Sustainable aquaculture in artificial ecosystems

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Abstract

The world's population is expected to grow to around 8.5 billion in 2030 which will lead to a dramatic increase of the demand for nutritious food. Giving the challenges to nutrition and food security which the world is already facing today, new solution will be required to satisfy the needs of 8.5 billion people. Food derived from the ocean can be part of the solution, due to its high nutritional value and efficient transformation of feedstuff to protein. But the increasing demand has led to overfishing causing destruction of habitats and loss of marine biodiversity why solutions with an innovative approach to the utilization of existing resources are required.

Aquaculture is already contributing to the global supply of foods and holds tremendous potential to mitigate some of the threats of overfishing on marine ecosystems. However, conventional aquaculture does not come without adverse side-effects for the local ecosystems, mainly related to pollution, and these will aggregate with more intense use of aquaculture.

The aim of this research was it to investigate how eDNA, sequencing and bioinformatic methods can be used for the development of symbiotic aquacultures, in which different species synergically live together and assume different functions to establish a self-sufficient, circular, and sustainable ecosystem. The methods will specifically be used to determine the species composition and the conditions under which the organisms live in natural balance. Information from this will be used to develop artificial ecosystems of aquaculture with the aim of ensuring a productive and sustainable use of marine areas.

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1. Introduction

It is estimated that global fish production in 2018 reached 179 million tons. Of this, 156 million tons were used for human consumption, corresponding to an average annual supply of 20.5 kg per year and person. The remaining 23 million tons were mainly used for the production of fishmeal and fish oil. *(FAO, 2020).* Today's fish production corresponds to an overfishing rate of about 30%. *(FAO, 2014).* Overfishing is defined as fishing harvest above the level at which fish stocks can reproduce sustainably and leads to the elimination of more of a natural aquatic species than what natural reproduction can support. Hence, overfishing is increasingly considered one of the most important processes leading to the global biodiversity crisis. Globally, overfishing is one of the biggest threats to the oceans and, above all, to developing countries. Over three billion people's livelihoods depend on marine and coastal biodiversity. Especially in the poorest parts of the world, fishing is the primary source of livelihood for coastal communities. If we want to preserve marine ecosystems, change is needed, which is why efficient and innovative management solutions are required.

This research project is founded in my curiosity of how we can make sustainable use of marine resources, including sustainable management of fisheries and aquaculture, and at the same time secure foods for a growing population and livelihood for coastal communities. Therefore, I will investigate how we, with the least environmental impact, utilize the sea's benefits in the development of an efficient and sustainable aquaculture that will contribute to a sustainable development of the aquaculture sector in Denmark.

2. Relevance and purpose

Aquaculture has a long history in many parts of the world. Around 500 BC, the Romans bred oysters and fish in Mediterranean lagoons and already about 1000 years earlier, freshwater aquaculture developed in China. *(Nash, 2010).* While farming has been extensively run in the past, modern methods are trying to intensify production due to increasing demand for fishery products. In developing countries, aquaculture is often grown at a subsistence level, whereas in industrialized countries it is more closely run for economic profit. Breeding facilities can either be set up on land in the form of fish farms or in the sea as aquaculture.

Fisheries play an important role in achieving the UN's sustainability development goals (SDG), in particular SDG 2. SDG 12 and SDG 14 which addresses the goal of conserving maritime environments and using marine resources in a way that promotes sustainable and responsible development.

Figure 1: 3 essential world goals that the project addresses. Source: un.org

Biologically, it is beneficial to grow aquatic organisms, as fish and other marine animals are more efficient at converting feed into nutrients than terrestrial animals and fisheries thus play a major role in the goal of eradicating hunger, achieving food security and better nutrition as well as the climate goal. But fishing alone cannot be the solution because the sea's sensitive ecosystems will collapse if overfishing continues. An alternative solution may therefore be the mentioned aquacultures, which cover controlled cultivation or farming of aquatic organisms such as fish, crustaceans, snails, and aquatic plants and include various populations in fresh, brackish, and salt water.

