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BIGH: a case of an urban aquaponics farm in the metropolis centre

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Obrigada.

"Sê todo em cada coisa. Põe quanto és

No mínimo que que fazes."

Odes de Ricardo Reis, Fernando Pessoa, 1933

Declaração de Honra

Declaro que, o presente relatório final de mestrado, é da minha autoria e não foi utilizado previamente noutro curso ou unidade curricular desta, ou de outra instituição. As referências a outros autores (afirmações, ideias, pensamentos) respeitam escrupulosamente as regras de atribuição e , encontram-se devidamente indicadas no texto e nas referências bibliográficas, de acordo com as normas de referenciação. Tenho consciência que a prática de plágio e auto plágio constitui um ilícito académico.

RESUMO

Inserido num contexto de estágio profissional, o presente relatório engloba a descrição pormenorizada das rotinas estabelecidas da piscicultura pertencente à empresa BIGH, localizada no centro urbano de uma das principais capitais europeias – Bruxelas.

É possível fazer uma distinção das rotinas da empresa dividindo-as em duas grandes categorias. A primeira é denominada como rotina de vigilância, e nela estão inseridas o controlo dos parâmetros físicos e químicos da água, estado dos tanques e das instalações da piscicultura, dos materiais e equipamentos (como por exemplo, os biofiltros), além do estado de saúde dos peixes, controlo do stock e de outros recursos, como a comida e também, estabilizadores de pH, ou até a medicação usada como sedativos e antibióticos.

A segunda categoria integra a rotina associada aos pedidos e às vendas da empresa que, para a equipa da piscicultura, apenas incluía a parte do processamento do peixe até obtenção do produto final.

O presente relatório tem como principal foco a rotina de vigilância, destacando as tarefas de controlo da qualidade da água, as respectivas condições do sistema, assim como o bem-estar dos peixes. Contudo, perto do final do estágio, a empresa tomou a decisão de realizar um processo de renovação e modificação da piscicultura e quarentena da empresa. Este processo inesperado e interessante é definido pelos peritos em aquacultura como um processo de "*shutdown*".

A este respeito, foi atribuída a tarefa de melhorar as rotinas já estabelecida pela empresa em termos de biossegurança, o que resultou no desenvolvimento de novos protocolos, que se encontram incluídos no presente relatório.

ABSTRACT

As part of a professional internship, this report contains a detailed description of the established routines of fish farming belonging to BIGH, located in the urban centre of one of the main European capitals - Brussels.

A distinction can be made between the company's routines by dividing them into two main types. The first is called daily routine, and it includes the control of physical and chemical water parameters, the condition of the tanks and fish farming facilities, materials and equipment (e.g. biofilters), as well as the health of fish, control of the stock and other resources such as food, pH stabilisers, or even medication used as sedatives and antibiotics.

The second category includes the routine associated with the orders and sales of the company which, for the fish farming team, only included the part of processing the fish until the final product was obtained.

The report is mainly focused on day-today routine, highlighting water quality control tasks, respective system conditions, as well as fish welfare.

However, near the end of the stage, with the company's decision to carry out a process of renewal and modification of the fish farm and quarantine of the company. This was something different and remarkably interesting and can be defined by the experts in aquaculture as a "*shutdown*" process. Regarding this, the task of improving the routines in terms of biosafety was assigned, which resulted in the development of new protocols for the company, all included in this report.

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ABBREVIATIONS AND ACRONYMS

AOB – Ammonia Oxidizing Bacteria

BCE – Before the Common Era

BIGH – Building Integrated Greenhouses

$Ca(ClO)_2$ – Calcium hypochlorite

$CaNO_3$ – Calcium nitrate

$C_{10}H_{12}O_2$ – Eugenol

CO_2 – Carbon dioxide

ºC – Celsius degrees

EDTA – Ethylenediamine Tetraacetic Acid

EFA – Essential Fatty Acids

EU – European Union

FA – Fatty Acids

FAO – The Food and Agriculture Organization

FCR – Food Conversion Ratio

g – Gram

IMTA – Integrated Multi-Trophic Aquaculture

Kcal – Kilocalorie

KJ – Kilojoule

L – Litre

LAP – Land Animal Products

LHO – Low Head Oxygenerator

MBBR – Moving Bed Biofilm Reactor

Mg – Milligram

m^2 – Square meter

m^3 – Cubic meter

mm – Millimetre

m/hr – Meter per hour

NaClO – Sodium hypochlorite

$NaHCO_3$ – Sodium bicarbonate

$Na_2S_2O_3$ – Sodium thiosulphate

NH_4 – Ammonia

NOB – Nitrite Oxidizing Bacteria

NO_2^- – Nitrite

NO_3^- – Nitrate

O_2 – Oxygen

P – Phosphorus

PUFAs – Polyunsaturated Fatty Acids

Psu – Practical Salinity Units

RAS – Recirculating Aquaculture Systems

RBC – Rotating Biological Contactor

HDPE – Black Polyethylene High-Density

TAN – Total Ammonia and Nitrogen

1. INTRODUCTION

Scientists revealed that humans have been producing fish for their consumption for millennia. Records have shown that some cultures would harvest tilapia from ponds in Egypt at 2500 BCE (Sneed, 1973) and, according to Basurco and Lovatelli, 2003, there was also coastal aquaculture in the ancient Roman civilization to farm fish and oysters.
Aquaculture is a form of fish farming which involves breeding, cultivation, and marketing of aquatic animals, as well as plants in a controlled environment (Swann, 1992), being this the major reason that led this activity to spread worldwide. Over the decades, with the emergence of new demands, environments and knowledge, new techniques have been developed.

The Food and Agriculture Organization (FAO) highpoints three main types of culture systems, starting with extensive, that includes seaweed culture, coastal bivalve culture, coastal fishponds and cage culture, which includes raising fish within fixed or floating net enclosures supported by frameworks, in eutrophic waters. Natural lakes and farm ponds are examples of extensive systems. For semi-intensive systems, FAO includes integrated agriculture-aquaculture, sewage-fish culture, meaning water treatment ponds and fresh and brackish water pond. Lastly, for intensive systems, the most common production is for carnivores fishes on freshwater, brackish water and marine ponds or in cages and also the production in raceways or tanks (Baluyut, 1995). One of the advantages of this kind of systems is its isolation from the external environment, meaning that all the water parameters are more easily controlled. This also allows for organic waste to be treated before being disposed of in nature, highlighting an environmental advantage, comparing to intensive systems that normally dispose of their water effluent directly. It is important to mention that the terms used to name each type of cultural production usually refers to the number of inputs that are associated with each activity and how extensive the production is.

Many types of production appeared along the years, such as commercial cage aquaculture, pioneered in Norway at the 1970s (Yan et al., 2007), mariculture of salmon and molluscs in Canada, Chile, Scotland in early 1970s and 1990 the rise of seaweed farming (Bostock et al., 2010), an intensive monoculture of salmon and shrimp in the 1980s (Bostock et al., 2010), polyculture with the co-production of six/eight different carp species in China (Edwards, 2015).

More recent was the development of Integrated Multi-Trophic Aquaculture (IMTA) systems, with focus on productivity, while being environmentally sustainable, and also Recirculating Aquaculture Systems (RAS) systems, that can be considered the most recent achievement but that had huge success spreading worldwide. IMTA systems can be found established all over Denmark, France, Germany, the Netherlands, Norway, and the UK, for example (F. Murray et al., 2014). RAS systems are designed to be as much as environmentally sustainable since it is possible to save 90 - 99 per cent less water, when comparing to other aquaculture systems. This is possible because the water is reused along with the system, avoiding water disposal, using machinery that is responsible to filter the water (Badiola et al., 2018).

Very briefly, an IMTA system combines, in the right proportions, the cultivation of fed aquaculture species (more commonly finfishes) with inorganic extractive aquaculture species (principally seaweeds) and organic particulate extractive aquaculture species (such as mussels or other aquatic invertebrates). It is aim is to increase long-term sustainability and profitability per cultivation unit (not per species, as practised in monocultures), by recapturing some of the nutrients and energy that are lost in finfish monocultures, and transforming them into additional crops with commercial value (Troell et al., 2009). A balanced ecosystem management approach having in mind site-specificity, operational limits, and food safety guidelines, as well as environmental quality and regulations, are essential for the good management of an IMTA system.

Nowadays, it is already common knowledge that a non-equal distribution of resources such as food and water created a scenario of developed countries more privileged than the non-developed ones. According to the International Food Policy Research Institute, Global Food Policy Report, 2017, allied to the expected exponential growth of the world population, an increase of demand for all kinds of food will also be predictable, meaning that the space available for food production may not be enough to answer to all the population requirements. When discussing the topic of mass food production, the space needed for this kind of activity is equally massive. This means that there will be a necessity for better management of space for these vast productions and all the basic life requirements such as water and even basic living activity. Adding to this, for the last several years overfishing became problematic, reflecting a bigger threat to the oceans, allied to the surge of the ecological concern associated with this activity. All of this led to a booming of aquaculture (Ahmed, 2019).

Aquaponics associates the use of aquaculture’s effluents, that provide a source of nutrients for plant growth, with the remediation of the water that nutrient absorption from plants achieves for aquaculture (Graber and Junge, 2009).

The symbiosis that is established on these kinds of systems allows using the nutrients resulting from the aquaculture wastes as nutrients for horticulture (Estim et al., 2019), growing fishes until they reach a size adequate for human consumption. The re-use of nutrients found in aquaculture effluents that are used in aquaponics as a source for plant growth will remediate the water and allows an efficient recirculation of water in the system (Suhl et al., 2016). Today, commercial aquaponic production exists primarily in controlled environments, such as greenhouses or outdoor locations with a controlled climate system (Love et al., 2015), since being established in these kinds of controlled environments reveals to be more beneficial as well as easy to manage for the producers.

Aquaponics are being highlighted for their versatility, as they can be very beneficial on metropolises, where space is limited, not only for space purposes since they can be implemented on unused spaces that were assumed not rentable for any activity, such as rooftops but also for saving and minimizing the energy/water costs usually associated to this kind of activity. Also, little wastewater is created in these systems, minimizing the environmental impact associated (Yep and Zheng, 2019). This kind of activity is considered an example of sustainable circular economy and an efficient way to economize energy (Love et al., 2015) since there is a well-organized “chain” where what may seem a waste product turns into main nutrients available in the system while trying to also optimize the available spaces (König et al., 2018).

Allying aquaculture production and horticulture allows creating an almost self-sustainable system that can be a beneficial response to socioeconomic issues – response for the overpopulation needs while also distributing the resources more equally. The strategy of aquaponics – linking two distinct activities such as closed systems aquaculture and hydroponics – reveals a substantial reduction on costs, since it promotes the reusing the water as fertilizer on plant and vegetable production. This underlines the capacity of water management. Therefore, it minimizes the possibility of aquaculture effluents contaminations that are usually associated with aquaculture and horticulture.

Although this utopian idea would be ideal, just like any other activity there are some difficulties associated with it. Due to the huge commercialization and aquaculture intensification aligned with the globalization of trade market, diseases and parasites are examples of problems tied to this kind of activity.

Diseases can have different pathogen sources such as bacteria, parasites, fungus or viruses and each express symptoms differently on fishes, although the diagnosis of them might be difficult sometimes due to similarities of some symptoms. When addressing salmon farming, sea lice (most common are *Lepeophtheirus* sp. and *Caligus* sp.) are the most common parasites, reaching levels of contamination so high that, in Chile, since 2008, was implemented a biweekly government-mandated monitoring program to assist in the management of this ectoparasite (Kristoffersen et al., 2013). Another example was the outbreak of the iridoviral disease that led to the eradication of Portuguese oysters originally imported from France (A. G. Murray, 2013).

The correct maintenance of a fish farm facility, as well as tanks, materials, food and correct sanitization of the operators and visitors, are simple responsibilities which, when properly controlled, play a major role on preventing possible contaminations. Currently, there are many mechanisms of preventing and minimizing the contamination risks in fish farms, such as temperature or light cycle changes to rupture parasites life cycle, targeting the most infectious phase.

When avoiding it is not achievable, controlling and containing any epidemic pathology becomes the main focus for the survival of the batch.
After the pathogen enters the system, the priority should be minimizing the mortality throughout antibiotics and autoimmune boosting components incorporated (Barman et al., 2013) on the food, such as vitamin C or E and even n-3 fatty acids.

For all that was mentioned above, the correct management, including cleaning and sanitation, of a fish farm should be the first step to reduce possible diseases. If this fails, correct use of possible treatments and medication is crucial, as the fish raised is often for human consumption, meaning that the treatments given should be not harmful to humans and are regulated by law.

1.1 INTERNSHIP'S FRAMEWORK

This internship was held in Erasmus+, in the capital of Belgium, Brussels, and was carried out on a business environment at BIGH's company, more precisely in their fish farm, also known as pisciculture. The internship lasted six months, starting on November 2019 and ending in May 2020.

For a start, the internship included two major stages: "before the shutdown" and "the planning of the shutdown". The "before the shutdown" stage, is the description of the normal routine at the beginning of the internship. During this period, the company had not decided yet that they wanted to do the shutdown. Subsequently, the shutdown planning was inserted more to the end of the internship and involved all the preparation and planning of such a process.

Piscicultures demand regular supervision and routines, which must be coordinated with all the needs and concerns of the aim product or products being manufactured. The daily tasks assigned during the internship consisted of chemical and physical parameters supervision, such as water analysis, including pH, temperature, conductivity and CO_2 levels analysis and also included biological control and maintenance – mortality count and the removal of the dead fishes, feeding, backwash and others, according to each week requirements, the fishes health and the overall system status. Since the fish species produced at BIGH were used for human consumption, orders were checked and listed, the fishes had to be prepared in advance so that they would be ready to ship accordingly to sanitary and health standards. Due to these procedures, the fish processing had to be done one or two days before the delivery time. The process of harvesting and processing was also one of several responsibilities during the internship. Other duties, such as fish transfers, tank maintenance, cleaning and sterilization were also conducted according to the BIGH planner.

Closer to the end of the internship, while still performing the daily tasks and the normal fish farm routine, a biosecurity protocol was developed, that would be applied for sanitation and cleaning the system during the shutdown. As far as the first step of the shutdown the dismantling process, it was delayed due to constraints and slow of product outflow. Therefore, it was not possible to register outcomes.

All the procedures referring to tasks performed at the BIGH's pisciculture mentioned above are described in detail, as well as explained on the section entitled as "Description of the routines in BIGH pisciculture" found further on in the report.

1.1.1 Recirculating aquaculture systems

Aquaculture enables high production of fish according to human demands, allowing not only to mass produce, but also to produce various species such as fish, crustaceans and, nowadays, even algae, one at a time – monoculture – or many at the same time – polyculture or integrated multitrophic aquaculture – on enclose and limited spaces, or in cages placed in the open sea (FAO, 2000). This reflects the extensive possibilities of ways to produce in the aquaculture world.

Recirculating aquaculture systems (RAS) are indoors, tank-based systems in which fish can be grown at high density under controlled environmental conditions (Zhang et al., 2011). These system principles are based on the re-use of water, meaning that RAS rely on different water treatment techniques to maintain optimal water quality for the fish (Timmons and Losordo, 1994).

Firstly, it is important to choose the right tank design. Factors such as size and shape, depth and self-cleaning ability can have a substantial impact on the performance of the production. These days, three (or four) types of tanks exist circular, square with cut corners, raceway and the "hybrid" between both circular and raceway, d-ended raceway tanks (Lekang, 2020). Each type promotes different conditions for the species chosen and it is the company and pisciculture manager's responsibility to make the right decision.

For circular tanks or square with cut corners, the water moves in a circular motion which creates a hydraulic pattern that gives certain "self-cleaning" ability, since the organic matter will more easily be drained. Besides, oxygen control and regulation become easier on circular tanks, since the water column is constantly being moved, distributing the O_2 equally in the tank. On the other hand, for raceway tanks the hydraulic pattern will not impact the organic matter washout; this can be influenced by the number of fish found inside the tank (Lekang, 2020). The oxygen content in raceways normally tends to be higher in the inlets when comparing to the outlets. Nevertheless, the O_2 content will depend on the amount of fish found inside that will promote its distribution (Bregnballe, 2015).

To reach optimal conditions for fish production while maintaining proper water quality, a cascade of water treatment steps need to be installed. Although this suffers some changes with the fish species and also with the facilities, the essential treatment processes consist of mechanical and then biological filtration, CO_2 removal, the addition of O_2 afterwards and then some form of disinfection such as, for example, UV lamps (Ebeling and Timmons, 2012).

Therefore, the mandatory equipment for this to be possible is mechanical and biological filters, followed by the disinfection system (that can also be installed at the end of the circuit to guarantee good water conditions), protein skimmer, degasser, also called as CO_2 stripper and then the O_2 supplier, the oxygenator. Heating and/or cooling systems are also vital as well as an efficient pipe and pump circuit. There is additional equipment that can also be installed according to each fish farm aim such as automatic feeding system as well as handling system (Helfrich and Libey, 1991).

