of offshore farming based on experiences in coastal farms (Holmer, 2010; Gentry et al., 2017). However if properly managed, aquaculture has great potential to provide multiple benefits – nutritious food and positive socio-economic outcomes – by minimizing negative externalities (Massa et al., 2017).

The rapid increase in human population leads to urban expansion and concomitant detrimental effects on wildlife biodiversity. Offshore farming could make a significant contribution to conservation of biodiversity. Finding ways to turn some negative conditions into positivity with the help of aquaculture should be among the main research topics in the future.

Offshore aquaculture could be successful if appropriate steps are followed, including:

a) renewable energy sources should be utilized,

b) the needs of the fish species farmed must be fully understood.

c) potential sites should be carefully selected for the species to be produced

d) operating costs should be carefully calculated, and self-sufficient integrated systems should be established.

e) energy production plants may be useful for integration with aquaculture sites as multidisciplinary production techniques, to obtain synergistic effects.

l. Water Quality Management in Aquaculture Research

This is the whole of policy making, development, planning, quality control, investment, permitting, inspection, sanction and coordination activities that will take into account the demands of living things, institutions and organizations in a way that will provide optimum benefit from water resources and control adverse situations (Kırtorun and Karaer, 2018). It is necessary to preserve the natural structure of fresh water used for drinking, utility, irrigation, industrial, recreational and aquaculture purposes. In order to determine for what purpose the aquatic ecosystem will be used, water quality characteristics should be determined (Mutlu, 2018). Sustainability of natural resources is possible with scientific research of these resources. Studies and future-oriented investments are made to meet the quality and reliable food needs of people, worldwide. With this basic principle and awareness, similar studies have been carried out by relevant institutions in order to meet the food needs of society. The most important approach accepted globally is to create new alternatives by using existing resources without damaging them (Yıldırım and Okumuş, 2004).

Since the health of aquatic organisms is highly dependent on water, deterioration of the quality is a major concern (Brönmark and Hansson, 2017; Hura et al., 2018). High stocking densities lead to deteriorating water quality. This impacts on increased susceptibility to stress and therefore to diseases. In short, adverse water quality is an ideal environment for the proliferation of potentially pathogenic micro-organisms, and, as a result, the development of disease conditions all too often leading to death of the cultured species (Lieke et al., 2020). Water pollution is inevitable in aquaculture because the cultivation process leads to the accumulation of waste, which causes pollution in the receiving waters and groundwater. This is because aquaculture wastes are released into natural water bodies (Lalloo et al., 2007).

m. Stock Enhancement

Ecological and economic changes may occur in water resources, including a decrease in fish stocks, reduction in reproductive success, disappearance of valuable species, and less productivity. Rehabilitation of damaged fish populations, establishment of stable populations in newly created resources, increasing species diversity and establishing an ecological balance or harmony between existing species by stock enhancement are among the frequently applied practices (Wondrak, 1994; Çetinkaya et al., 1999; Yılmaz et al., 2011). Fish suffer substantial losses in reproduction due to natural reasons, such as floods, overflows, turbidity, and predators. In order to compensate for these losses, broodstock from nature are caught initially. Then, offspring are produced by stripping the male and female broodstock in hatchery conditions with a much higher survival rate (>90%) than would ocur in the natural environment from which the broodstocks were taken. In this way, the continuity of the existing population is ensured (Aksungur et al., 2006). Stock enhancement studies may be done for various purposes, such as aquaculture, rehabilitation and development, enrichment, supplementation of natural stocks, stock enhancement of new water sources, sport fishing and biological control (Çetinkaya, 2006; Yılmaz et al., 2011).

Reducing Pressure on Natural Stocks and Protecting Species

Fish populations decrease as do fish gene resources because of overfishing. The biggest threat to aquatic organisms is overfishing. There are other potential threats including the movement of aquatic organisms that are part of the food chain away from their natural environment. This may occur because of the closure of waterways resulting from the construction of new dams inhibiting fish passage. This would prevent migration leading to adverse effects on breeding and feeding. Also, there are issues, such as the deliberate or accidental introduction of invasive species and the factors that may cause diseases, the increase in water pollution, and the deterioration of the natural habitats of fish as a result of human activities. Two main events are used to eliminate the negative effects of all these threats. The first is the preservation of the existing gene structure, and the second is the protection of genetic diversity (Demir, 2017).