3. Scope

The complexity of the mixed fisheries in the North Sea and the Baltic Sea as well as the biodiversity make it impossible to target catches at a single species. My project therefore is, to the extent possible, tailored to reflect this, partly by covering different stocks and partly by combining breeding and conservation. Aquaculture for salmon farming is, for example, to a large extent in Norway. China makes use of modern industrial recirculating aquaculture systems (RAS technologies), where five systems has been established along the coast. In Denmark, there are 19 aquaculture farms that are primarily used for breeding rainbow trout (*Oncorhynchus mykiss*).

Intense aquacultures, however, come with multiple challenges. The high density makes the fish population sensitive to parasites as for example salmon louse, (*lepeophtheirus salmonis*) and infectious diseases. Medication against these parasites and diseases pollutes the water, with uncontrollable effects on the wildlife around the aquaculture. Likewise, spill of excess feed and large amounts of excrements end up as pollutant to the maritime environment, causing overfertilization of the water. A special challenge are chemical additives in the feedstuffs. Today, only consumer fish is produced in aquacultures, whereas traditional fishing involves fishing for pelagic species intended for human consumption and industrial fish species, which are widely used in the production of fishmeal, which is used in fish feed and other animal feed *(Skov, 2019).*

The focus of my project is the development of an aquaculture system where different organisms live in symbiosis. The problem statement can be summarized as “How can we develop a symbiotic multi-species aquacultural system where organisms like mussels, algae and cultivated fish live in a nature-like ecological system, as alternative to traditional fishing and conventional fish farms, and how can the sustainable effect of the system be monitored?”.

4. Sustainability Aspects

Two of the biggest environmental challenges in Denmark are emissions of nutrients and loss of biodiversity, which is largely due to pressure on land and freshwater resources. Two things that are, in fact, closely linked. Most of the sea areas in Denmark are surrounded by land where intense cultivation takes place. To get a higher yield of the crops, farmers use large amounts of fertilizers and manure that contain important nutrients for plants such as nitrogen and phosphorus. However, the crops in the fields cannot absorb all the nutrients that are being spread. Instead, a large part of the excess nutrients is carried into the sea by rainwater, groundwater, and rivers. Here, they contribute to eutrophication, which means that the sea receives too many nutrients. Over a longer period of time and in larger quantities, this results in algae blooms, which make the water cloudy, with which the sun's light cannot reach the bottom. Thus, it becomes more difficult for benthic plants and organisms to survive and therefore they risk dying or withering. The dead material then sinks to the bottom, and everything collects as a thick layer of dead, organic material. The organic material is broken down by decomposers such as bacteria via respiration. As respiration is an oxygen-demanding process, there will be a lack of oxygen at the lower layers of the seabed, which causes oxygen depletion. The fish and benthic animals that do not have time to move away from the area thus risk being suffocated. When species disappear from areas with oxygen depletion, biodiversity also decreases. When the sea is supplied with too many nutrients which partly come from food production, wastewater from the cities and agriculture, the eutrophication takes place, which leads to oxygen depletion, which causes loss of biodiversity. An additional major problem is that the Danish coastal waters have for decades been excessively fished, and many domestic species are threatened or even extinct.

Production of fish in aquaculture affects the environment in different areas, however, the degree of pollution depends on the size of the aquaculture and on the location, as among other things water currents can affect the spread of the pollution. Intense fish farming near the coast, especially in the shallow waters of the Baltic Sea and Kattegat with little water circulation, could even aggravate these effects locally. However, recent advances in marine aquaculture have relieved the pressure on the natural environment. Today, modern aquaculture has a very low environmental impact in Denmark due to high degree of recycling, which reduces the environmental impact.

Still, those aquacultures are not self-sufficient and sustainable because they require external supply of feedstuff and emit pollutants. My project therefore aims to establish a process for establishing and monitoring an aquacultural system which assimilates the environment and food chain of a natural marine environment.

5. Methods

5.1 Introduction

Based on the theory of the biology and ecology of aquatic organisms and experimental determination of the species composition in specific biotopes using metagenomic environmental DNA (eDNA), I will use 16S rDNA and 18S rDNA sequencing to investigate how technologies to grow fish and plants in it the same system can be symbiotically assembled by imitating natural biotopes. eDNA is defined as genetic material obtained directly from environmental samples such as water. This is an effective biomonitoring tool, which makes it possible to monitor that the aquaculture facilities meet environmental objectives, and that biodiversity is maintained. Thereby, future aquacultures could follow the principle of long-term, sustainable utilization, which is necessary to ensure healthy fish populations. The goal is maximized sustainability, including no use of the chemical ethoxyquin in feed processing and storage, no use of pesticides, no artificial additives as well as better space conditions for the organisms. These requirements must ensure that the system has the least possible impact on the nature around the aquacultures.