More importantly than to know the dynamics is to understand the role of each equipment in this cascade. The mechanical section traps solid matter that accumulates gradually originating a sludge at the end (Mirzoyan et al., 2010). This sludge can have several uses, such as biogas production, generating heat or electricity or agricultural uses, such as fertilizer (Van Rijn, 2013). Also, the water resulting from this mechanical treatment can be used in aquaponics after the right treatment (Diver and Rinehart, 2000).

Secondly in the system, the biological filtration, that depends primarily on the water temperature and the pH. This process is responsible to strip dissolve compounds such as nitrogen that can be toxic when in free form NH_3, also known as ammonia (T. Yang and Kim, 2019). So, in the biofilter, there are chemical reactions (Figure 1) that naturally occur and need to be understood so that water chemistry remains ideal.

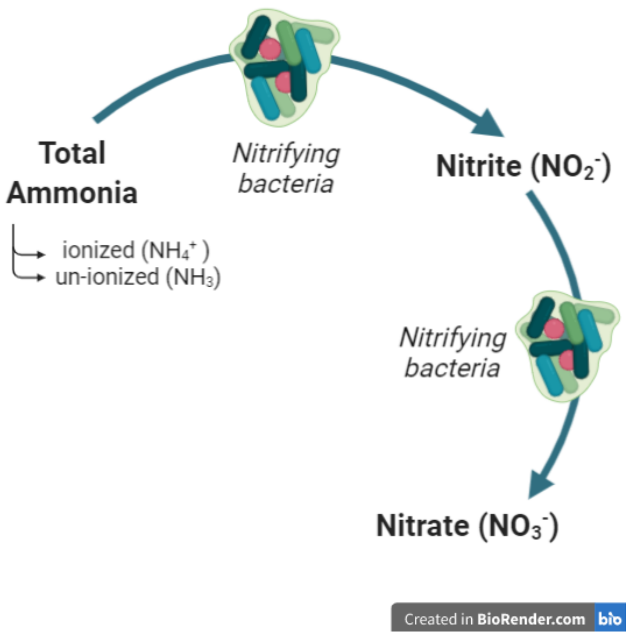


FIGURE 1 - NITRIFYING BACTERIA CHEMICAL CHAIN

Next, for the degassing process of aeration, there is a removal of carbon dioxide, resulting from the fish respiration, bacteria activity, and organic matter disintegration. This is important because the accumulation of carbon dioxide and nitrogen will have detrimental effects on fish welfare and growth. Meanwhile, for the oxygenation, it consists of the injection of oxygen in the system. Although it is an especially important step, this needs to be controlled, because high saturation levels of O_2 can also turn to be dangerous to the animals.

Regarding the UV lights, these will be responsible for the disinfection process. These type of lights release wavelengths that cause mutations and in time destroy DNA in biological organisms. Still, to maintain the efficiency of these kinds of lights, it is advised to change the lamps every year. This step should be integrated after all the filtration process, avoiding the shadow effect caused by debris and residue found in the water, maximizing the process.

Overall, it is also important to guarantee monitorization of pH and water temperature, avoiding the possibility of poor water quality and maintaining the right functioning of the entire equipment and system. For this is necessary to install systems that either automatically controls or demand manual intervention, regulated using probes.

This way, fish farming in a closed system such as RAS results in increased biosecurity and higher flexibility in site selection, while producing stock and decreasing depletion of natural resources.

1.1.2 Aquaponics

The emergence of this kind of technology came as an answer to the high demand for food since there has been a “booming” on the world’s population. Not only that but also the fact that energy and water are becoming more limited makes the need for good management of these vital resources crucial. On 2014, FAO reported that aquaculture is one of the fastest-growing food production areas providing just about 50% of all fish and fish products for human consumption (Suhl et al., 2016). The scientific community classifies aquaponics as the generated activity between two other big activities: Aquaculture and Hydroponics. This reflects on culture and production of fish and plants in recirculating systems, at the same time, minimizing space and costs associated with these types of production.

Nowadays commercial aquaponics uses methods and equipment from both the aquaculture and hydroponics industries (Love et al., 2015) to create symbiotic systems that provide the production of edible vegetables while growing fish, which can be also consumed, minimizing wastes during all this process.

Aquaponics can be operated on freshwater and seawater aquatic species, depending on their compatibility (Estim et al., 2019). The species exploited, the major aim of production and all the characteristics of the system, as well as the surroundings and resources available, are the most relevant aspects that will dictate how the aquaponic system will be established. Due to all that was mentioned, there are many and distinct aquaponic systems structured differently all over the world.

Although there is a large spectrum of combinations that can be settled, from the different species used to the engineering of the system, some core factors need to be present to guarantee the equilibrium of the production. Since aquaponics include many and diverse areas such as aquaculture, microbiology, ecology, horticulture, agriculture, chemistry and engineering (Yep and Zheng, 2019) many challenges may appear until a beneficial equilibrium is finally accomplished. Lastly, as any productive activity as agriculture and fisheries, and industry trends study should support the project of the aquaponic system proving that the activity will produce a profit.

Some examples of already established combinations for freshwater are Tilapia and African catfish cultured together with fruiting vegetables, such as tomatoes and cucumber, or leafy vegetables such as cabbage or kale (Estim et al., 2019).
On the other hand, for marine systems working with saltwater species, seagrass or algae can represent a good option for aquaponics.

1.1.3 The system design

The design of aquaponic systems generally mirrors recirculating systems, adding together a hydroponic component, while never disregarding the possibility to allow the correct elimination of fine, dissolved solids and organic material using mechanical filters, biofilter and even foam fractionator.

In aquaponics, the alliance between aquaculture and hydroponics major goal is to re-use the involved resources as much as possible while avoiding pollution and not forgetting also to have a rentable polyculture production (Diver and Rinehart, 2000). Also, when it comes to space management, aquaponics can play an important role, while still being profitable to have a production on urban or unoccupied areas, for example.

The basic thought behind aquaponics is an integration of fish and plants production and, at the same time, water and nutrients are re-used through biological filtration and recirculation, resulting on a biological system that produces waste that will serve as nutrients for the second biological system (Figure 2).

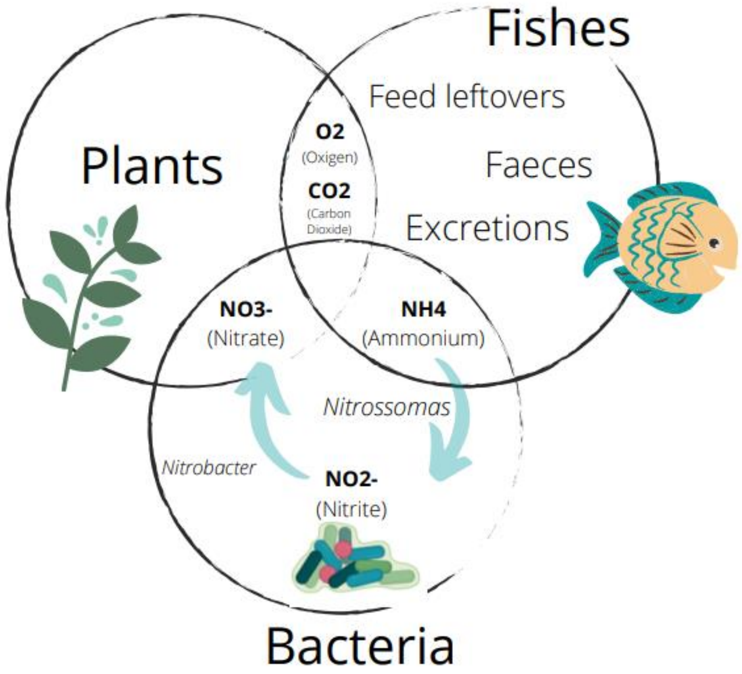


FIGURE 2 - AQUAPONICS' SYMBIOTIC INTERACTIONS

On other words, the nutrient-rich effluent from fish tanks is used as fertilizer for the plants. Afterwards, the nitrifying bacteria living in the gravel and association with the plant roots drain out the nutrients, acting as a biofilter and allowing for this cycle to be fully self-sufficient (Mullins et al., 2016).

1.1.4 NITRIFYING BACTERIA

Nitrifying bacteria are primarily obligate autotrophs, meaning that they consume carbon dioxide as their primary carbon source and obligate aerobes which need oxygen to grow. Ammonia oxidizing bacteria (AOB) obtain their energy by catabolizing un-ionized ammonia to nitrite and include *Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus*, and *Nitrosovibrio*. *Nitrobacter, Nitrococcus, Nitrospira*, and *Nitrospina* are nitrite-oxidizing bacteria (NOB) that transform nitrite into nitrate (Wheaton et al., 1994).

The essential groups of bacteria integrated into aquaponic systems are known as *Nitrobacter* sp. and *Nitrosomonas* sp. In biofilters, nitrifying bacteria often form interactions with heterotrophic organisms, such as *protozoa*, *micrometazoa* and heterotrophic bacteria that may play a role in boosting nutrient availability for the plants, to metabolize biologically degradable organic compounds (Yep and Zheng, 2019). More importantly, these two groups of bacteria are responsible for the nitrification that mostly occurs in the biofilter found in the systems (Diver and Rinehart, 2000).

Nitrification is a two-step process carried out sequentially, that starts with the oxidation of ammonia to nitrite and subsequently to nitrate (equation 1). Nitrite is constantly produced as the intermediary step between ammonia and nitrate (equation 2). Nitrite on high levels is toxic for fish due to its effect on the oxygen-carrying capacity of the blood haemoglobin (Farrell, 2011) so, it is important to avoid high concentrations of this compound in the system. However, for some species, the accumulation of nitrate could also be harmful even though, for plants, this compound can be used as a nutrient. Nevertheless, nitrate concentrations can be regulated adding a denitrification reactor (Boxman et al., 2018)

$$NH_4^+ + O_2 \rightarrow NO_2^- + H^+ + H_2O \ (Equation\ 1)$$

$$NO_2^- + O_2 \rightarrow NO_3^- \ (Equation\ 2)$$

Though, it is important to have in mind that heterotrophic bacteria grow significantly faster than nitrifying bacteria and will prevail over them if there is competition for space and oxygen in biofilters, when concentrations of dissolved and particulate organic matter are high (Carmignani and Bennett, 1977). So, the right cleaning regimen of the filters need to be implemented so this can be avoided.
Adding to this, factors including pH, alkalinity, temperature, oxygen, ammonia, and salinity need to be supervised because they can disturb the rate of nitrification (Shammas, 1986).

1.1.5 Fishes

Fish production allows species variation but needs to be always in sync with the system design and local markets. For aquaponic systems, if there is an equilibrium between fish and plant ratios, toxic nutrients produced will be absorbed by the plants, cleaning the water.

However, fishes are the most common choice of species to be produced, although other aquatic animals such as shrimps or crayfishes are also a possibility, even in aquaponics (Simon Goddek et al., 2019). Tilapia is well known in aquaponics for being tolerant of fluctuating water conditions such as pH, temperature, oxygen, and dissolved solids (Diver and Rinehart, 2000). This warm-water species is one example of a species that grows well in recirculating tank culture. However, in Europe, these kinds of indigenous species have no market potential or economic value and usually have lower consumer acceptance. So, other species that are suitable and are common in European markets are trout, perch, and bass.

Planning what species are going to be included in the system and produced is crucial since they represent the start of the "nutrient chain" established in aquaponics. Therefore, because fishes are the main nutrient source to plants (Simon Goddek et al., 2019) the right choice of species and the right management of it will impact all the production and even the system stability.

A quick way of explaining this is understanding that fishes produce important nutrients (although some are found in their waste as well as in-feed degradation) such carbon dioxide (CO_2), ammonia (NH_3) and phosphorus (P). The feed composition will be intrinsically related to the degradation process of the feed leftovers, so the number of nutrients produced will fluctuate and change from case to case.

Some estimate that approximately half a kilo of fish per 40 litres of water is a common final density at the time of harvest for new or inexperienced growers (Mullins et al., 2016). Although this can and will vary with experience, system design and, most importantly if there are stable waste and solid management.

Factors such as temperature can affect the natural nutrient chain since at higher temperatures, fish metabolism normally accelerates, resulting in more excreted nutrients and faeces (Licamele, 2009). Not only this, but the feed quality and digestibility of the diet may also impact the proportion of nutrients produced (Simon Goddek et al., 2019).

1.1.5.1 HYBRID STRIPED BASS (*MORONE CHRYSOPS* X *MORONE SAXATILIS*)

Morone species, commonly identified as bass, belonging to the *Moronidae* family, are characterized by their medium to large size, compacted elongated body, greenish grey with silvery reflexes and white colouration on the belly. It is equipped with small teeth on the jaws and two dorsal fins (the anterior one is supported by 8 spiny rays while the rear one has a spiniform ray followed by soft rays (Kardong, 2012)). According to FAO, the two European-North African species, *Dicentrarchus labrax* and *D. punctatus* are also included in this family.

The small group of freshwater fish, the white bass and yellow bass, the anadromous estuarine (striped bass) and also the marine striped bass are endemic species found in the Mississippi River drainage system to the East Coast of the United States and Canada. (Guner et al., 2016).

All the species in the *Morone* family are eurythermic, turning the grow-out process to market-size a much more flexible procedure in terms of biological requirements. Also, important to mention, these species are dioecious and group synchronous.

Crossbreed can be beneficial for the producers since it originates more resistant and resilient fish that grow faster than the antecedent generation, a characteristic crucial for fish production (Guner et al., 2016).

1.1.5.2 HYBRID STRIPED BASS PRODUCTION

According to the FAO Fishery Statistics, hybrid striped bass is mainly produced in the United States, Israel, and Italy. Nevertheless, other countries, such as Portugal, France, Germany, Vietnam (mostly on the South), China and even Russia are considered producers of this fish. Most recent FAO FishStat shows that in 2016 the Global Aquaculture Production for *Morone chrysops* x *M. saxatilis* was around 5382 tonnes.

Also, the ability of this species to be produced all year long makes the hybrid striped bass a good candidate for an aquaculture fish. According to FAO, the process of production includes three phases starting with hatchery, fingerling production (nursery) and finally grow-out. The production cycle starts when there is a cross between striped bass and white bass to originate a hybrid. The type of hybrid can change and depends on the parent's sex and in *Morone* case, the possibilities are palmetto bass (striped bass ♀ X white bass ♂)

and the sunshine bass (white bass ♀ X striped bass ♂). The palmetto bass has exhibited superior growth and survival and, at the same time, lower metabolic energy needs in comparison to striped bass. For the sunshine cross, it has shown positive attributes, such as rapid growth and disease resistance (Nematipour et al., 1992).
Regardless of the cross, hybrids' eggs must be manually stripped from the female and then manually fertilized with sperm from the male of choice, meaning that hybrids do not reproduce by themselves.

Since all *Morone* spawns in freshwater, this can be achieved increasing spring water temperature from 12 ºC to 24 ºC. It is important to mention that in nature, striped bass is a pelagic spawner while the other *Morone* spawns near shore, typically near to vegetation or rocky substrates. The peak of spawning is reached around 18 ºC – 20 ºC.

This species fingerling production is considered the most delicate process due to the food requirements and risk of predation (Ludwig, 2004). Egg development for this species is fast, requiring about 36 hours to hatch when at 20 ºC (R. M Harrell, 2016). After hatching, it begins their life journey and five days after hatching the larvae are ready to start to receive food such as artemia and rotifers. FAO states that larvae of *Morone* hardly accept artificial food as their first feed. In addition to this, being particularly small, the fry of *Morone* cannot reserve many nutrients in their yolk sacs. Not less important is the fact that the small size of the fry makes them much more vulnerable to predation by invertebrates than the larvae produced with striped bass eggs (R.M. Harrell, 1997). Maybe because of their small size and vulnerability, to increase the chances of larvae survival it is recommended to implement feeding regimes with sources that contain high levels of unsaturated fatty acid enrichments such as EPA and DHA.

Larvae are stocked until they become fingerlings with 30 - 40 mm, when they are ready for the last phase of the grow-out that ends when the fish reaches the market size from 0,5 to 1 kg (Figure 3).


FIGURE 3 - HYBRID STRIPED BASS PRODUCED AT BIGH. YEARLING (LEFT FIGURE) AND ADULT (RIGHT FIGURE).

1.1.6 Plants

Plants are the major key that enables the removal of waste products from recirculating aquaculture systems (RAS). They are capable of using the CO_2 originated from the fish and the uneaten feed as their source to growth (Diver and Rinehart, 2000). Plants as an element are ultimately related to climate considerations when starting an aquaponic system. This means that there is an intrinsic association between the place where the aquaponic system settles and the choice of plants to be produced (Bernstein, 2011).

Regarding farm location, there are different kinds of approaches to this topic. There is the possibility of growing indoors during the summer, growing outdoors and when winter strikes bring the aquaponics indoor, using greenhouses or, for the last option, limiting the production only to summer or winter depending on the case (Simon Goddek et al., 2019). It is important to never forget that the production of plants is always connected to the fish production, since the wastewater from the fishes is used for the plants, providing the essential nutrients (Licamele, 2009).