The decrease in the production of many fish species by fisheries in recent years shows that the natural stocks of aquatic organisms should be supported by stock enhancement. According to Okuzawa et al. (2008), natural populations of inshore fish and invertebrates are being overexploited because of the increasing demand and prices (Pauly et al., 2002). In addition to fish species, stock enhancements of other aquatic species are made in some countries. For example, the Aquaculture Department of the Southeast Asian Fisheries Development Center in the Philippines has been implementing stock enhancement of donkey's ear abalone (*Haliotis asinina*), mud crabs (*Scylla* spp.), giant clam (*Tridacna gigas*) and seahorses (*Hippocampus* spp.) (Okuzawa et al., 2008).

The artificial breeding of fish species is the main application of aquaculture for many aquatic species used by humans as food sources. Apart from fish and other aquatic organisms (including mussels, oysters and shrimp), which are mainly produced for human consumption, there are aquaculture conservation practices developed for non-commercial fish species (Turkowski et al., 2008; Ciesla et al., 2014; Nowosad et al., 2016; Kucharczyk et al., 2019; Kujawa et al., 2019). These activities produce fish or invertebrates for rivers, lakes, seas and oceans (Kucharczyk et al., 2020).

Sustainable aquaculture plays a crucial role in the active conservation of endangered fish species. Rearing of offspring under controlled conditions is one of the most effective methods of producing stock for fisheries. This is related to the effectiveness of fishing operations, which is directly dependent on the release of the appropriate size and quality product to nature at the appropriate time (Sarkar et al., 2006; Ross et al., 2008; Zarski et al., 2011).

Importance of Aquaculture for Sustainable Stock Enhancement

For several decades, fisheries production has been declining as aquaculture increases, worldwide. Approximately half of the seafood consumed results from aquaculture. Globally, natural populations of inshore fish are under threat, primarily due to overexploitation and habitat degradation. Stock enhancement of hatchery-reared seed is regarded as an alternative approach to enhance regeneration (Okuzawa et al., 2008).

Conversely, aquaculture relies on a comparatively few species. Thus, it is necessary to introduce new fish species into aquaculture. Fish breeding is generally focused on two main issues; commercial fish production for aquaculture facilities and fish production to support natural populations. Fisheries and aquaculture activities make significant contributions to food supply, food security and the economy, both locally and globally. Climate change and related environmental changes affect fishing and aquaculture activities. Due to climate change, the feeding, migration and reproduction behaviors of fish are directly affected, and accordingly, their growth, mortality and reproductive performance are also affected. Because of direct or indirect effects, climate change can affect aquatic organisms positively or negatively (FAO, 2021). There are two important situations faced by aquaculture as a result of changing environmental conditions. First, the populations of some fish species have started to decrease due to climate change, and this situation has been overcome by stock enhancement. Populations needed for fisheries may be produced by aquacultural methods, and natural stocks can be supported. Second, as a result of climate change, the increase in water temperature causes some problems in the fish species that are reared. The fact that the cultivation of cold water species, such as trout, will become difficult in countries located in the temperate climate zone in the coming years will lead to the search for new alternative species. It is likely that breeding warm water rather than cold water species will begin to gain increasing importance.

Although stock enhancement is important in terms of supporting natural aquatic stocks, it carries some risks. Kitada (2018) stated that “hatchery release is one of the most popular management tools in fisheries, forestry and wild life management, but its negative impacts on wild populations are a global concern. Research and monitoring of its impacts are generally lacking, and the usefulness of hatchery release for fisheries and conservation objectives is unclear”. For this reason, it will be important to carry out stock enhancement studies.

n. Spatial Planning

Floating cages account for >80% of the total production of marine finfish, and is currently the primary production method for European seabass and gilthead seabream. The growing trend of marine aquaculture in the Mediterranean and the Black Sea is primarily connected to this technology (FAO, 2019). It is anticipated that this trend will continue, highlighting the need for marine and coastal space for the development of aquaculture in the years to come. This need is a direct result of the aquaculture development plans that have been drawn up for the region, as well as the steadily rising demand for fish and other seafood, together with the continuous improvements that have been made in farming technology.