The research project is based on the deductive method, which investigates whether the theoretical knowledge about the biology and ecology of aquatic organisms can be used to build a multi-species aquaculture as an alternative to fish farms. Quantitative data are used to develop the aquacultures using metagenomic eDNA, making it possible to compare the geographical representation of previous species richness studies in marine ecosystems. The project thus depends on the combined application of a number of quantitative methods, to first and foremost investigate under which conditions different organisms live, as well as how they adapt to each other in the ecosystems in question. Each organism has a unique DNA sequence or 'barcode' associated with it. This DNA barcode is a highly variable region scattered among conserved genomic regions. eDNA metabarcoding involves target-specific amplification and sequencing of these barcodes. The method is relevant for distinguishing between higher orders of eukaryotes.

5.2 Sampling and DNA-extraction

Well-functioning ecosystems in coastal areas of the North Sea and the Baltic Sea are examined by collecting 0.5-1 liters of water samples, which are filtered to concentrate environmental DNA (eDNA) from the filter (Figure 2). DNeasy PowerWater Kit is used to isolate genomic DNA from filtered water samples. In the laboratory, the filter is pulverized, the extraction buffer from the DNAeasy PowerWater kit is added and DNA is extracted as prescribed.

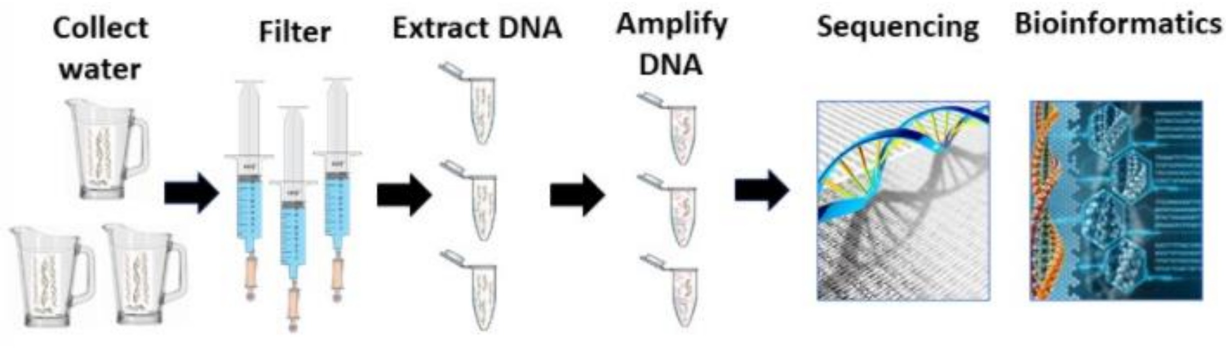

Figure 2: eDNA sampling and processing. Source: AquaBiota.se

5.3 PCR amplification and sequencing to identify species

Metagenomics NGS (Next-Generation Sequencing) can be used to assess biodiversity. Hereby, the genetic composition of a stock is determined by eDNA analysis. eDNA sequencing allows simultaneous analysis of thousands of species from the same water sample. Variable regions in prokaryotic 16S ribosomal RNA (rRNA) gene and eukaryotic 18S rRNA gene from eDNA are amplified using specific primers. For the production of sequencing libraries, the “16S and 18S Library Preparation Kit” is used and libraries are sequenced on a sequencing machine, which can be MiSeq, Illumina, for example, to keep costs low. Sequencing is a three-step method consisting of library construction, sequencing on a specific NGS platform and bioinformatics analysis. The methodological review of the sequencing is shown in figure 3.