Starting with the seasonal production scenario, which is less beneficial to the manufacturer, the planning is crucial when starting aquaponics but, in this case, it is even more since the producer depends on the seasons and has to be prepared in advance, so there no possible mishaps may happen. The other thing to have in mind is to choose a specific species that will certainly grow on the chosen season, leaving no space for miscalculations for the production (Gears, 2017).

Growing indoors is also possible but such, as any other, has benefits and some difficulties associated. When choosing to grow indoors, some concerns have to be considered in advance such as lighting, in some cases heating, the weight associated to the substrate of the aquaponic systems, as well as the weight of engines and water of the fish production and the possible water spilling and humidity that are unavoidable in aquaponics (Diver and Rinehart, 2000). Having in mind that water weighs about 1 kilo per litter and, depending on the substrate, but using gravel as examples, weighting 1682 kilo per m^3 (Bernstein, 2011) adding the weight of the fish stock and all the support equipment associated to it, when growing indoors the weight is a very relevant subject. Although there are not so traditional soil-free options nowadays (Somerville et al., 2014), these are normally applied when the aim is an extensive production. Adding to this, unless there is some source of natural lighting, there is a need to install a lighting system according to the species necessities and set alight with a cycle suitable for them. Depending on the species, choosing the wavelength of the light also changes, not only according to each species needs, but the canopy levels are also a factor that could dictate which, how and where the system of lights has to be constructed.

The other possibility, according to the author of "*Aquaponic gardening: a step-by-step guide to raising vegetables and fish together"*, exchanging sites or the production out and indoor, depending on the season and the size of the system. When the approach is for a commercial purpose, this option is not suitable since it involves a lot of moving and changing in the middle of the production. This case is only considered when starting a small project or a personal project.

Lastly, starting to be more and more common, are the greenhouses. For production on a larger scale, this is the most favourable way, since it provides a controlled environment (Wu et al., 2019) built all around the main focus of supplying plants, vegetables and/or fishes. Not only this, but the greenhouses can naturally hold heat, decreasing the costs associated with heating. Condensation is also naturally created on greenhouses, reducing the water use on the majority of cases. This might be common, but for some species, complementary watering can also be needed (Gears, 2017). All the details mentioned must be settled after the selection of plants that are going to be produced. There is a large variety of possibilities depending on the pH, temperature and even luminosity (Wu et al., 2019).

In 2014, Somerville categorized plants in aquaponics according to the nutrient requirements as follows:

- Plants with low nutrient requirements, such as basil.
- Plants, such as cauliflower, with medium nutritional requirements.
- Plants with high nutrient requirements, such as strawberries or other fruits.

Nowadays, there is some knowledge about the plants that are possible to produce in such systems, for instance, lettuce (*Lactuca sativa*) with variants such as iceberg, loose-leaf or even romaine. Basil (*Ocimum basilicum*), tomatoes (*Solanum lycopersicum*), cauliflower (*Brassica oleracea*), eggplant (*Solanum melongena*), peppers (*Capsicum* sp.), beans (from *Fabaceae* family), peas (*Pisum* sp.), broccoli (*Brassica oleracea*) and even berries such as strawberries (*Fragaria virginiana*) and even blueberries (*Rubus loganbaccus*) are other possibilities of plants that can be provided in such system (Diver and Rinehart, 2000). At BIGH, the main production was centred in microgreens, such as basil, parsley (*Petroselinum crispum*), and cherry tomatoes (*S. lycopersicum* var. *cerasiforme*).

All the previously mentioned depend on certain nutrient needs that cannot be under or oversupplied, so it is important to retain that producing different plants can be challenging.

The right combination of fish and plants, as well as the right stocking density, needs to be established from the begging to obtain positive results.

The control of macro and micronutrients such as nitrogen, phosphorus and even carbon should also be guaranteed so the equilibrium of the aquaponic system can be maintained (Wu et al., 2019). Luckily, up to 100% of the phosphorus found in the water originated from the production of fish can be recycled by the plants (Figure 4), but for other nutrients such as potassium, not so easily originated by fish production, sometimes need tighter control when cultivating for example fruits (Mullins et al., 2016).

FIGURE 4 - PLANT EXAMPLES PRODUCED AT BIGH. CHERRY TOMATOES (LEFT) AND BASIL (RIGHT)

2 BIGH

BIGH - Building Integrated Greenhouses - the rooftop aquaponic farm, can be considered a brand-new enterprise since it only started their production very recently, in 2017.

The innovative vision led to the idea of trying to reuse the roof of an old food market building found on the *Ferme Abattoir,* giving it a purpose to something that otherwise would not be used. This is the first aspect of the circular economy that distinguishes this company.

BIGH Farm also has a handful of synergies installed, such as recycling the heat originated from the refrigeration retailers' cold rooms, electricity reimbursement from the solar panels installed as well as using water from the well, besides collecting water from the rain, leading to better water management and minimizing the costs, reusing water.

BIGH can be divided into three main areas: fish farm, greenhouse, and outdoor garden (Figure 5).

FIGURE 5 - BIGH'S OUTSIDE VIEW (LEFT) AND OUTDOOR GARDEN (RIGHT)

The BIGH outdoor garden can be considered one of the largest rooftop productive gardens in Europe and a very innovative farm with some new farming techniques on rooftop outdoor substrates.

It is on BIGH's greenhouse that herbs, tomatoes, and microgreens manufacture can be found, and those three different areas can add up to 2000 m^2 production area.

Certainly not less important, the fish farm on BIGH can produce almost 35 tonnes of hybrid striped bass per year and the water used in the aquaculture system enriched with nutrients and solid waste is used to fertilise the vegetables produced. With all of what was mentioned it is possible to consider BIGH an aquaponic system.

2.1 HUSBANDRY FACILITIES

As explained before, BIGH's farm was built on the rooftop of an already existing building in the core of Brussels city. Therefore, the space management for this project was always a huge component since all the areas had to be maximized as possible for the demands of BIGH. The pisciculture was located after the outdoor garden and near to the tomato production since the water from the pisciculture was being channelled to the tomato farm as served as a water source as well as fertilizer.

Using the designed layout plan (Figure 6), after entering from the main entrance (1) and before entering the pisciculture, the monitoring panel (Figure 7) was located on the wall next to the pisciculture. When inside the pisciculture, the first thing that could be found was the quarantine (2a), which was already dismantled since the beginning of the internship. At the same point, it was already possible to see the tanks of the pisciculture and both waterways (8) between the two tanks rows. Fish processing occurred on the point marked as 4 and, according to sanitary and biosecurity regulation, next to it was the freezing room (3). Also, according to the standards, the changing room (5) where both visitors and operators should sterilize and clean their hands and equip themselves according to the rules with hairnets, white lab coats or full overalls, white boots, and latex gloves.

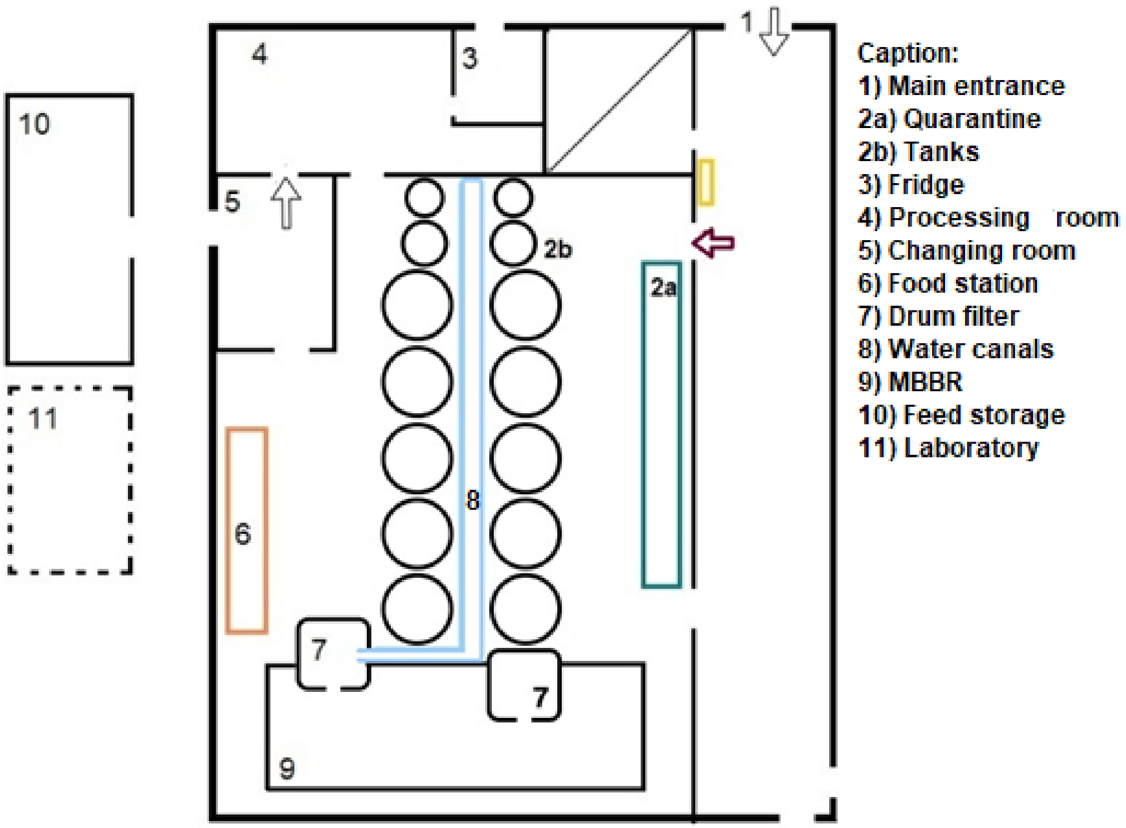


FIGURE 6 - SKETCH OF BIGH'S PISCICULTURE FACILITIES

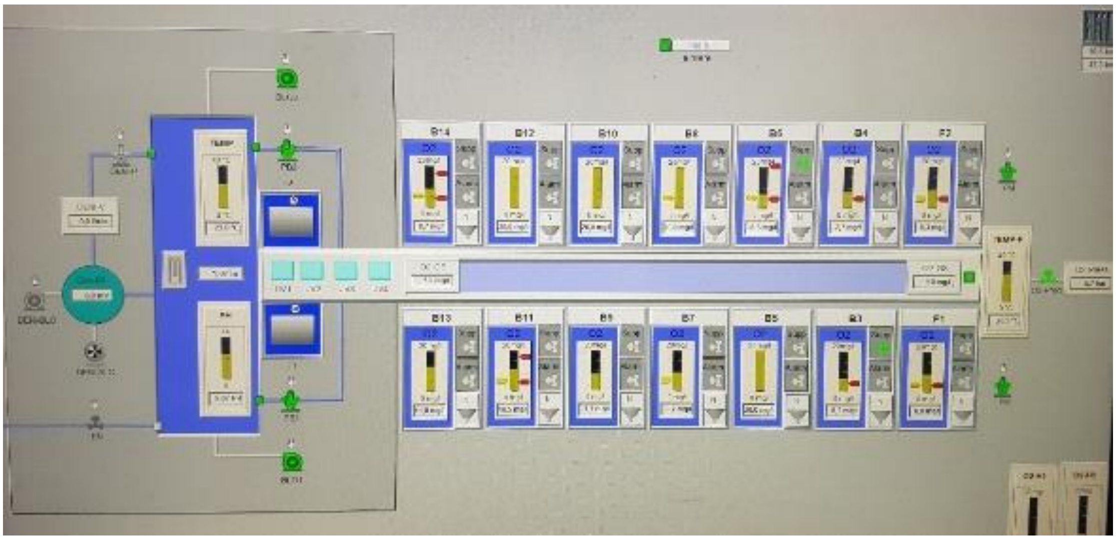


FIGURE 7 - BIGH'S PISCICULTURE CENTRAL CONTROL PANEL

The feed preparation station was located on the point marked as 6 on the diagram, but all the food was stored outside the pisciculture on a separated room (10). At the end of the pisciculture, it was possible to find the equipment station. First were the drum filters (7) and at the very end was the Moving Bed Biofilm Reactor (MBBR) (9). To access this station, it was needed to go upstairs and the MBBR machine was on a level that almost reached the ceiling, turning into a difficult task if the operator was a taller person.

The lab (11), used for the daily analyses, was not on the same building of the pisciculture but rather located on a different building near to the pisciculture and it had to be accessed by stairs.

2.2 QUARANTINE

Before the shutdown, the fish were imported from Israel and grown from 1 gram to a market size, which includes weights between 300 and 600 gram.

The quarantine installations included six tanks, the first four with 0,8 m^3 and the other two with 1m^3. The system was installed almost on a cascade way, meaning that the water stream was going unidirectionally - from Q1 to Q6 (Figure 8). Since the very beginning, there were some troubles in the system, not only because of the unidirectional water stream but also because the water flow created was not strong enough, leading to serious problems with accumulation of residue and sedimentation of waste while oxygenation levels decreased along with the system. The correct maintenance of oxygen levels is crucial especially in the early stages of life and low levels of oxygen can represent high levels of offspring mortality (Huet et al., 1986). The task of controlling oxygen levels is more difficult when chemical treatments are running that will end up consuming even more oxygen.

FIGURE 8 - BIGH'S INITIAL QUARANTINE SYSTEM

Also, there was an inefficient organic matter evacuation and this can be explained by the way the waste exit was built, not accounting the fact that weak or no stream existed on the more distant tanks, representing a loss of capability of expelling the waste to the proper evacuation exit, leading to the accretion of organic waste and proving another malfunction on the system.

An even more aggravating miscalculation was the misplacement of the biofilter on the quarantine since this was assembled before the mechanical filter which is often the first step in the recirculation loop, reflecting on a lack of filtration, increasing the organic loading and bacterial growth while reducing the biological filter performance. All these combined decreased the overall quality of the water in the system.

Since the beginning of the internship, the quarantine was not functioning and in fact, was already being dismantled. The information gathered was collected on the BIGH’s archive and information from the aquaculture manager.

2.3 GROWTH-OUT

The BIGH system was assembled by *Spranger®*, the german company, with 117 m^3 of volume destinated for the fish production, with a total of fourteen tanks distributed by purpose starting with two tanks of 3.3 m^3 for purging purposes, two more tanks of 5 m^3 and ten tanks of 10 m^3 each.

The tanks were round-shaped and made of black polyethene high-density (HDPE) and were installed in two rows separated by the canals – organized the even number on one side and the odd on the other side (Figure 9). With 1.65 meters high and with a water depth of approximately 1.25 meters, there was a sloping bottom, integrated with a small sump found on the centre of the bottom, protected with a perforated disk to avoid fishes from being pulled into the system. Each tank also had a surface pipe to remove the floating foam.

FIGURE 9 - BIGH'S GROWTH-OUT SYSTEM

BIGH was equipped with a low head oxygenator (LHO) responsible for infusing oxygen into the system's water effectively. Each control panel was set up on the side of the tank (Figure 10). A direct ratio exists between the amount of oxygen in the system's water and the quantity of fish produced, so more oxygen means more fish that will translate on an increase in profits. To help the distribution, these LHO's were supplied with plates with large holes and in the oxygenation chamber, there were inserted floating plastic balls to assist oxygen transfer. For BIGH, the control and management of oxygen were made per tank with the inlet concentration around 150%. In the case of CO_2, it was measured in the system and at times it reached 30-40 ppm.

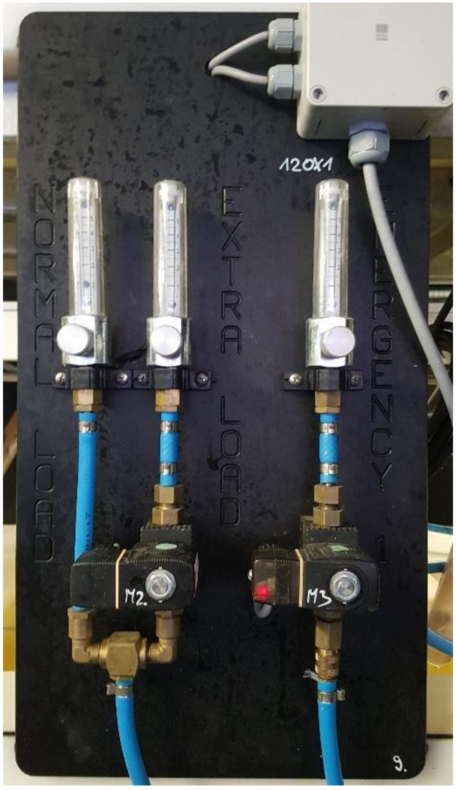


FIGURE 10 - OXYGENATION CONTROL PANEL AT BIGH

The effluent used in the pisciculture was led to the water treatment area via a drain canal made from PHDE sheets and supported by a steel frame. The wastewater was drained using a canal, especially for this purpose, leading into two drum filters covered with 65-micron mesh fabric. The drum filters flow in a large rectangular moving bed filter with a water depth of around 1.05 meters and are 5.4 meters wide and 10.5 meters long. The 55 m^3 MBBR water volume contains about 27 m^3 black curler media. The MBBR is heavily aerated with a set of two centrifugal blowers that receive up to 450 m^3/hr clean air from outside the building. In the middle of the MBBR, there are two vertical shaft propeller pumps, each mounted in a 1-meter diameter screen cylinder. The pumps pump the water into the supply waterway via a UV unit.