The Marine Spatial Plan (MSP) enables the formulation and execution of an overall coordinated management plan that takes an ecosystem-based perspective. Thereby, MSP acknowledges that activities, such as the development of aquaculture, the production of oil and gas, tourism uses (such as marine parks and conservation areas), and services (such as ecology and habitats) will continue to call for coordinated management. The implementation of MSP may be accomplished by using the necessary tools or engaging in the necessary activities. Regulations, integrated coastal management (ICM), zoning, mapping and gathering data, databases, software packages, and other tools and information that contribute to the construction of marine spatial planning are examples of the types of activities that might fall under this category (FAO, 2016).

The rapid growth of aquaculture needs the implementation of an integrated coastal zone management (ICZM) plan in order to guarantee the industry's sustainability. The deployment of aquaculture facilities in coastal areas needs enough space, and there has been a long-standing recognition of the necessity to more effectively integrate aquaculture with other businesses in order to minimize potential conflicts (Massa et al., 2021).

The use of geographic information systems (GIS) has been adopted in a very wide range of habitats and to a large variety of cultural systems. On multiple occasions, the significance of using tools such as GIS, has been highlighted to address the geographical and spatial aspects involved in marine aquaculture. These aspects include the location, description, and selection of areas of interest for aquaculture (FAO, 2007). GIS is totally expandable, and is able to encompass ecological, governmental and social borders. The capacity to spatially integrate and analyze natural and human elements as components of ecosystems is one of the strengths of GIS.

The requirement for allocated zones for aquaculture (AZA) is “a maritime region where the growth of aquaculture has priority over other uses and will consequently be predominantly committed to aquaculture”. The establishment of an AZA will be the outcome of zoning procedures via participatory spatial planning, where administrative authorities legally define that specified spatial regions within a region have development priority” (Sanchez-Jerez et al., 2016). The Aquaculture Zone Effected (AZE) may be established while measuring the environmental carrying capacity and nutrient flow coming from the cages. Environmental monitoring must occur outside and within the permissible AZE.

o. Climate Change

With increasing world population, technology development increases industrialization, and with this increase, natural life diminishes. While people benefit from technology and live in prosperity, they are faced with the deterioration of the natural balance and pollution of the environment as the price of some values they neglect. Currently, climate change, which is one of the leading environmental problems, seriously threatens the future of the world, and exposes us to ecological dangers. Climate change has been occurring very slowly with natural events that have taken place in nature since the formation of the world, and all beings living in the world could adapt to this change. However, with the industrial revolution that started in England in the second half of the 18th century, the human factor came into play, and unfortunately, the world had a hard time keeping up with the technology that was developing with increasing momentum. Since the Industrial Revolution, human activities have seen a 47% increase in atmospheric carbon dioxide concentration, from 280 ppm in 1750 to 412.5 ppm in 2019. Scientists estimate that the most recent time when the atmospheric carbon dioxide concentration was this high was 3 million years ago (Lindsey, 2020). This increase has occurred despite the absorption of more than half of the emissions by various "carbon sinks" in the natural carbon cycle, including vegetation, oceans, and soil. Studies show that the temperatures of ocean, sea, river, and lake waters increase with global warming and depending on this temperature increase, causes the decrease or even extinction of plant and animal species (Fleming et al., 2014; Blanchard et al., 2017; Troell et al., 2017; Zolnikov, 2019). All these changes pose a risk to the global food production, and future generations face the prospect of hunger (Myers et al., 2017). For this reason, aquaculture is critical in meeting the healthy food and especially protein needs of the increasing population. Alread, the aquaculture sector significantly contributes to global food security, nutrition, and employment. Therefore, the effects of climate change on the sustainability of aquaculture have received increaasing attention (Blanchard et al., 2017; Dabbadie et al., 2019). Various studies show that the entire aquaculture chain is vulnerable to climate change, and of course, there are prejudices that poorly planned and uncontrolled aquaculture causes environmental pollution. All of these prejudices create an adverse reaction to the aquaculture sector (Cochrane et al., 2009; Fleming et al., 2014; Bueno and Soto, 2017; Barange et al., 2018; Dabbadie et al., 2019). Despite all this, the contribution of the aquaculture sector to the global economy and healthy food is undeniable. Of course, every sector, even every individual, has duties for the future of the world in which we live. The duty of the aquaculture sector is to produce zero-waste and zero emissions.

p. Use of Ozone in Aquaculture

The FAO Code of Conduct for Responsible Fisheries outlines principles and international standards for sustainable aquaculture practices. Among these to achieve the goal of sustainable aquaculture, we note:

- minimizing adverse ecological changes and related economic and social consequences resulting from water extraction, land use, effluent discharge, the use of drugs and chemicals, and other aquaculture activities.