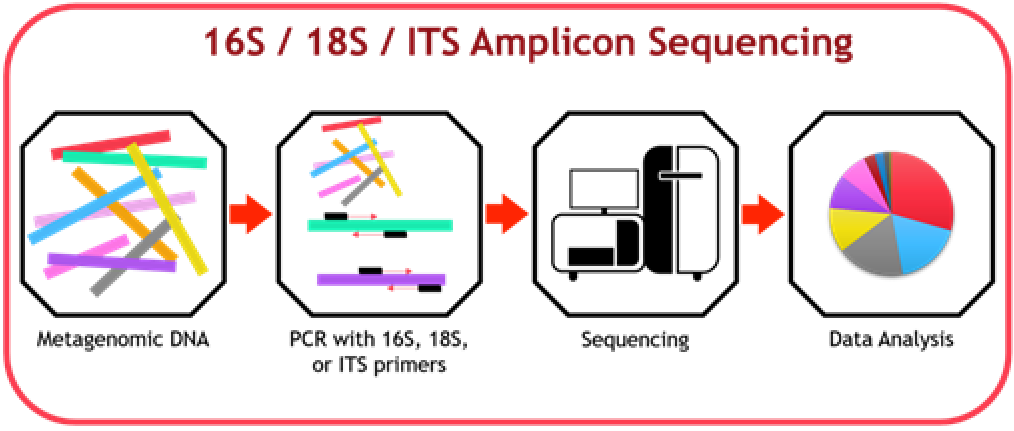

Figure 3: 16S, 18S sequencing. Source: biobasic-asia.com

For the identification of species and their phylogenetic classification, bioinformatics methods are used, which make it possible to compare sequences with reference genomes, i.e., identified 16S and 18S rRNA gene sequences which are available in databases, such as "Excavata EukRef databases" *(Kolisko, 2020).* The databases contain a large number of genes from organisms in which their DNA has previously been found in other specific environments.

6. Approach

First, I will examine the most important water quality parameters relevant to fish farming. These include salinity, temperature, oxygen, and pH measurements as well as levels of organic matter and are measured using marine biological field techniques using a CTD probe, which is typically equipped to measure temperature, conductivity, dissolved oxygen, pH, turbidity (water turbidity) and depth. Such a CTD probe is available on AU's research vessel Aurora, which is why, through my research contact, Peter Grønkjær, it has become possible for me to collect water samples. At five different locations based on well-chosen coastal areas in the North Sea and the Baltic Sea, four water samples (biological replicates) will be collected. The collected data are analyzed with computer programs and used to determine the occurrence of certain biological processes such as algae and plant growth, the species distribution in the water column and abiotic factors. Next, the species composition is determined using eDNA metabarcoding, whereby DNA from certain groups of organisms is amplified using specific primers, which using NGS sequencing and bioinformatics analyzes lead to a species list of species from the selected group of organisms whose DNA is found in the samples. This makes it possible to create the aquacultures so that they mimic the biotopes to which the species originally belonged. The schematic overview of analyzes and setup of artificial ecosystems is shown in figure 4.

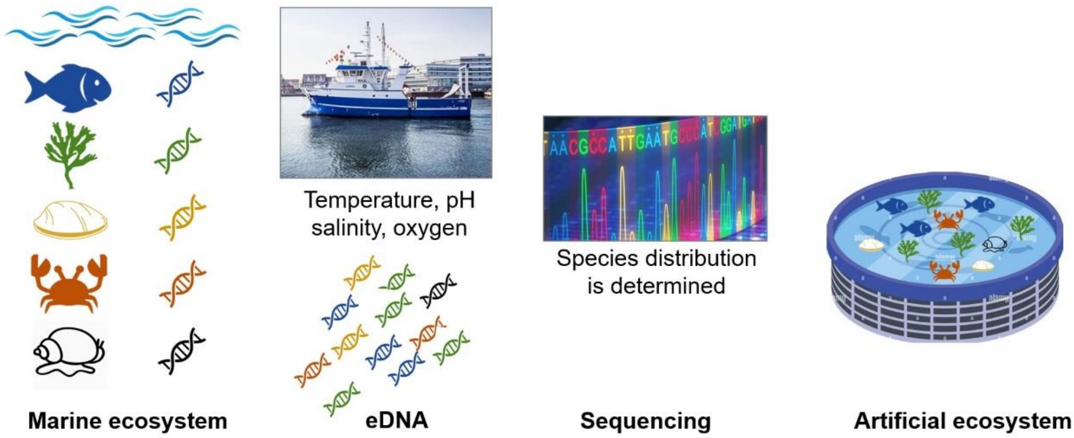

Figure 4: The use of eDNA to determine species compositions in existing ecosystems. Source: Karolin Janina Demtröder, 2022