The circulation flow was set to reach a maximum of 250 m^3/hr (125 m^3/hr per pump). The total system volume is approximately 170 m^3. Make-up water is supplied at a rate of up to 25 m^3/d from a buffer tank that is installed on the roof, which receives water from a well. The system water is heated through a heat exchanger coil that is installed in the MBBR.

Finally, the photoperiod represents a huge part of any aquaculture system. Light serves as a guide for many biorhythms in nature – seasonal or diurnal – both related to the periodicity of light (Calado et al., 2017). Many animals, including fish, show a 24-hour cycle in their activities being more active in light, less active in darkness, or vice versa, depending on the species. Fishes light receptivity changes with the developmental stage (Gilles and Le Bail, 1999) but it is ultimately linked to feeding. These natural cycles are influenced by changes in other factors, such as temperature or oxygen availability.

According to the species produced at BIGH, the light cycle settled at the fish farm was a light period of 18 hours and a dark period of 6 hours. The light period started at 5 in the morning until 11 at night.

2.4 BIGH'S PISCICULTURE ROUTINE

Just like in other piscicultures, the routines in BIGH's pisciculture were crucial to maintaining the system functional. Piscicultures require full-day tracking of what is happening since small differences could lead to a malfunction on the system, compromising the entire production. This means that there is supervision all day, all week and weekend, even at night-time, although it is not expected to be as busy and could be considered just a monitoring stage.

BIGH's routines could be divided in three, the morning routine, starting at 8H until 12H, followed by the afternoon routine, between 14H and 18H/19H. After that time, it may be considered the night routine. During the internship, all the routines were done, except for the night routine. It was also required meticulous planning of tasks and coordination between them. In BIGH, each task had its periodicity: some tasks had to be done every day and others each week or even monthly, as described in table 1. All the tasks mentioned were crucial for the performance of the pisciculture.

TABLE 1 - SUMMARY OF TASKS PERFORMED AND EACH PERIODICITY

Task name / Periodicity	Daily	Weekly	Other
Water analyses	✓		
Laboratory analyses	✓		
Mortality count	✓		
Backwash	✓		
Feeding	✓		
Fish processing			✓
Brushing the tanks		✓	
Facility cleaning		✓	
Changing the water from foot washers		✓	
Changing sodium hydroxide (NaOH)			✓ (checking every 3 to 4 days)
Sensor calibration		✓	
Cleaning drum filter		✓	
Fish weighing		✓	
Tank transfer			✓
Tank disinfection			✓
External analyses			✓
Fish processing facility analyses			✓

For a better understanding of the previous table, for the periodicity, it is possible to find a third column identified as “Other”. On this, it is included monthly and whenever it was needed, meaning that there was not an established period. For instance, the fish processing would fluctuate with the orders and demands, sometimes more than once a week, other weeks there may be no orders. At the same time, tanks transfers occurred whenever the fish reached the optimal size to be moved for another tank, while the tank disinfection only occurred if there was a transfer of the entire batch, or if there was any tank needing to be emptied. Therefore, this could occur every two weeks or monthly, so there was not a stipulated period to do it. The external analyses were performed by *BRUCEFO®* laboratories. Although not mandatory, for quality and management reasons, BIGH decided to ask for water external analyses monthly. For the after shutdown routine, the same control measure was applied for the fish processing facility aiming to detect bacterial presence in the room.

Regarding the changing of the sodium hydroxide solution, it was also dependent on another factor, the pH. This solution was used for pH control, meaning that if there were big instabilities of this parameter, the amount of the solution disposed of would change with it. When the pH reached low values, more of the substance would be introduced into the system, reflecting on the regular sodium hydroxide's monitoring.

2.4.1 Water quality routine

The start of the routine begins with the three major factors measurement in any aquaculture – pH, O_2 and the water temperature.

All the above mentioned represents an important part of maintaining the proper functioning of the system. Starting with the dissolved oxygen, this parameter is essential for the fish to grow, while being vital for the nitrifying bacteria that convert fish waste into nutrients that plants can use. Whenever the oxygen level is too low, there is a need to increase aeration and this is possible by adding more air stones.

The temperature usually is easily verified on computers, but it is important to always stay alert to small fluctuations since this can be a signal of malfunction or some issues on the heat generators. It is important also not to forget that the temperature is related and can influence other parameters such as pH or oxygen levels.

The measurement of these physical parametric elements was made when possible by using the *MultiLine® Multi 3510 IDS*. Each morning, between 8H30 and 9H30 and, more importantly, before feeding (since the stress associated with feeding and the suspended materials may interfere with the levels of the parameters), the pH, O_2 and the water temperature values being collected.

Two sample points were the water supply channel – where the water came after the drum filter, ready to supply each tank and the system's water outlet – as far as possible of the end of all tanks and right before entering the drum filter. So, with this assortment of sample points, it was possible to compare the values coming in and out the drum filter, making it possible to control the effectiveness of the filter itself, while at the same time controlling the quality of the water.

When hazardous values were detected, some processes had to be performed. There were pH paper strips available on BIGH that were used to check the pH value whenever some unexcepted situation occurred, such as machine malfunction. If there was sudden acidification of the water, meaning the pH will be lower than 7, the staff needed to act. These procedures can be found later in this report (Troubleshooting guides section).

For oxygenation or temperature problems, the emergency O_2 rings were triggered by the operators, or from the central computer of BIGH that monitored the temperature of the system.

2.4.2 Laboratory routine

During the internship, the analyses were performed every morning between 8H30 and 9H30 before feeding the fishes since the fish would be stressed and due to food degradation, that creates suspended solids, as this could alter the values being studied. Gathering all these parameters information allowed to evaluate the state of the system, since for each test there is a permissible range of values (Table 2).

Table 2 - Requirements set by the company regarding water quality

Water quality requirements established in BIGH	
Parameter	**The acceptable range of values**
Temperature	24 – 26 ºC
Ph	6,75 – 7,25
O_2	5 – 12 mg/L
CO_2	< 15 mg/L
Nitrite (NO_2^-)	< 1 mg/L
Nitrate (NO_3^-)	< 400 mg/L
Ammonium (NH_4^+)	< 2 mg/L
Total hardness	> 100 mg/L $CaCO_3$
Carbonate hardness	> 75 mg/L $CaCO_3$

The kits used to measure all the parameters were *visocolor*®ECO, which performs a colourimetric test on each ion found in each water sample. The results from these tests were read through a compact photometer PF-12Plus. Likewise, the water analysis regarding physical parameters was performed directly from the system. For the lab analyses, it was needed two water samples, both on the same point, differentiating only between the inlet and outlet waterways.

For each sample point in the pisciculture, tests were conducted twice, totalizing twelve tests. Adding the water samples also needed to the CO_2 tests, it was needed at least 650 mL to execute all the laboratory tests. All the results were written on a daily report and archived on an online database.

For the ammonia as well as nitrate and nitrite the final values were obtained doing the average between each test results and multiplying by the respective dilution factor.

It is important to mention that, because of a malfunction on the pH probe, during the last months from January until the actual shutdown the pH was controlled using *Macherey-Nagel*® pH strips 0-14 which represent less exact control equipment.

2.4.2.1 CARBON DIOXIDE

The instrument used for measuring carbon dioxide calculates the amount of free dissolved carbon dioxide concentration directly in the water sample. In other words, the probe evaluated the amount of free dissolved CO_2 – partial gas pressure – that affects the fish. The *OxyGuard*®CO_2 provided continuous and direct measurements at the moment and salinity adjustment was possible although for BIGH minimal quantity of salt was in the system. The salt used was only added to the purging tanks to enhance the after taste of the processed products.

The *OxyGuard*®CO_2 Portable Dissolved CO_2 Analyser consists of a probe and a battery-powered transmitter. This probe was essential since it measured the carbon dioxide content of the water directly by detecting the carbon dioxide partial pressure in the water. It is important to note that this quantification was not based on a pH measurement meaning that the machine was not prepared to detect any pH changes. The range of the instrument was 0-50 mg/L. While laboratory analyses were conducted, CO_2 tests would be running. Therefore, the water sample used for these tests was collected before feeding time.

During the internship, the company allowed testing various scenarios with different parameters that could affect CO_2 concentration. The first trial was performed on the first month in BIGH and it only served to develop some hypothesis to where to test after the machine returned from the supplier. The first focus was to see the differences in CO_2 between tank densities.

After the return of the machine, the CO_2 control was strictly made only on the upper and water outlet. Although there was an attempt to try to determine CO_2 peaks during the day in the purging tank, it was not possible to collect data over a substantial period and with the correct consistency, this made it impossible to draw any conclusions or predict the CO_2 peaks.

Regarding the first trial, focused on the differences in CO_2 between tank densities, it is important to highlight that it was necessary to make a random selection of 20 cases since there was a difference between the number of data collected (N=20 low density and N=25 high density). By interpreting graph 1 it is possible to denote that there is no exact symmetry.

There were involved 20 replicates in low density were included and 25 replicates in the high-density case. For this reason, only 20 cases of the 25 replicates available were randomly selected for the high-density tanks.

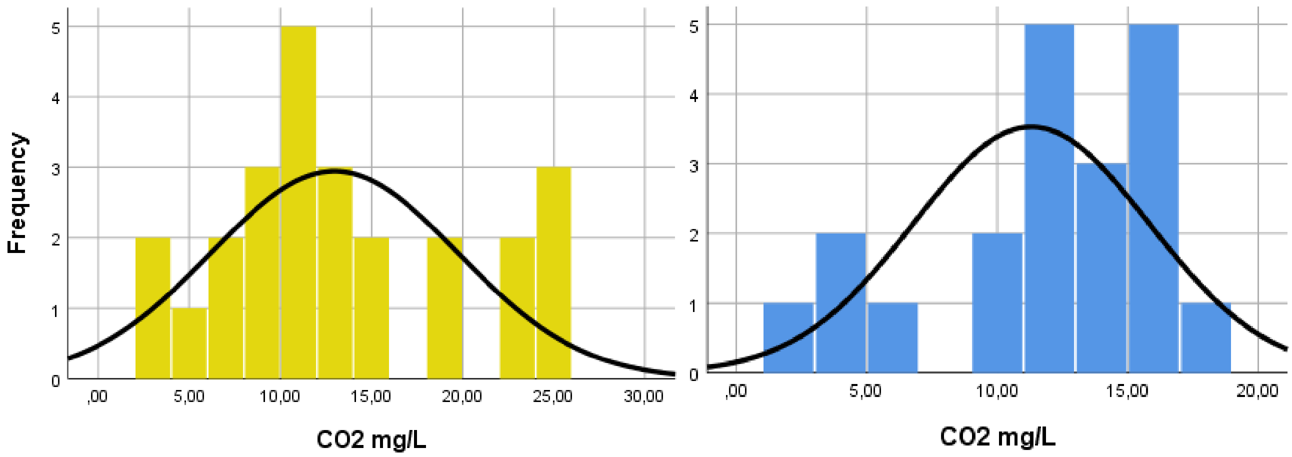


FIGURE 11 – FREQUENCY GRAPHS FOR CO_2 LEVELS. LEFT – HIGH-DENSITY TANK AND ON THE RIGHT LOW-DENSITY TANK

After that, an analysis with 20 replicates in each level of the single factor was performed. Through *IBM SPSS®* software according to the statistical analysis, the value of kurtosis is negative for both cases, low-density tank -0.27 ± 0.992 and for high density -0.72 ± 0.902. When running a one-way ANOVA test to evaluate the hypotheses, it is assumed that the density of the tank will not be able to affect the CO_2 concentration values - this will be the null hypothesis in this case. According to the test of homogeneity of variances (Table 3) $F(1{,}38) = 3{,}124, p - value = 0{,}085$. Since the p-value obtained is higher than 0,05 this proves that there is no significant difference, although at first sight through the interpretation of graph 1, there are more values within the acceptable range of CO_2 concentration (< 15 mg/L) at BIGH.

TABLE 3 - TEST OF HOMOGENEITY OF VARIANCES FOR CO_2 (TANKS)

		Levene Statistic	df1	df2	Sig.
mg/L	**Based on Mean**	3,368	1	38	,074
	Based on Median	1,626	1	38	,210
	Based on Median and with adjusted df	1,626	1	28,596	,212
	Based on trimmed mean	3,124	1	38	,085

Secondly, there was a statistically significant difference between groups as determined also by the one-way ANOVA test $F(1{,}38) = 0{,}410, p - value = 0{,}526$ (Table 4). Since the p-value obtained is greater than 0,05 it is possible to conclude that there is no significant between tanks. Therefore, the null hypotheses must be accepted. For this trial, this reflects on the assumption of tanks density will not be able to affect the CO_2 concentration values.

TABLE 4 - ANOVA TABLE FOR CO_2 (TANKS)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14,400	1	14,400	,410	,526
Within Groups	1335,200	38	35,137		
Total	1349,600	39			

The second trial was mainly aimed to understand the efficiency of the aeration of the system. There are gas interchanges that take place as standard between the surface of the water and the air. As a result, the concentration of carbon dioxide in the water decreases.

Although this occurs, in aquaculture additional aeration systems are necessary as normal gas exchange is not sufficient with the amount of fish produced.

High levels of CO_2 usually can be related to poor quality of the water. Adding to this, studies point to a relation of additional stress on fish and higher levels of CO_2 (Carmichael et al., 1984). The data was collected during the internship, although it was not possible to collect the CO_2 concentration with the same consistency. For this experience, only the values collected at the same time of the day were used.

A boxplot graphic was made with the data collected (Figure 12). At first, analysing the size of the boxes of both waterways, there is a larger difference between the first and third quartiles in the case of the system's water outlet . This means that the range of distributions is higher for the lower canal. However, it should be noted that the mean and median are significantly closer for the system's water outlet when compared to the upper. For both waterways, the same amount of data was gathered, resulting in an equal number of cases registered N=53.

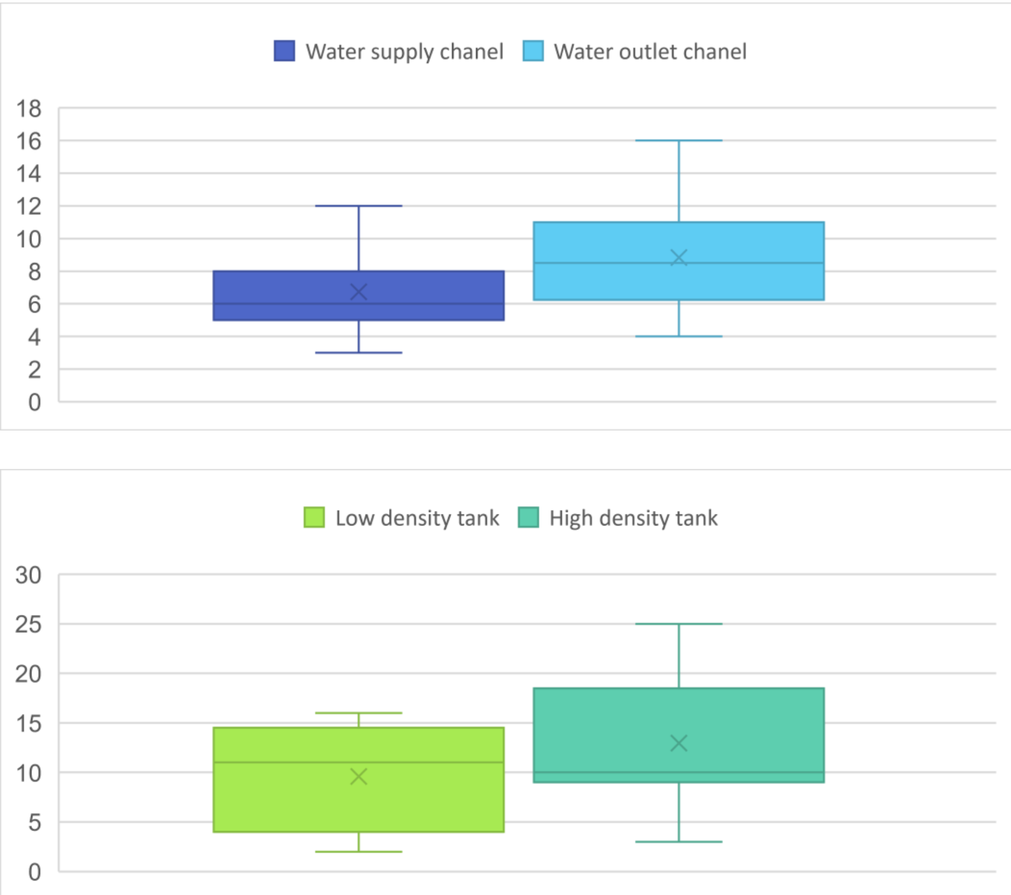


FIGURE 12 - A COMPARISON BETWEEN THE AMOUNT OF CARBON DIOXIDE LEVELS

When comparing the two frequency graphs (Figure 13), it is possible to identify that for the water supply channel the data collected follows the trend curve in opposition to the water outlet which shows a larger record of more distributed cases from the mean value µ= 8,85. When comparing both mean values, it is revealed that the mean of the water supply channel is lower ($\mu = 6{,}74 \pm 1{,}98$) compared to the water outlet ($\mu = 8{,}85 \pm 2{,}85$).