- promote effective farming and fish health management practices favoring hygienic measures and vaccines. Safe, effective and minimal use of therapeutants, hormones and drugs, antibiotics and other disease control chemicals should be ensured.

Ozone is an active solution that can help to meet these guidelines in aquaculture. Ozonation may be used to:

- reduce water extraction and discharge of effluents through water recirculation

- reduce discharge of effluent problems related to fish-carrying vessels and farms

- limit the absorption of micropollutants, drugs, chemicals and hormones by fish and ultimately end consumers

- ensure best practice fish health practices with minimal use of antibiotics and other disease controlling chemicals

In hatcheries, the quality of the water is of primary concern. Thus, it is essential that influent waters are clean and free from contaminants and micro-organisms that may infect and destroy a complete hatching. This applies to recirculating as well as single pass systems. Aquaculture needs systems that reduce micropollutants, particulates and dissolved organic waste as well as control microbial activity (including those of viral origin that are unable to be controlled with antibiotics). Conventional means of solids removal, such as microscreen filters and sedimentation tanks, remove coarse, settleable and filterable solids, but do not remove micropollutants, fine and colloidal solids. Similarly, biofilters remove dissolved ammonia and nitrite, but not other dissolved wastes.

A wide range of chemicals, including formalin, hydrogen peroxide, peracetic acid and sodium chloride, are used to control microbial growth and prevent disease outbreaks (Noble and Summerfelt, 1996; Pedersen et al., 2010; 2013; Pedersen and Pedersen, 2012; Verner-Jeffreys, 2015). However, high concentrations of chemotherapeutants may impair biofilter performance, affect fish welfare, and create risks for worker safety, and endanger ecosystems when non-degraded residuals are released into nearby aquatic sources (Hohreiter and Rigg, 50 2001; Masters, 2004; Wooster et al., 2005; Pedersen et al., 2010).

Ozone (O_3 or trioxygen) is an allotrope of oxygen that is less stable than the more common atmospheric oxygen - the diatomic allotrope O_2. Ozone is a powerful oxidant and antimicrobial agent, and unlike other agents, it does not leave any ozone residual in the system due to the rapid decay to oxygen. For this reason, it is produced on site through generators that transform the oxygen present in the air into ozone. Once the ozone has been produced, it is mixed with water through micro-bubble or venturi systems. Depending on the substances present in the water, i.e. how refractory or likely they are to generate reaction by-products, ozone may be combined with other AOP technologies (advanced oxidation), with absorption (GAC: granular activated carbon) processes or other technologies.

The conventional use of ozone in aquaculture may be divided into three main categories:

(1) treatment of effluent

(2) pretreatment of incoming water

(3) ongoing water quality control inside aquaculture systems, particularly in Recirculating Aquaculture Systems (RAS).

Ozone may be toxic to aquatic organisms. For this reason, after the required contact time for oxidation and sanitation, excess ozone is removed from treated water prior to entry into tanks holding stock animals. However, a growing body of research has studied the direct application of ozone, which may be defined as exposure of residual ozone and ozone-produced oxidants, to cultured species of finfish, shellfish and live feeds across various life stages. Regardless of whether sea water or brine is used, it is recommended to take particular measures to reduce the formation of bromates, such as reducing contact times and using low bromide salt, thereby limiting stress for the fish.

Some of the main reasons for using ozone for water treatment of aquaculture systems are mentioned below:

- To reduce fish pathogens (Bullock et al., 1997; Summerfelt et al., 2009). Ozone is effective against bacteria, virus, fungi, protozoa, algae and unlike other chemicals, does not create resistant strains. The reduction of pathogens results in fewer diseases and better survival rates.
- Unlike antibiotics, ozone does not create antimicrobial resistance (AMR). AMR has emerged among bacterial fish pathogens associated with the rise in antibiotic use in medicine, veterinary and aquaculture as part of therapy and prophylaxis (Stentiford et al., 2017; 2020). Ozone is able to oxidize antibiotics, both in the influent water, recirculated water and in the effluent (Choi et al., 2020; Kye et al., 2020).
- To remove micropollutants present in rivers or the sea. Hormones, drugs and other chemical substances could be absorbed by fish, and their removal guarantees a higher quality of the aquaculture product to the consumer. The removal of estrogens (or analogues) reduces possible immunoreactivity.
- Ozone oxidizes dissolved and particulate organic compounds, thereby improving solid settling and separation and decolorizing the water (Krumins et al., 2001). Of particular note, it eliminates the yellow/brownish color as a result of Dissolved Organic Carbon (DOC)/ humic substances.
- The accumulation of dissolved organics has been implicated as a possible cause for reduced fish growth rates and reduced nitrification efficiency (Hirayama et al. 1988; Morrison and Piper 1988; Easter 1992; Nunely 1992; Bosworth 1994). In fact, improvements in growth have been reported (Good et al., 2011) using ozonation.
- No storage and handling of chemicals: the necessary ozone is generated on-site thus eliminating the need for chemical storage and handling.
- Few harmful by-products compared to other chemicals used for oxidation or disinfection.
- It removes odors and taste. Some algae and bacteria produce toxins and metabolites (e.g., geosmin) that give fish and seafood an unwanted taste and may impact the health of fish and final consumers. Ozone removes taste and odor from water (Bullock et al., 1997; Tango and Gagnon, 2003; Summerfelt et al., 2009).
- It reduces the need for chemical treatments.
- It has rapid reaction rates: fast reaction rates equate to reduced treatment times and footprint of the water treatment line.

It is appropriate to examine results detailed in the scientific literature, and applications in aquaculture sites: In fish eggs, embryos and larvae, ozone increases hatching rate (Forneris et al. 2003), reduces egg mortality, reduces bacterial or virus load on eggs (Can et al. 2012), and improves larval survival (Forneris et al. 2003). Water disinfection with ozone in place of certain chemicals is used as a sustainable practice (Can et al 2013).

In fish farming, ozone improves survival rates (Bullock et al. 1997), improves growth and feed conversion (Good et al., 2011), disinfects incoming hatchery water (Tipping, 1987), disinfects hatchery wastewater (Majumdar and Sproul, 1974; Conrad et al., 1975), reduces diseases in the population, reduces the bacterial load on the fish (Bullock et al., 1997; Summerfelt et al., 2009), and improves the chemical and physical characteristics of influent and effluent water (Krumins et al., 2001; Summerfelt et al. 1997).

In crustacea and mollusks, ozone reduces bacterial load or viruses on bivalves with suggested advantages including lower running costs (Lees et al. 2010; Goncalves and Gagnon 2011). It disinfects incoming water (Crisp and Bland, 1990), improves survival, increases growth and production, improves the chemical and physical characteristics of influent and effluent water, and removes odors and taste from water (Bullock et al., 1997; Tango and Gagnon, 2003; Summerfelt et al., 2009).

In addition, ozone is used in live feeds for bacterial decontamination (Allen Davis and Arnold, 1997; Suantika et al., 2001; Watanabe et al., 2005) and to improve culture water physico-chemical characteristics.

q. Plant-Based Anesthetics

Anesthetic or sedative substances are used for many applications, such as calming and/or immobilizing living things, handling, examining, catching, transporting and measuring. Currently, anesthetics including methane sulphonate MS-222, benzocaine and 2-phenoxyethanol are used widely in aquaculture. MS-222, which is commercially marketed as "TricaineS" or "Finquel", may be used legally in fish produced in the USA and UK. The time needed or MS-222 to be excreted from the fish’s body has been specified as 21 days by the Food and Drug Organization (FDA) in the USA. This situation causes delays in the marketing of fish under some conditions (Coyle et al., 2004). However, in Canada, the use of MS222 is prohibited. Because oils from herbal products are thought to be good options as anesthetics, their use has increased and the search for new natural compounds is continuing (Aydin and Barbas, 2020). In this context, anesthetic properties have been described for essential oils derived from clove (*Syzygium aromaticum*) (Keene et al., 1998; Aydın et al., 2015) mint (*Mentha piperita*) (Ghazilou and Chenary, 2011; Can and Sümer, 2019), rosemary (*Rosmarinus officinalis*) (Roohi and Imanpoor, 2015), lavender (*Lavandula officinalis*) (Metin et al., 2015) zaater (*Zataria multiflora*) (Sharif Rohani et al., 2008), bushy matgrass *Lippia alba* (Cunha et al., 2010), basil (*Ocimum gratissimum*) (Silva et al., 2012), espanta pulga *Hesperozygis ringens* (Gressler et al., 2014), chamomile (*Matricaria chamomilla*) (Can et al., 2017), geranium (*Pelargonium graveolens*) (Can et al., 2018), peppermint (*Mentha arvensis*) (Can and Sümer 2019), lemon balm (*Melissa officinalis*), lavender (*Lavandula* sp.) and coriander (*Coriandrum sativum*) (Can et al., 2019), and spurge (*Euphorbia rigida*) (Alagoz et al., 2021).