7. Implementation

The aquaculture sector is the world's fastest growing animal food production. In addition, the Danish producers of fish feed and recycling technologies for fish farming are among the world leaders in exports to the whole world *(Brogaard, 2017), (NaturErhvervstyrelsen)*. Therefore, this project has great potential to contribute to a sustainable expansion of aquaculture production in Denmark, as well as strengthen Danish exports. The project therefore has great future potential, but a realization would require collaboration with larger companies, funds, organizations, or other investors.

8. Discussion and perspective

Regardless of the results of the project, further research is planned. If the project shows desired effects, potential is seen within further development of the systems. Here it will be interesting to investigate how a continued low environmental impact from farming is ensured, and what measures must be taken to further ensure resource efficiency. In addition, it may be of interest to probe, by using eDNA, how the impact of the multi-species models on marine ecosystems is measured or treated. The use of eDNA can be used precisely for future biological monitoring and thus for assessing the impact of aquaculture on the surrounding biota. In addition, the use of eDNA can also be used to evaluate the efficiency of engineered plants. On the other hand, if the project does not have the desired effects, it becomes essential to look at why the effect is absent and what can instead be done, developed or otherwise so that growth and development go hand in hand with protection of the environment and nature.

As further improvement step, additional organisms could be integrated into the aquacultural system to remove any by-products such as excessive feedstuff or excrement, creating an artificial ecosystem which maintains clean water without the use of filtration and water pumps. Clean water improves the living conditions for both the cultivated species and other organisms, hence improves the efficiency of the system. Again, eDNA can be used to monitor that these additional organisms do not invade the natural ecosystems around the aquaculture.

The goal of my project is to avoid detrimental impact of aquacultures on the marine environment. I intend to do this by taking an eDNA footprint of the environment before establishing an aquaculture and monitor any impact by regular eDNA sampling afterwards. This implies that my method is limited to conserving the state of the local ecosystem before an aquaculture is build, but the method can of course not evaluate whether this local ecosystem is genuinely “natural” or whether it already was influenced by environmental impacts before the first samples were taken.

It is likely that the composition of species in a local ecosystem will change with time of the year, currents, or other natural influences. It may therefore be necessary to measure such natural variations by long-term observations of eDNA-samples before establishing an aquaculture. Thereby, I can evaluate whether changes of biodiversity in subsequent monitoring are related to establishing the aquaculture or have natural causes.

By using aquaculture, it is conceivable that we can ensure nutritious food for the growing world population, as the global food demand increases. Aquacultures have a high ecological efficiency and low environmental impact as well as low carbon dioxide and methane emission, especially because fish permute a higher degree of feedstuff into protein than mammals.

However, for utilizing the full potential of aquacultures, their resource efficiency and their impact on marine environments needs further improvements. This can be achieved by creating an artificial ecosystem with accurately selected aquatic organisms which live in symbiosis. For example, such an artificial ecosystem with a circular food chain could in the future combine breeding of fish with growing mussels. Maintaining such an artificial ecosystem whilst minimizing the impact on the environment requires to constantly monitor and adjust the conditions to the needs of the organisms. For both land-based and sea-based aquaculture facilities, the optimal location of the facilities in relation to production conditions applies as a central area for continued research.

9. Conclusion

This project has examined possibilities of linking knowledge from eDNA drawn from well-functioning ecosystems to the further development of aquacultures with high biodiversity and low environmental impact to ensure the growth and sustainably use of the oceans, seas, and marine resources. It appears that the use of eDNA and bioinformatics assays can be used to develop symbiotic aquaculture systems that mimic natural ecosystems. In addition, the project has made a design proposal for how these multi-cultural aquacultures can be developed and it has been explained why aquacultures have a great opportunity to be part of the future greener transformation. It concludes that sustainable aquaculture in artificial ecosystems is an innovative approach to conventional fish farming in favor of ensuring marine biodiversity as well as the protection and sustainable use of the oceans and their resources.

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