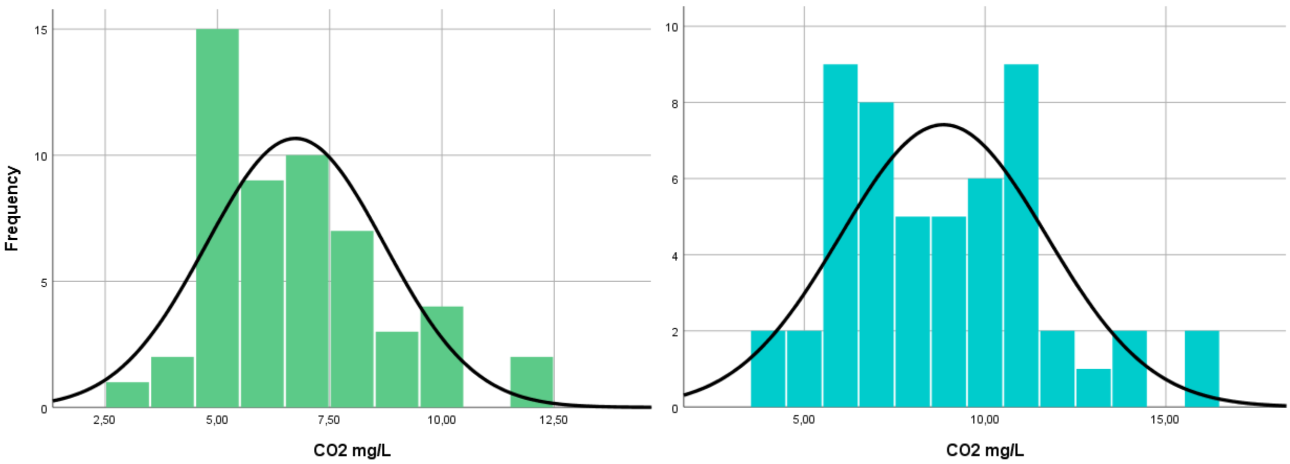


FIGURE 13 – FREQUENCY GRAPHS FOR CO_2 LEVELS. WATER SUPPLY CHANNEL (LEFT) AND WATER OUTLET CHANNEL (RIGHT)

Using the *IBM SPSS®* software it was possible to make a statistical description. Results show that it is possible to infer that in both cases there are few outliers since the value of kurtosis is less than 1. This is also supported by the boxplot which shows no outliers.

For the experiment concerned, the null hypothesis contends that it is not possible to establish a relationship between the amount of CO_2 and the chosen location, in this case, upper and water outlets.

There was a statistically significant difference between the two groups – upper and water outlet – demonstrated by one-way ANOVA (Table 5), $F(1{,}104) = 19{,}626, p - value = 0{,}00002$. Since the p-value obtained was lower than 0,05, the null hypothesis associated with this trial must be reject proving that the level of carbon dioxide differs between the channels of the systems. Variances were heterogeneous according to the Cochran's C Test, so a square root transformation was applied. The subsequent analysis proved a statistically significant result in terms of CO_2 levels being larger in water outlet than in higher.

TABLE 5 - ANOVA TABLE FOR CO_2 (BIGH'S WATERWAYS)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	118,340	1	118,340	19,626	,000
Within Groups	627,094	104	6,030		
Total	745,434	105			

This result was expected as the bottom canal collects the wastewater directly from the tanks while the water supply channel the water to the system after the filtration process.

2.4.2.2 AMMONIUM

Monochloramine can be originated from ammonium ions as a result of the effect of chlorine in the alkaline range and when combined with thymol, forms a blue indophenol dye. This is the active principle for the parameter test that was used. The measurement range for this test was 0,2 – 3 mg/L NH_4^+. Water temperature should never be lower than 18ºC or higher than 30ºC since this would affect the chemical reaction. Low temperatures would decrease the reaction rate affecting the results.

After pipetting 5 mL of the water sample taken from the system, ten drops of the first reagent NH_4^{-1} were added, sealing, and shaking the provided recipient. This was followed by the addition of 1 measuring spoonful (70 mm) of NH_4^{-2} and repeating the sealing and shaking processes. The waiting time after this was five minutes.

Finally, after that time, four drops of NH_4^{-3} were added to the mixture. Only after seven minutes, the product could be interpreted by the photometer.

2.4.2.3 NITRITE

Sulfanilamide is diazotized by nitrite in an acidic solution and the diazonium salt is coupled with naphthylamine to form a reddish-violet dye. The measurement range for this test was 0,02 – 0,5 mg/L NO_2^- and the dilution factor was 1:4.

On a test tube after taking 1 mL of water sample and prefacing the rest with distilled water until the total of 5 mL, four drops of the first reagent NO_2^{-1} were added. Following with 1 measure of the kit spoon (70 mm) of the second reagent, NO_2^{-2}, shake and let it react for ten minutes, after that period it was possible to make the readings on the photometer.

2.4.2.4 NITRATE

The kits used performed a colourimetric test that used nitrate ions reduction to nitrite ions in an acidic medium. When combined with a suitable aromatic amine, an orange-yellow dye appears. The measurement range for this test was 1 – 120 mg/L NO_3^{-1}.

After adding five drops of NO_3^{-1} and 1 level measuring spoonful (70 mm) of NO_3^{-2} into the water sample, with the dilution factor of 1:10, the mixture had to be sealed and shaken. The reaction time for the nitrate was five minutes and after that period, the product was ready to be read by the photometer. Some considerations to have in mind are that nitrite could interfere with this test, but this could be circumvented by adding amido sulphonic acid. Temperatures lower than 28ºC would slower the reaction rate. Also, depending on the concentration, oxidizing substances cloud reduce the measurement readings.

2.4.2.5 TOTAL HARDNESS

What is known as water hardness is caused by magnesium and calcium ions which in this test are combined by the complexing agent EDTA forming chelates. The test was carried out by titration using a metal indicator that was capable of changing colour when all the hardness-producing substances have combined.

On a test tube, two drops of GH^{-1} would be added to 5mL of the water sample, until the water turns red. Holding carefully the recipient, the addition of GH^{-2} should be done drop by drop, shaking at the same time the tube, until it changes colour to green. Each drop corresponded to one degree of total water hardness.

Finally, with the information mentioned above, the following calculation was made:

$$Total\ hardness = number\ of\ drops\ (^{\underline{o}}D) * 17{,}8$$

2.4.2.6 ALKALINITY (CARBONATE HARDNESS)

Alkalinity is a measure of the buffering capacity of an aquatic system and refers to the part of calcium or magnesium ions which is present in the form of carbonate or hydrogen carbonate.

The test for carbonate hardness carried out by titration with hydrochloric acid, using a mixed indicator that turned when the pH reached 4,5.

Just like the other tests mentioned before, 5 mL of the water sample was needed. Two drops of the reagent labelled as CH^{-1} was added and the water sample would change its colour to blue. After this, the reagent CH^{-2} was added drop by drop in a vertical way to the test tube, while shaking, until the sample changes its colour to red.

The carbonate hardness should be normally lower than the overall hardness. If this value reveals to be higher, it is possible to conclude that the ratios were abnormal.

With the information mentioned above, the following calculation was made:

$$Alkanity = number\ of\ drops\ (ºD) * 17{,}8$$

2.4.3 Overall analysis of the values

For the entire internship, mortality control was mandatory and one of the most important tasks in the fish farming routine.

According to the graph obtained presented on a logarithmic scale for better analysis (Graph 4), it is possible to see that the mortality was high at the beginning and the values were inconsistent. Despite this, from 30/12/2019 onwards, the values dropped significantly and remained between the range of 1 dead to a maximum of 20 per day. This can be related to the success of the treatment used, minimizing the main pathogens causing the disease found at BIGH. It is important to point out that the number of fishes in the system also decreased gradually and this meant fewer fishes in the system. The mortality may, therefore, have decreased as the fish population also dropped.

The peaks observed on the figure 14, are associated with the slaughtering processes of the tanks decided by the company since the fish were not healthy and could be a possible source of infection to the remaining tanks.

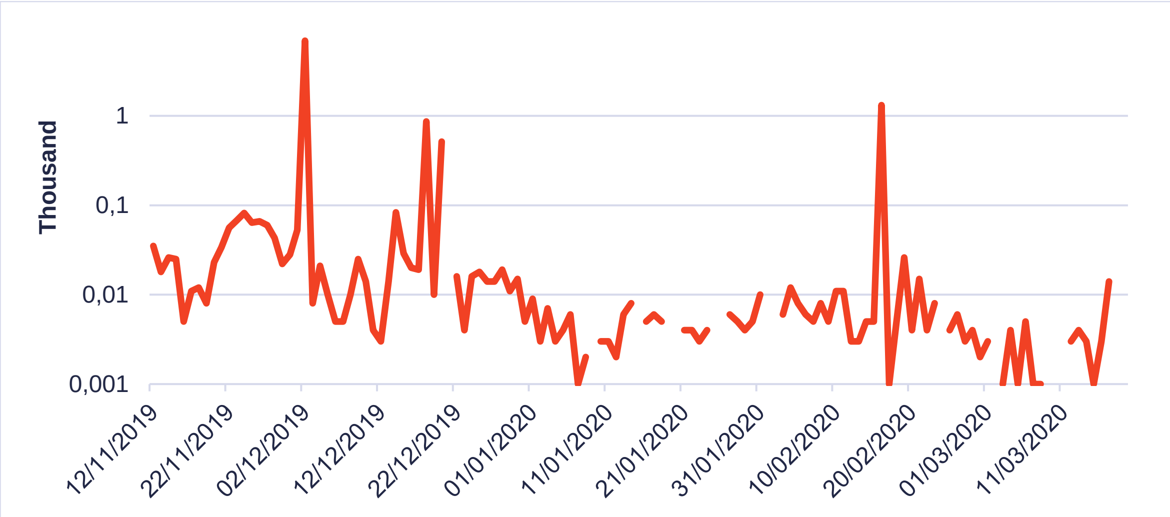


Figure 14 - Fish mortality rate

Moving on to the analysis of some of the most important factors of water chemistry and starting with alkalinity, as previously mentioned, alkalinity can represent the capacity of water to respond to acidity and can be related to other factors such as pH and temperature. Thus, a Pearson correlation was performed through the *IBM SPSS®* software to examine possible relationships between alkalinity, pH, and temperature for both water channels in the system.

Interestedly, it was possible to see a relation between alkalinity and pH for both waterways. For the water supply channel as seen in table 6, it was possible to conclude that both alkalinity and pH are positively related $r(77) = 0{,}05, p < .01$. Although it can be considered this a correlation weak since $r = 0{,}05 < 0{,}1$ (Akoglu, 2018).

TABLE 6 - ALKALINITY AND PH CORRELATION TABLE (WATER SUPPLY CHANNEL)

		pH
Alkalinity TAC [mg/L CaCO3]	**Pearson Correlation**	,500**
	Sig. (2-tailed)	,000
	N	77

**Correlation is significant at the 0.01 level (2-tailed).

For the water outlet (Table 7), a positive relation between pH and alkalinity was also demonstrated since $r(79) = 0{,}505, p < 0{,}01$. This value represents a strong correlation since $r > 0{,}5$.

TABLE 7 - ALKALINITY AND PH CORRELATION TABLE (WATER OUTLET CHANNEL)

		pH
Alkalinity TAC [mg/L CaCO3]	**Pearson Correlation**	,505**
	Sig. (2-tailed)	,000
	N	79

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

Therefore, it was possible to reject the *H0* hypothesis for both cases, meaning that there is a correlation between alkalinity and pH even though it appears that for the system water outlet these parameters are more strongly correlated when comparing to the water supply channel.

In this way, it is possible to find a relationship that shows that the two parameters are related. According to both R-values that are positive, the variables are directly proportional. Subsequently, an analysis of the possible relationship between the three measured ammonia components found in the system was performed. Similarly, to the alkalinity (Figure 15), an independent analysis was performed for the upper and lower channel of the system.

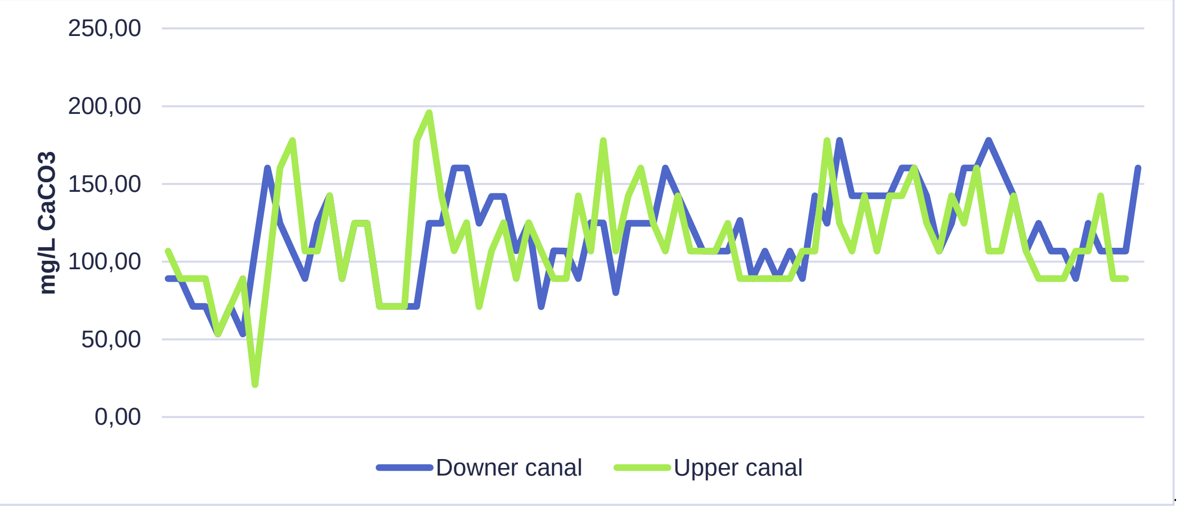


FIGURE 15 - VALUES FOR ALKALINITY IN BOTH BIGH'S SYSTEM WATERWAYS

Regarding the concentration of N-NH_4 on the upper and water outlet, the number of replicates for each canal was different. For this reason, only the first 79 samples of the water supply channel and all the water outlet replicates were selected. According to the analysis, it was possible to conclude that variances were homogenous $F(1{,}156) = 0{,}002, p - value = 0{,}965$.

Using a one-way ANOVA (Table 8), it was possible to reject the null hypothesis since $F(1{,}156) = 5{,}301, p - value = 0{,}023$ and the p-value are lower than 0,05. On other words, it is possible to reject that the concentration of ammonium will not variate between the upper and lower canal.

TABLE 8 - ANOVA TABLE FOR N-NH_4

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,226	1	,226	5,301	,023
Within Groups	6,647	156	,043		
Total	6,873	157			

However, for the other two components, the results were not as clear.

For the nitrite, in terms of results, there was no need for transformation since variances were homogenous. Then, again by using a one-way ANOVA method (Table 9), there was no significant difference between upper and system water outlet since $F(1{,}156) = 0{,}088, p - value = 0{,}767$.

TABLE 9 – ANOVA TABLE FOR N-NO_2^-

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,003	1	,003	,088	,767
Within Groups	6,170	156	,040		
Total	6,173	157			

In nitrate case (Table 10), the results show also that there is no significant difference because $F(1{,}156) = 0{,}755, p - value = 0{,}386$. For both cases, the p-value is greater than 0,05 meaning that the null hypothesis cannot be rejected, and this leads to conclude that the levels of both nitrite and nitrate will not vary between the upper and lower waterway.

TABLE 10 – ANOVA TABLE FOR N- NO_3^-

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	598,245	1	598,245	,755	,386
Within Groups	123684,532	156	792,850		
Total	124282,777	157			

Moreover, a detailed analysis of the possible correlations between the components was then made. For this purpose, a Pearson's correlation test was conducted on the same software.

Using the critical value of Pearson's correlation for 79 degrees of freedom as a reference it was possible to recognize the possible correlation between all three components: N-NH_4, N-NO_2^- and N- NO_3^- found in both waterways.

Whenever the Pearson correlation value (R) obtained is higher than the correspondent critical value of Pearson's correlation there is a correlation between the two components.

It is possible to establish two correlations (Table 11). N-NH_4 and N-NO_2^- on both upper and water outlet are positively linked with $p - value < 0{,}05$. However, there is no established correlation on the supply waterway, for the system water outlet NO_2^- and NO_3^- shown that are negatively linked with $p - value < 0{,}05$.