Essential oils are aromatic oily compounds obtained from different parts of plants, i.e. flowers, buds, seeds, leaves, branches, bark, wood, fruit and roots. These natural products are widely used in various fields including perfume, cosmetics, aromatherapy, phytotherapy, spices, nutrition and agriculture. Recently, studies involving the antibacterial, antiviral, antifungal and anti-inflammatory effects of essential oils have made significant contributions to the field of health (Misra and Srivastava, 2010; Sharapov et al., 2014). Although essential oils are derived from plants, it does not infer that their use as anesthetics is completely harmless because of possible toxicity and genotoxicity (Slamenova and Horvathova, 2013). Recently, it has become important to determine the histological effects of essential oils used as anesthetics in fish. In these studies, the gills have been considered as one of the most prominent indicators of the effects due to anesthetics (Brandão et al., 2021). In addition, the anesthetic substance may cause changes in the biochemical profile that could lead to oxidative stress (Velisek et al., 2011). Therefore, it is critical to determine the safety of these anesthetics with regard to possible DNA damage as determined by histological and antioxidant analyses. Also, because the anesthetic response and toxicity are dose-dependent, more comprehensive studies on specific fish and other aquatic species, medicinal extracts and anesthetic protocols need to be studied before recommendation for commercial use in aquaculture species.

Conclusions

There has been rapid and sustained development in aquaculture in many countries since the end of World War 2. Aquaculture has become recognized as an important food source to the expanding global human population, and is vital for food security. Notwithstanding the economic benefits of this expansion, there is a need to be aware of the demands of other users of the aquatic environment, including commercial and sports fishermen. Therefore, aquaculture needs to fit into overall aquatic management regimes, including the impact of welfare issues especially for the farmed species. With increases in production of a diverse range of species, there have been key developments in production methods, including the use of aquaponics and land-based recirculation systems, both of which are more frugal users of water. Certainly, there is increasing investment in land-based aquaculture with significantly reduced to essentially zero water usage leading to production levels of over million metric tonnes per hectare of water per year. With mariculture, there is a move to using offshore sites rather than coastal floating cages. This has brought new challenges to develop robust structures to contain the farmed aquatic organisms. Any potential impact on biodiversity needs to be considered, especially with regard to mitigation of any negative effects. Problems have arisen, and aquaculture has not been slow in adopting new technologies, including nanotechnology, to find effective solutions. The enormous amount of new informaton from aquaculture research has resulted in improved management strategies for natural fishery populations. The overexploitation of aquatic species [= trash fish] as sources of fish meal and oil for incorporation into diets for carnivorous species, e.g. salmon and trout, is nonsustainable. Alternative sources of key ingredients have been researched, including the use of plant-based and single cell protein (including bacteria, microalgae and yeast) ingredients resulting in replacement of fish meal in farmed aquatic animal diets with increased production and body protein and decreased body fat. This will result in not only making the farmed food healthy and sustainable, but also provide high quality protein for the rapid increases in the global human population. Towards this aim, efforts have been and will continue to be made to reduce waste. One impact is the effect on pollution, namely through uneaten feed and feces. The adverse impact on water quality is an ongoing issue that needs close attention. In this regard, many micro-organisms, notably in bioflocs, contribute to cleansing water, reducing water use, and moderating nitrogen levels. Disease remains a problem for monoculture systems whether in aquatic or terrestrial habitats. Yet, there has not been any shortage of innovative approaches to improve health with new vaccines, prebiotics and probiotics and innovative new biosecurity programs. From reliance on antimicrobial compounds, attention has focused on specific [= vaccines] and nonspecific immunostimulants, probiotics and prebiotics, and natural plant products used singly or in combination, that have been successful in combating a wide range of infectious diseases. The effects of environmental pertubations, which are more commonly labelled as climate change, will impact all aspects of human activity, including agriculture/aquaculture. Thus, it is essential for future generations to ensure the sustainability of aquaculture to secure the needs of society for high quality food. Nevertheless, we believe that the future for sustainable aquaculture is certainly bright!

Ethical approval

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