TABLE 11 - CORRELATION TABLE BETWEEN COMPONENTS

Local			$N\text{-}NO_2^-$	$N\text{-}NO_3^-$
Water supply channel	**$N\text{-}NH_4$**	**Pearson Correlation**	,431**	-,113
		Sig. (2-tailed)	,000	,319
		N	79	79
Water outlet	**$N\text{-}NH_4$**	**Pearson Correlation**	,343**	,047
		Sig. (2-tailed)	,002	,683
		N	79	79

**Correlation is significant at the 0.01 level (2-tailed).

As seen before, for the water supply channel R-value for $N\text{-}NH_4$ and $N\text{-}NO_2^-$ is positive. This proves a reasonable correlation since R-value is approximately equal to 0.5.

In contrast, the R-value between $N\text{-}NH_4$ and N- NO_3^- is negative. This shows that when $N\text{-}NH_4$ and $N\text{-}NO_2^-$ concentrations increase, N- NO_3^- drops or, the most likely scenario for the trial, $N\text{-}NH_4$ and $N\text{-}NO_2^-$ concentration decrease and N- NO_3^- is increasing. This may support the idea that a well-matured biological filter is eliminating the ammonium and nitrite, fulfilling its function. At the same time, there is an accumulation of nitrate, resulting from the natural nitrogen cycle. This should be expected if there is no denitrification process.

With all of this, it should be noted that it was expected to see an interaction between the three components since they are all included in natural chemical chain reactions including the nitrification (Equations 3 and 4).

$$NH_4^+ + O^2 \rightarrow NO_2^- + H_2O + H^+ \text{ (Equation 3)}$$

$$NO_2^- + O_2 \rightarrow NO_3^- \ (Equation\ 4)$$

2.4.4 TROUBLESHOOTING GUIDES

Being an aquaponic farm it was important to guarantee several essential macronutrients and micronutrients for plants to grow. Luckily, the majority of these nutrients are supplied to the plants by the fish feed and by-products produced in the fish component of aquaponics systems.

At any time, if there were problems detected on the system there were some steps that should be followed. This topic addresses that.

ADJUSTING THE PH

If the pH was too low, this needs to be resolved as soon as possible because the natural chemical chains such as nitrification will slow down meaning that the ammonia will start to accumulate in the system. This scenario will be lethal since ammonia is toxic to fish. At BIGH, whenever pH levels lower than 7 were detected, this meant that the base solution in a form of calcium - NaOH or $NaHCO_3$ – might be running out.

The first step was to check if there was a solution in the solution container. The next step was to control the amount of solution added to the system. This needed to be slowly added over several days because adding a large amount of base all at once will severely affect the pH that subsequently will shift most of the TAN (Total Ammonia and Nitrogen) into NH_3. For this, there was an automatic system that was controlled by the staff (Figure 16) that was mainly used to control pH levels whenever necessary.

FIGURE 16 - AUTOMATIC DROPPER LOCATED AT THE BEGINNING OF THE WATER SUPPLY CHANNEL

AMMONIA

Possible causes for higher levels of ammonia include overfeeding, too higher densities for the volume of water or not enough aeration. So, the first thing to check is the levels of O_2, secondly, adjustments in feeding rates can be beneficial and afterwards, maybe fish density can be altered with transfers. On the other hand, low levels of ammonia can be harmful to an aquaponic system since plants need ammonia to grow. Likewise, what was mentioned previously, this can be solved by adding more fish to your system, changing the feeding strategy or to increase the density in the tanks.

NITRATE

When in an aquaponic system, the excessive levels of this nutrient can indicate that not enough plants are being grown, so there is an overflow of nitrate that is being overproduced by the nitrifying bacteria. This can be easily tackled by adding more plants or harvesting more fish to reduce the amount of ammonia produced.

At BIGH since there was a harvest every week, adding more plants was the more suitable solution although this situation was rarely registered.

2.4.5 Mortality

When arriving at the pisciculture, examining the amount of dead and visible sick fishes in each tank was essential. The mortality count was the first daily task to be performed, before feeding the fishes. Before the night routine, if needed, the mortality count had to be repeated. For juveniles, the mortality rate could reach one hundred since at this stage fishes are more vulnerable and weaker.

Figure 17 - Specimens with a head malformation

For instance, when they reach the adult stage, the mortality rate should not exceed more than two or four per tank (should not exceed 2 to 3,5 % per tank). High density as well as malformations (Figure 17 and Figure 18), diseases, a food competition and cannibalism were reasons for higher levels of mortality. Although not overly complicated, this task was relevant because, by removing the dead animals, it reduced a possible source of diseases, while preventing organic matter excess that resulted from the dead fish bodies decomposition. There was always a particular attention to the fact that the tanks had a slope in the middle where dead fishes accumulated. Removing them avoided tank overflowing and stagnating water.

After removing the dead animals, the collected remains had to be stored on a freezer. *RENDAC®* was the company responsible for the weekly collecting of the fish waste, including the dead fishes and the fish processing waste. Although it is usual to collect the pisciculture waste and incinerate it or produce ingredients for pellets, in BIGH's pisciculture the waste was not used for that purpose.

In Brussels, *RENDAC®* is one of the few biofuel companies responsible for the transformation of finished products such as animal fats in a biological fuel for energy production at Belgium's power plants. This was another way that BIGH chose for reinforcing the circular economy, one of the philosophies of the company, minimizing the waste while re-using it for other purposes.

FIGURE 18 - SPECIMENS WITH OCULAR MALFORMATION

2.4.6 BACKWASHES

This process was crucial for the good functioning of the system as well as it guarantees a quick cleaning of the sedimented residues at the bottom of the tanks and more importantly in the pipes.

This procedure had to be performed after the analyses and the mortality rate determination but before feeding as it could trigger stress on the animals.

The "*quick-flush*" can be also considered a backwash since it involved opening the main water valves of each tank. This technique allows a quick exit of water from the system and tanks creating a backwards quick stream that will carry the waste.

At the same time, the water outlet of the system was cleaned since all the organic residue accumulated on it could flow out the outlets.

For the water supply channel, this would be cleaned according to the number of tanks used for the growth-out. This means that, when the system was fully functioning with many tanks in this phase and not on purging, the cleaning of both gutters was more frequent and sometimes had to be done almost every day. Gradually with the increase of purging tanks and emptied tanks, this was only performed once or twice a week due to low levels of organic matter accumulated on the canals.

Generally, for the system water outlet cleaning, only one person was needed as opposed to the water supply channel that needed two people to perform this task.

Brushes and pipe brushes were essential to perform this chore. Due to the system design, the effectiveness of the cleaning process at the water supply channel could only be considered average.

2.4.7 FEEDING

In aquaculture, the nutrition has major importance because a well-balanced diet, adapted to the chosen species and the system, allows maximizing the fish growth, maintaining health and avoiding nutrient deficiency diseases and, even more, specific aspects, as minimizing lipid deposition, important for sturgeon production for example. The size, sex, genetics, reproductive stage, temperature and activity regime are examples of characteristics that influence composition that changes between and within species (M Jobling, 1987).

According to Brody, S. (1945), animals get their needed energy from the oxidation of complex molecules found in the feed. The energy is obtained when the complex molecules are converted to simpler molecules by the digestion. Although energy metabolism in fish can be considered similar to mammals and birds the fact that fish do not spend energy to maintain their body temperature, allied to the fact that nitrogen excretion requires less energy than homeothermic land animals, are two remarkable exceptions which grant more efficient energy management for fishes.
Having this in mind it is important to identify the main energy sources responsible for biochemical reactions as well as how they occur. Fats, carbohydrates and proteins are the three components that are responsible to supply fish their energy needs (Malcolm Jobling, 1995).

2.4.7.1 BIOENERGETICS

The relationship between feeding and metabolism is denominated bioenergetics. All the energy obtained through the ingestion will be transformed and deposited into new body tissues and primarily lost in the form of faeces in the meantime (Rankin and Jensen, 2012). Fishes like any other animal, have a natural energy flow that can express the link between energy needs for maintenance, voluntary activity, and their energy losses.

Accordingly, with Samuel Brody in "Bioenergetics and growth" 1945, fishes lose their energy through the gills, excretions such as faeces, urine and as heat. For the heat lost there are three different sources. The standard metabolism (SM) that includes the energy required to keep the animal alive. Then the voluntary physical activity representing the energy consumed by a fish swimming, moving for seeking food and the energy use for maintaining their position. Lastly, the heat of nutrient metabolism, also known as heat increment or specific dynamic action (SDA), which is the heat emitted by the chemical reactions associated with the processing of ingested feed. In this category, all the energy used for digestion, absorption, transportation, and anabolic activities is included. The cost of excretion of waste products is in the heat increment source.

2.4.7.2 ENERGY SOURCES FOR FISH

It is particularly important to understand that all the energy ingested as food will have to represent the sum of all the fish energy losses with the energy retained (De Silva and Anderson, 1994). Therefore, as seen in figure 19, the ingested energy (I) it is the sum of energy loss as faeces (F), excretory products (U), the energy lost in metabolism (M) and the fish growth (P). Going into this topic in more detail, the fish energetics chain, the partitioning of the ingested feed, starts with the food intake energy so-called the gross energy (GE) that will originate digestible energy (DE) while the first energy loss as faecal excretions and excretory loss on products such as mucosa, enzymes and even bacteria are also produced. The DE will generate metabolizable energy (ME) and at the same time, there will be energy loss associated with the gill and urine excretions.

After this, comes the net energy (NE) that becomes available for what is known as recovered energy (RE) that includes growth, reserves and reproduction and the loss in this process is called maintenance energy – basal metabolism and voluntary energy. Between the ME and NE, there are energy losses such as heat increment, basal metabolism, and

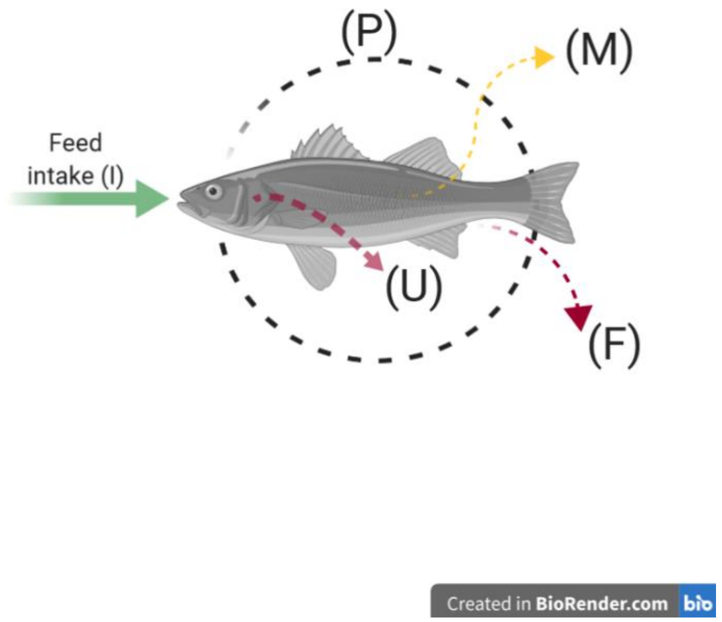


FIGURE 19 - ENERGETIC DIETARY FISH DISTRIBUTION

fish activity. The heat increment includes heat production and the heat necessary for the complex molecule's transformation into simpler molecules (Rankin and Jensen, 2012).

After explaining the fish distribution dietary energy, it is possible to enter on the topic of which are the providers for this energy chain. The Food and Agriculture Organization (FAO) considers that the three most important energy suppliers in fishes are fats, carbohydrates and proteins meaning that other molecules included in feed might not be used for the basic fish energy requirements. According to the species, sex, stage, and others, the energy requirements will change, so for better management of pisciculture, the understanding of this topic can result in the success or fiasco of one entire production.

CARBOHYDRATES

In this category are included sugars that are considered essential for all living organisms. Most mammals use carbohydrates as a major energy source but for fishes, this is not the case since they do not use them efficiently (T. Lovell, 2012). The basic unit of carbohydrates is the monosaccharides that are frequently synthesised as a result of a process such as gluconeogenesis or photosynthesis in case of plants.

In fishes, glycogen is used to satisfy energy demands. This polysaccharide is stored in tissues such as the muscle and, in some fish species, can be used during food deprivation (S. P. Lall and Dumas, 2015).

Carbohydrates as an energy source are considered one of the least expensive components to incorporate in feed although they have some limitations as an energy mediator in dietary aims for fishes. Nonetheless, carbohydrates should not be excluded. Moreover, the percentage included should be controlled since the excess can be deposited as glycogen and can be subsequently less reachable for fishes to use as energy (Kamalam J and Panserat, 2016).

When incorporating this component in fish feed, it is better to use carbohydrates that require some level of digestion, for example, starch rather than the basic units monosaccharides (such as glucose), leading to a time gap between the consumption of the carbohydrates and the appearance of monosaccharides in fish blood (De Silva and Anderson, 1994).

Consequently, for fish, there are still strict dietary requirements for carbohydrate. Whenever these are not included in the diet, studies have shown that other nutrients, such as protein and lipids, are catabolized for energy (S. P. Lall and Dumas, 2015). It has been also proved that carbohydrate may affect fish disease and stress tolerance (Aires, 2012) and this can carry an important role in biosafety matters.

Lastly, this component helps to maintain feed stability due to their binding properties. However, some resources can be more beneficial than others, for example, cereal grains serve as inexpensive sources but most of the fish species can synthesize cooked starch better than raw starch.

PROTEINS

Proteins are one of the most important components in the feed since this supply is considered one of the key factor of fish productivity (S. P. Lall and Dumas, 2015). On the other hand, this is also the most expensive component to incorporate in the feed.

According to Steven Craig of Virginia-Maryland College of Veterinary Medicine in “Understanding Fish Nutrition, Feeds, and Feeding” in 2017, for hybrid striped bass, the protein levels in aquaculture feeds should have preferentially 38 to 42 per cent of proteins. Although the protein percentage appears to be express on the feed, the metabolic source used for energy is, in fact, the amino acids. Fishes do not have a specific protein requirement, instead, they have an amino acid requirement (Aires, 2012). Twenty different amino acids can be found in proteins and these can be divided into two categories: essential and non-essential. The main difference between them is the ability to be synthesized (or not) by animals. The non-essential amino acids are those who can be synthesized by an animal.

In the case of the amino acids in an animal, there are only three possible outcomes: protein synthesis, they can also be structurally converted to another compound and finally can be consumed. In fishes, they are most likely to consume the amino acids meaning that they degraded them for energy (De Silva and Anderson, 1994). This way it is possible to better comprehend the importance of proteins in fish feed.

Before entering on the specific case of BIGH, it is important to mention that there is a wide spectrum of meal possibilities, many categories to assort the food such as food type – natural including “trash” fish or artificial also known as pellets – the type of ingredients used – animal sources (worms, snails, larvae, insects and even fish) or plants (micro-algae, algae, wheat or rice bran and even soy).

LIPIDS (FATS)

Lipids have about twice the energy density of proteins and carbohydrates and usually correspond to roughly 7 to 15 per cent of fish diets (Craig et al., 2017).

As shown earlier, the use of carbohydrates is inefficient for fish, leaving lipids the possibility of being the conventional energy sources in fish diets (Aires, 2012). Of course, different species will use differently the energy sources available, but specialists considered that lipids perform an important role as a source needed for normal growth and development in fish. Not only this, but lipids also support the absorption of fat-soluble vitamins (S. P. Lall and Dumas, 2015) that are also important for fish development.

In this category, different kind of lipids can be included, such as fatty acids (FA) that play an important role in the maintenance of optimum growth and reproduction, health, and even flesh quality (R. T. Lovell, 1991) which is very important for aquaculture producers. Polyunsaturated fatty acids or PUFA such as omega-3 and omega-6 (also known as n-3 and n-6 series) are not synthesized by the majority of fishes so they must be supplied in their diets (Wilson, 2017).

These kinds of fatty acids also represent an important role regarding the sales of the final product since the general public for health reasons prefer foods rich in omega-3 fatty acids that usually are related to health benefits such as improved cardiovascular health.

Fish PUFA requirements vary between marine and freshwater species and there are limits on the maximum lipid levels that can be incorporated in the diets without affecting fish growth performance or body composition (Aires, 2012). Studies have proved that essential fatty acids not only have an important role in growth and survival rate but especially n-3 highly unsaturated fatty acids (HUFAs) can change the tolerance of larval fish to induced stress (Lim and Webster, 2001).

Although lipids are very important for the correct fish development, as well as an important growing and reproductive source when the correct amount of this component is exceeded fish fatalities can occur as it can lead to fat deposition in the liver (Farrell, 2011).

MINERALS AND VITAMINS

Vitamins are required by animals for the growth and maintenance of normal cells and organ functions. These elements can be inserted in two different groups: fat-soluble such as vitamins A and E (usually acting as an integral part of cell membranes) or the second group, that may have the hormone-like function and are water-soluble (vitamin B12 and C that can act as coenzymes accelerating enzymatic reactions) (S. P. Lall and Dumas, 2015).

Just like in other groups of animals, fishes also can suffer from general vitamin deficiency syndromes. Avitaminosis and hypervitaminosis syndromes can also be diagnosed, meaning that vitamins have an important role in fish nutrition (Halver, 1954). When exposed for a long time this deficiencies might lead to death.

It was been shown that vitamin A can be implicated in disease resistance in fish. Studies have demonstrated that whenever there was vitamin A supplementation a consequential decrease in susceptibility to various diseases were detected. Fish tend to have a higher percentage of long-chained HUFAs such as vitamin E. This vitamin can enhance proliferation, chemotaxis, and bactericidal activity of phagocytes at moderate dosages but higher doses can also reduce the intracellular killing ability (Blazer, 1992).
Other studies prove that vitamin C can result in increased survival of infectious diseases of fish (Aires, 2012). Some specialists not only link vitamin C to wound healing mechanisms but also reported interactions between dietary vitamin C and Atlantic salmon's metabolism of trace elements related to stimulation of the humoral defence system (Waagbø et al., 1993).

Many studies have also shown that vitamin C in fish can lead to a reduction of negative impacts caused by stress and environmental factors upon health and disease resistance (Hilton, 1989).

Vitamins play an important role in the nutrition of fish, but minerals also deserve special attention. These are required for normal metabolism and, such as vitamins, there are two types of minerals – macro and microminerals – and in fishes, these elements usually are detected in their tissues (Watanabe et al., 1997).
Some examples of mineral deficiency signs in fish include reduced bone mineralization, anorexia, cataracts or even skeletal deformities (S. P. Lall and Dumas, 2015).

Mineral requirements, such as any other, will always depend on the species, sex, life stage and other factors such as water quality but there are some minerals considered essential that should be always included for the right biological cycles to occur.

Starting with, calcium and phosphorus that are commonly related to the correct development and maintenance of the skeletal system. For example, it has been shown that fish scales are a vital location of calcium metabolism and deposition (Rankin and Jensen, 2012). Calcium it is one of the most abundant elements found in a fish body not only for skeletal-related necessities but also for other functions such as muscle contraction, blood clot formation, nervous transmission, maintenance of cell membrane integrity and also enzymes activation related reactions (Santosh P. Lall, 2003). Another essential mineral is phosphorus that can be located in every cell of the body and it is responsible for crystalline material of bone (Aires, 2012).

For the microminerals, iron is the most known due to the importance of the cellular respiratory process. Copper is an example of a trace element that has an important role in fish since it is a vital component of several enzymes such as ceruloplasmin, that are involved in oxidation-reduction reactions (Santosh P. Lall, 2003).

Symptoms associated with minerals and vitamins deficiencies usually can be detected if there is a daily observation routine. Even though symptoms might and will vary between species there are common indicators such as reduced growth, poor feed conversion, anorexia and skin or fin erosion (Santosh P. Lall, 2003) that are easy to detect by the staff in the fish farm that can sound the alarm for mineral and vitamin deficiencies.

Although vitamins and mineral defects are easy to avoid in fish diets, these are the most frequent category of deficiencies detected in commercial aquaculture (Aires, 2012) mainly due to the high cost of integrating this category of nutrients in the feed.

2.5 BIGH'S FEEDING STRATEGY

At BIGH the chosen feed was artificial – pellets. Starting with the fingerlings, after they arrive at the pisciculture the food given was *Alltech®COPPENS*. Depending on their growth rate, the pellets given were different. The first feed they received was the ADVANCE 0.8 – 1.2 mm, followed by START PREMIUM 1.5 mm and STAR ALEVIN with 2.0 mm. The range of feed not only differentiates itself on the pellets size (Figure 20) but more significantly the composition (Table 12) of each size changes with the growth rate and the necessities of each life stage. The four main features that are present are the sinking capability, high digestibility, LAP free and they all are RAS suitable.

FIGURE 20 - BIGH'S FEED DIFFERENCES IN SIZES

Fishes usually were hand-fed but during the early stages, since it was more beneficial to distribute food during all day, 12H automatic belt feeders were programmed for feeding the offspring. The amount of food for each tank was calculated and changed weekly according to the growth, for the food ratio, middle weighting was crucial.

TABLE 12 - *ALLTECH®COPPENS* NUTRIENT COMPOSITION ACCORDING TO PELLET SIZES

Pellet size		0.8 – 1.2 mm	1.5 mm	2.0 mm
Protein	%	56	54	54
Fat	%	15	15	15
Crude fibre	%	0,1	0,3	1,1
Ash	%	12	10,3	9,0
Total P	%	1,99	1,73	1,32
Vitamin A	IE/kg	14000	12000	27000

Vitamin E	mg/kg	280	240	240
Vitamin C	mg/kg	700	600	675
Gross Energy	MJ/kg	21,0	20,7	21,1
Digestible Energy	MJ/kg	19,4	18,2	17,9
Characteristics		High energy starter diet. Phase feed for optimal efficiency with high survival rate.	Medium energy mini pellet for high performance.	High protein diet for high performance. Good for restocking.

The food given to adult fishes was *BioMar®*, EFICO Sigma 840. This food is ideal for low growth periods and it is formulated with ideal levels of amino acids, vitamins, and omega-3 fatty acids, which are particularly important for the rapid growth of the hybrid species farmed in BIGH. This food included the required pellet size from small fry to adult size with minor nutritional differences (table 13). The fish were hand-fed twice every day. The food conversion ratio supposedly should be 1.65 but had a drop to 1.25-1.40.

TABLE 13 - *BIOMAR®* NUTRIENT COMPOSITION ACCORDING TO PELLET SIZES

Pellet size		3mm	4,5 mm	6,5 mm
Crude protein	%	47	44	43
Crude lipid	%	14	16	18
Carbohydrates (NFE)	%	21	21,3	21,0
Crude cellulose	%	4	4	4
Ash	%	8,4	8,0	7,9
Total P	%	1,2	1,2	1,1
Gross Energy	MJ/kg	20,7	20,8	21,4

Classical digestible energy *	MJ/kg	17,9	18,1	18,6
Typical content of Nitrogen (N)	%	7,5	7,0	6,9

* Classical digestible energy is calculated on proteins, lipids and NFE

2.5.1 Case study on the feeding strategy

A 30-day trial was conducted between February 21 and March 21, 2020, focusing on identifying the best and the most beneficial feeding strategy for BIGH. This study was done mainly to explore the possibilities of feeding scenarios and whether there would be any noticeable results. Since it was not possible to keep the same person responsible for feeding all the time during the trial, this could affect the consistency of the study, thus no statistical accuracy can be guaranteed. Nevertheless, as this study was the basis for the selection of the feeding regimen at BIGH, it was considered important to mention in this report.

For the object of study, four tanks were selected, two with a high density of fish – B6 and B8 – and the other two – B5 and B9 – with low density. Two different strategies of feeding were implemented.

According to table 14, the first (Type *A*) involved the distribution of feed one time per day while the second (Type *B*) involved a three-phase distribution during all day, both for the low and high-density tanks. The total amount of feed was distributed equally according to density.

Table 14 - Feeding trial description

Feeding Regimen	**Type**	**Per day**	**Tank density**	
			High	**Low**
	A	1 time	Tank B6	Tank B5
	B	3 times	Tank B8	Tank B9
	Total amount of feed (*kg*)		6	4,2

The schedules established for feeding the animals were previously defined in agreement with the aquaculture manager to guarantee compatibility with the commercial requests and the whole fish farm routine. Water quality monitoring, as well as the laboratory routines, were not altered during this trial.

Firstly, and before starting the study, it was crucial to collect information such as initial biomass and the initial number of fish per tank to reach reasonable conclusions. There was tighter visual control of satiety and wellbeing during this period of trial.

The shoal or higher presence of animals on the same space leads to a higher demand for food, this could be expected since studies have shown that feeding rate is one factor intrinsically linked to shoal behaviour (Krause et al., 1998). This was taken into account before starting the trial, aiming to see if this applied to fishes at BIGH.

Although the biomass gain it is significant in the case of the high-density tanks (B6 and B8) as seen in figure 21, when comparing the mean fish weight gain it seems that has been a higher weight gain in the tanks B8 and B9 (Figure 22). In both tanks, a three-phase supply was established proving that this approach might be more advantageous.

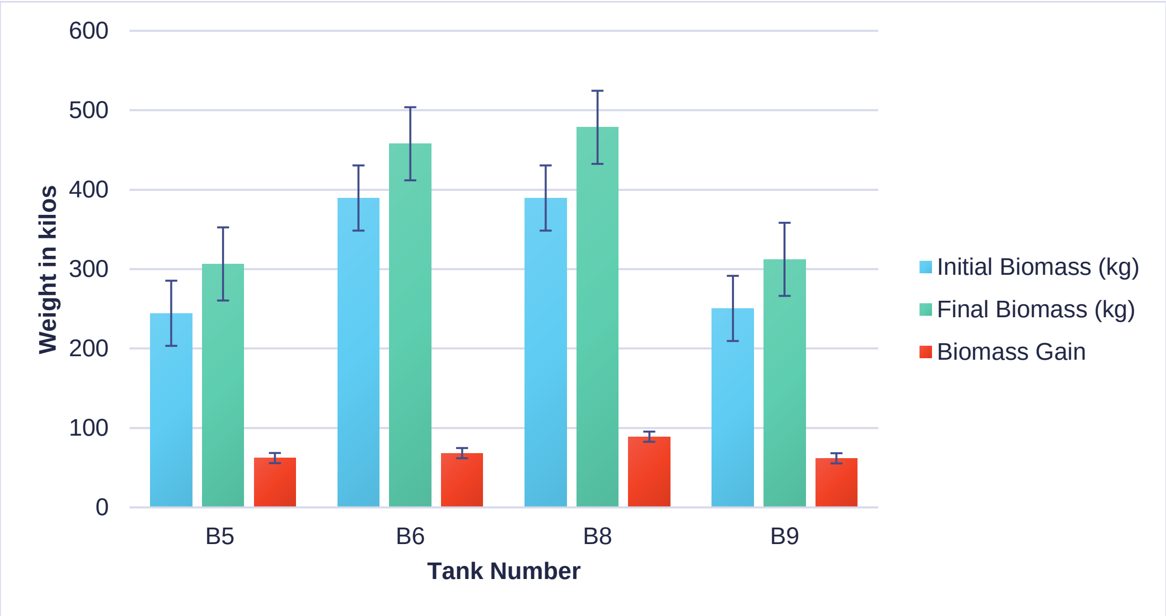
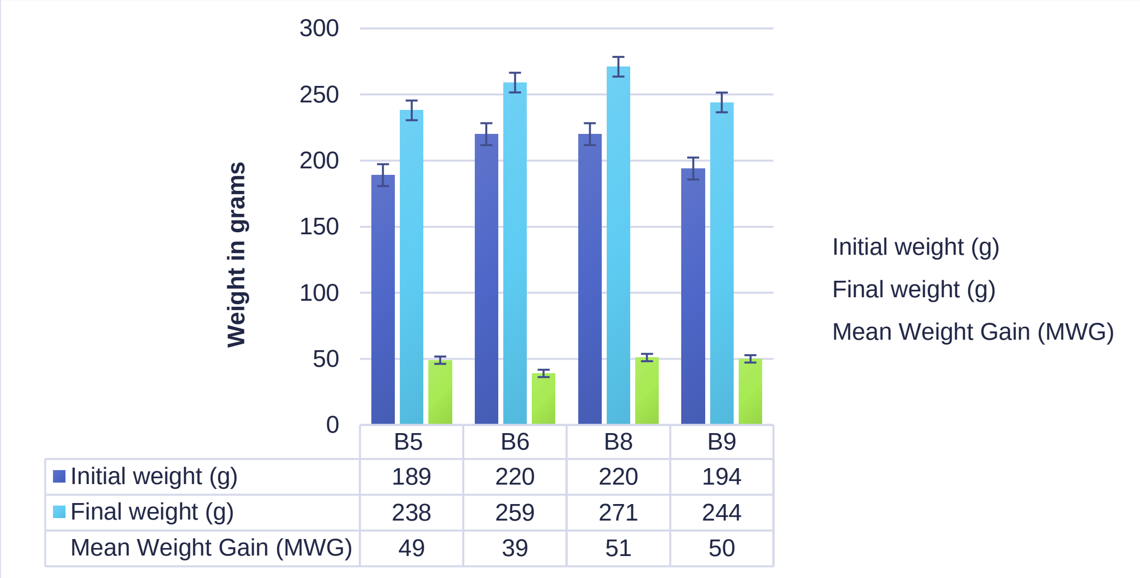


FIGURE 21 - BIOMASS COMPARATION GRAPH



	B5	B6	B8	B9
Initial weight (g)	189	220	220	194
Final weight (g)	238	259	271	244
Mean Weight Gain (MWG)	49	39	51	50

FIGURE 22 - WEIGHT COMPARATION GRAPH

Despite the mortality rate was low (Figure 23), during this experiment, some of the fish showed a loss of appetite. It is important also to highlight the fact that no previous sorting was done and there was a big difference between the sizes of the individuals in the tanks.

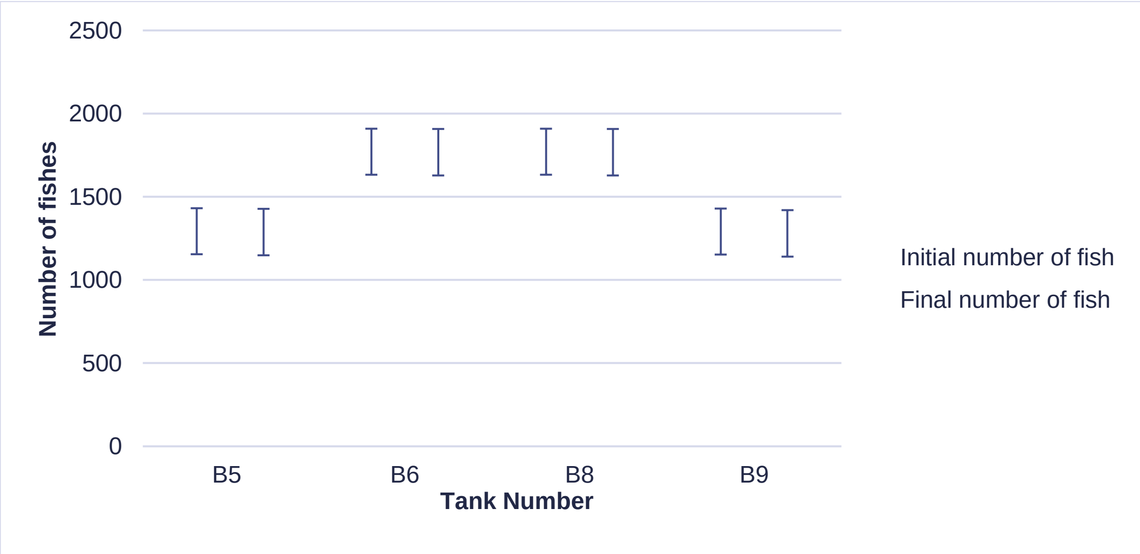


FIGURE 23 - FISH POPULATION COMPARATION GRAPH

This could be the reason to explain the values found in table 15 for the specific growth rate (SGR) that seem to be remarkably close between each tank.

Another important parameter that can be affected by what has been mentioned above is the feed ratio conversion (FCR), as this calculation can be influenced by several factors, including the health status of the animal, gender and age (Parker, 1987).

In conclusion, and although some values might seem to be pointing in different directions (for example according to the table there was an increase of 25 % of growing in B5 when comparing to B8, with 23 %), as mentioned at the beginning, there were gaps during the experiment and for that very reason, some incoherence's were found through the analysis.

Despite that, it was important to highlight this study as the results obtained *in situ* were considered positive by the administration of BIGH. It was BIGH's understanding that fishes seem to be more receptive to food while in higher densities tanks and when fed three different times during the day.

It should be considered that factors such as temperature, light period and even the nutritional composition of the food given may affect the animals' willingness to eat.

TABLE 15 - HYBRID STRIPED BASS PERFORMANCE TABLE

Tank	B5	B6	B8			B9		
Initial Biomass (kg)	244,4	389,5	389,5			250,5		
The initial number of fish	1293	1771	1771			1291		
The final number of fish	1288	1768	1768			1280		
Final Biomass (kg)	306,5	457,8	478,5			312,3		
BIOMASS GAIN	62,1	68,3	89			61,8		
Initial weight (g)	189	220	220			194		
Final weight (g)	238	259	271			244		
Mean Weight Gain (MWG)	49	39	51			50		
Average daily growth (ADG)	2,042	1,625	2,125			2,083		
Weight Increase %	25,926	17,727	23,182			25,773		
Specific Growth rate (SGR)	0,010	0,007	0,009			0,010		
Number of deaths	5	3	5			11		
Mortality rate %	0,387	0,169	0,282			0,852		
Survival rate %	99,613	99,831	99,831			99,148		
Feed hour	12:00	12:00	09:00	12:00	15:30	09:00	12:00	15:30
FR%	1,64	1,54	0,51	0,51	0,51	0,56	0,56	0,56

Amount of feed	4,2		6		2	2	2		1,4	1,4	1,4
The total amount of feed	96		144		144				96		
Feed efficiency	51		27		35				52		
Feed conversion ratio (FCR)	1,959		3,692		2,824				1,920		

2.6 TANKS AND THE FACILITY MAINTENANCE

Sometimes the tasks incorporated in the topic of sanitizing, cleaning and maintenance are not taken as seriously as they should for a fish farm, as ensuring biosafety is critical. For this, some easy task such as brushing the tanks or cleaning the fish farm facility were mandatory. For the first chore, tanks should be brushed as quick as possible to avoid high levels of stress in the fishes and just to remove the accumulated organic matter.

The facility cleaning was normally done before the weekend and included rinsing and scrubbing the floor and feeding station with detergent. When the aquaculture manager gave instructions, the floor would be cleaned with hydrogen peroxide.

On the main entrance for the fish farm as well as inside the locker room before the processing room was two feet cleaning stations with *Virkon*®, a powerful oxidizing product that uses a mixture of peroxygen compounds. It is aldehyde-free and at a dilution of 1 % represents no threat to the environment and the staff operating with it. It was effective to eliminate the majority of microorganisms and the active principle of the product lasted for five days. This means that every five days all the water from the feet cleaning stations had to be changed. Another chemical that had to be changed and supervised was the sodium hydroxide, but this implied regular observation by the staff since this chemical would be injected drop by drop in the MBBR by a pump. Constant supervision was needed since if changes in the pH were detected, the amount of this chemical had to be controlled as well.

The calibration of all probes as well as sensors found in the fish farm and the lab ought to be performed weekly, although sometimes, due to some unexpected problems such as accumulation of organic matter in the probes inside the tanks, the calibration had to be done with different regularity.

2.7 WEIGHING AND GRADING

This process is especially important for the right maintained aquaculture since it can express the development and growing of the fishes. Not only that but also it is with the middleweights that the proportion of food for the next week was estimated.

The middleweight was only performed when the fishes reached 350 grams since BIGH did not aim for a bigger size than that. Depending on each size of the batch, this weighting process changes. In BIGH, the fishes, for this process, are divided into three categories. First of all, for fishes between 1 and 5 grams and 5 to 30 grams, the weighting should be done once a week. In the case of fishes found in the range of 30 until 150 grams the weighting should be done every two weeks and for bigger fishes, above 150 grams, the weighting must be done triweekly.

When describing this process, it may seem that there is little difficulty, still, as any handling, it causes stress to the animals and when not correctly done it may lead to unexpected mortality. The process starts with the harvest of the fishes with nets suitable for each range – mesh net between 3 mm and 4,5 mm for smaller fishes and 15 to 20 mm for the bigger ones. Before the harvesting process, there should already be prepared a suitable container with some $C_{10}H_{12}O_2$ (the tranquillizer named as *Eugenol*®). For every 100 L, it would be necessary 0,75 mL of *Eugenol*®. The containers used in BIGH held around 45 L, meaning that the dosage of *Eugenol*® indicated to that amount of water was 0,3375 mL (approximately 0,40 mL), but never more than that since the overdosage could kill the animals. It was necessary to wait 15 seconds for the *Eugenol*® to react.

While one of the operators harvested, the other had to be responsible for catching any fish that came out of the container and to keep track of the number of fishes inside the container. After gathering the volume of water needed and the fishes started to show symptoms of the effect of sedation, the total weight (A) could be noted down. For the last step, the return of the fishes to the tank, the process should be as quickly as possible before the tranquillizer effects start to wear off and both operators should participate in it. Although the process itself is the same for all tanks there are also small variations in procedures depending on the fish size.

For the first range, the weighting process must be repeated three times while for the upper two categories it must only be done once. This can be supported by the fact of much higher density on the tanks with younger and smaller fishes. Adding to this, on earlier stages growth rate may be indicative of some abnormality so, repeating this process can give vital information also regarding this. Also, it is important to notice that there are some differences in the number of total fishes (B) needed on each weight (Table 16). After all, this process is finished, and all the data is gathered, some calculus had to be done.

TABLE 16 - NET SIZE RECOMMENDATION IN RELATION TO WEIGHT AND NUMBER OF FISH COLLECTED

Size range	1-5 g	5-30 g	30-150 g	> 150 g
Recommend mesh size	3 mm - 4,5 mm		15 - 20 mm	
Total number of fishes (B)	30	30	30	15
Number of repetitions	3	2	1	1
The total amount of fish	90	60	30	15

To calculate the middleweight corresponding to each tank the following equation must be used:

$$Average\ weight = \frac{Total\ number\ of\ fish\ (B)}{Total\ Weight\ (A)}$$

As mentioned before, the sample weight can reveal the growing rate of the fishes and can also be useful to obtain other information such as the feed conversion ratio or the estimative of the food intake for the next week. The specific growth rate (SGR) is also possible to calculate with the information gathered with the sample weighting.

$$Specific\ Growth\ Rate\ (SGR) = \frac{\ln final\ weight\ - \ln initial\ weight}{Number\ of\ days} * 100$$

Food conversation ratio (FCR) can indicate the efficiency of the adopted feeding strategy and also reveal new information. For instance, the amount of distributed feed and its actual consumption or confirm some concerns such as mortality rate (all deaths occurring between the initial weighing and the final weighing are to be deducted from the final biomass - their effect on the FCR is negative).

The feed conversion ratio changes between species and the type of fish's diet. Interestingly, tropical shrimps have FCR considered high that varies between 1,6 to 2,0 (Wyban et al.,

1995) omnivorous fish alternate from 1,4 to 1,8 (Pandit and Nakamura, 2010) and salmonids present the lowest FCR ranges from 1 to 1,2 (Nordgarden et al., 2003)

$$Food\ conversation\ ratio\ (FCR) = \frac{Feed\ intake}{Weigth\ Gain}$$

For the feed estimation or the calculation of the feed intake, the sample weighting provided vital information. It is important to establish that food intake typically has two meanings, on the individual level, such as the food intake for the fish of a certain size, and secondly the food intake on a population level, such as the food intake of a population of a certain age structure and biomass, usually expressed as the intake per unit of biomass during a certain time frame (Hassan et al., 2016).

According to the National Research Council in "*Predicting Feed Intake of Food-Producing Animals*" (1987), there is a variety of factors that can impact the feed intake, starting with environmental factors, such as water temperature, or water chemistry or even the lighting photoperiod. The authors considered factors such as hormones especially important. Lastly, dietary factors are also decisive for the success of the feed intake. These include food particle size and composition, feeding stimulants – gustatory and olfactory stimuli – and the feed quality. Studies indicated that feed that included oxidised fish or vegetable oil leads to low intake levels, meaning that the right storage of the feed is crucial.

To estimate de feed intake each week the total number of fishes (F) and the middleweight (MW) of each tank are necessary. With these two variables, it is possible to calculate the total biomass (B). When multiplying that by the feeding rate (FR), the total quantity of food per day for each tank was obtained.

$$Total\ Biomass\ (B) = \frac{Number\ of\ fishes * Middle\ Weigth}{1000}\ kg$$

$$Estimated\ Feed\ intake/day\ = Total\ Biomass\ (B) * Feeding\ Rate\ (FR)$$

FAO's estimate that the most optimal growth rate can be easily achieved by feeding the fishes two or three times a day so, after calculating the amount of feed per day, the total amount should be divided by two or three whatever the scenario.

2.8 TANK TRANSFERS

The main goal of tank transfers was to separate and sort the batches gradually and according to the size of the fishes. When the fishes arrived (supposedly with 10 grams), they were grouped until they reached approximately 45kg maximum per tank to avoid high levels of mortality at this early stage.

The separation by sizes is essential during all the grow-out to avoid the size differences that could lead to growth issues and, in the worse scenario, to cannibalism. The tank density should not surpass 80 kg/m^3 but, during the internship, when tanks reached the capacity of about 60 kg/m^3, transfers were performed.

For the correct selection of the fish sizes bar graders were used. In BIGH, three different bar graders were used, meaning that there were three options of fish size differentiation (12 mm, 25 mm and lastly 30 mm). Before each grading, fishes had to be sedated with *Eugenol*® (clove oil). For each 100 kg of fish weight, 1,5 mL of this sedative was used. After the fishes started to become numb and to show symptoms of dormancy, such as slow opercular movement, slow swimming and finally the body reverse turning the belly upwards, the procedure had to be done as fast as possible since long exposure to this sedative could also be lethal.

Harvesting was done by hand by lowering the water level and then netting out the fish. The sorting of fish was made by grading using manual bar graders. Although there was a large *Faivre*® roller bar grader, it was never used during the internship.

More importantly, these procedures should always be done in a way that minimized potential contamination with chemical or microbiological exposures. So, this task should be performed as quickly as possible.

All the equipment used in harvestings such as nets and containers had to be cleaned with a *Virkon*® solution (potassium monopersulphate) and well stored after the use.

2.9 TANK CLEANING PROCESSES

First, it is necessary to explain the difference between the cleaning and the disinfection processes. Cleaning includes strongly scrubbing the area or/and tools, with the main goal of solid waste and residue removal (Yanong and Erlacher-Reid, 2012). After that, a pressure washer was used to clean (although this can be also done by hand), using, cleaning products such as detergent. On the other hand, for the disinfection process, chemicals were used to sterilize (Torgersen and Håstein, 1995), meaning that the main purpose is to eliminate microorganisms such as fungi, bacteria, viruses or parasites. Usually, this process eradicates most of the common life-forms, leaving a new and disinfected environment.

At BIGH these procedures were performed before any new entry of fish in a tank or one day after a transfer was completed. The first thing was to empty the tank. After that, the process of cleaning can start using a water pressure machine. All the rough areas as pipe entries and spots that accumulated more waste were scrubbed with brushes. Generally, the brushing started from the top of the tank going to the bottom and fragile materials such as the oxygen ring, probes and the slope protection were given special attention since they usually had more tendency to accumulate solid waste. No cleaning product or detergent was used in this phase. After cleaning, the disinfection process (Figure 24) can start harnessing the fact that the surfaces were still wet. At BIGH the chemical compound chosen for the disinfection was hydrogen peroxide 20 % (*H_2O_2*).

FIGURE 24 - TANK DISINFECTION PROCESS

When added to water, hydrogen peroxide breaks down into oxygen and water over time, and the formation of these by-products is one reason that hydrogen peroxide is reasonably safe for the environment. Hydrogen peroxide's highly reactive nature makes it ideal to use in aquaculture against numerous external fish-disease-causing organisms (Yanong, 2008).

The product should stay for 30 minutes and only after that rinsed with water. Hydrogen peroxide is considered highly reactive, strong oxidizing and bleaching that is classified as corrosive at concentrations higher than 20%. Because of that in BIGH, the chemical was diluted doing a ¼ H_2O_2 for ¾ of water solution.

As mentioned before, regarding the corrosive properties of the chemical, safety equipment such as mask and high gloves are recommended and were used to avoid possible side effects if there was contact with the eyes and skin.

After the waiting period, it was possible to do the last rinse, followed by 24 hours of drying. Only with all the surfaces of the tank finally, dry the process can be considered finished, is then possible to introduce new fishes.

2.10 DRUM FILTER CLEANING PROCESSES

The biological filter found at BIGH was a Rotating Biological Contactor (RBC), a type of aerobic biological filter (Figure 25), capable of creating an environment that promotes healthy growth of the microorganisms that are essential to maintain water quality.

FIGURE 25 - INTERNAL DRUM FILTER'S STRUCTURE

This task had to be performed at least once each week but if there was an opportunity to do it twice this would reveal numerous benefits for the system since the amount of organic matter accumulated would decrease substantially.

This should not be performed by one staff member alone since this kind of machinery is very robust and massive.

Before starting, for safety reasons, it was mandatory to stop the machinery. Although there was another biofilter in the system at BIGH's fish farm, this task had to be performed as rapidly as possible, but still efficiently, so the accumulated organic matter could be washed away. Right after that, it is possible to start to disassemble the big pipes that were inside. Those had to be firmly brushed and after rinsing with water.

Also, especially important was to clean the surfaces inside of the filter and its mesh with high-pressure water. The water sprinklers found above the drum filter should also be strongly scrubbed to avoid the loss of water pressure, because of the accumulation of residues, since they were responsible to automatically remove the organic waste when the machine was connected.

When and after the confirmation of the existence of bacteria, viruses, or other microorganisms, for biosecurity reasons, the use of hydrogen peroxide (H_2O_2) was required. The same steps should be followed but all the surfaces as well as parts, pipelines and sprinkles had to be exposed to H_2O_2. The solution must act for at least ten minutes before being rinsed and let it air dry for thirty minutes, meaning that the entire process had to be carefully organized. The rinsing after the exposure to the chemical is also important to avoid lethal contamination of the fish.

2.11 FISH PROCESSING

While fishes were on the purging tanks, all the orders were noted and organized. Only after knowing that, fishes were harvested. This process was conducted the day before the orders fulfilment, to maintain the final product as fresh as possible. When harvesting the fishes, containers of 45 L were filled with water and ice, causing a thermal shock on the fishes, numbing them, for the electric shock that they were subjected to after that.

The Organisation for Animal Health (OIE) defines stunning as a method that should guarantee the immediate and irreversible loss of consciousness. When the process of stunning fails, fish should be killed before consciousness is recovered.

According to the World Organisation for Animal Health and the European Food Safety Authority, the two best methods to provide a humane slaughter for most of the species farmed in the EU such as Atlantic salmon (*Salmo salar*), Rainbow trout (*Oncorhynchus mykiss*), European sea bass (*Dicentrarchus labrax*), European eel (*Anguilla Anguilla*), and Atlantic Bluefin tuna (*Thunnus thynnus*) include percussive stunning and electrical stunning (Daskalova, 2019). Another method mentioned is spiking, which includes driving a sharp spike into the fish brain. The way used at BIGH for killing the animals can be considered one of the most humane since the ice would daze the fish and the spiking should produce immediate unconsciousness so that the animals would not suffer during the bleeding process.

After these processes, the bleeding takes place. This consists of cutting the gills, putting the fishes in saltwater causing an osmotic shock. All these procedures can take at least one hour, and after, the cleaning of the entrails can occur. The preparation of the final product – filets or just entire fish – closes this stage.

The final stage includes the correct cleaning and disinfection of all the preparation room. Not all the duties in the fish processing were performed regularly during the internship due to personal beliefs, although all of those were attend at least once.

The nutritional values for 100 grams of fish produced on BIGH were approximately 124 Kcal; 518,82 KJ; 22,730 grams of proteins; 2,501 grams for lipids; 0 grams of carbohydrates and 88,00 milligrams of salt. BIGH offered two ranges of fish for selling in terms of size, being the small fish – 300 grams until 450 gram and big fish 450 grams until they reach 600 grams. Gutted or entire fish was also a final product produced by BIGH (Figure 26).

FIGURE 26 - FINAL PRODUCT EXAMPLES - FILET (LEFT) AND ENTIRE FISHES (RIGHT)

3 FISH WELFARE AND HEALTH MANAGEMENT IN AQUACULTURE

Aquaponics nowadays can be sustainable as well as innovative but, like any other productive activity, there should be a concern about the conditions provided to the animals housed in the systems. This way, fish welfare, as well as their correct health management, play an imperative role in the success of aquaponics business operation.

First, it is important to understand what fish welfare is. Having a unique definition for this term it is difficult since it is used in different circumstances and by many, all with different qualifications and experiences. According to personal experience, the best way to describe this concept was that fish welfare includes all physiological, behavioural and cognitive experiences and should lead to a good adaptation, while being capable to let the animals having their natural biological systems working (for example, the immune system), combining with living in an environment free (as much as possible) of negative experiences such as pain, fear and hunger and, at the same time, providing them access to as much as positive experience such as social interactions (Branson, 2008).

Of course, this utopic view is sometimes difficult to achieve in environments that are mainly production focus such as fish farms. Inside these environments, fish welfare concerns should not only include the grow-out process but also the transport and even the killing process. Fish welfare on a fish farm can be adversely affected by factors such as stocking density, diet, feeding technique and water quality (Branson, 2008). Poor control or misconduct regarding these factors usually causes stress and health issues and can even encourage aggressive behaviour.

Moving from a small-scale to large-scale production can be more complex than initially apparent as, sometimes, having more animals to take care might uncover challenges not foreseen in the beginning.

Nevertheless, to improve product quality and increase the market value while achieving positive public perception, unhealthy and weakfish should be avoided, and healthy animals must be raised under welfare standards.

As mentioned above, fish welfare and health control are crucial to avoid stressor factors that can lead to bigger issues such as diseases that might be infectious or non-infectious. Non-infectious diseases are not contagious and are caused by non-living factors, either congenital (such as genetic anomalies) or iatrogenic (induced by external conditions such as nutritional problems)(Ashley, 2007).

On the other hand, infectious diseases are caused by living factors - pathogenic organisms (viruses, bacteria, fungi, or parasites) present in the aquatic environment or carried by other fish. Stressors can reduce fish natural resistance leading to infectious diseases to appear (Figure 27).

FIGURE 27 - FISH WITH SKIN ULCERS AND SMALL ULCEROUS PROLIFERATION ALONG THE BODY

Bacteria can become a problem when fish are exposed to stressors. Bacterial diseases are considered the major cause of mortality in aquaculture (Ashley, 2007).

Among the most common bacterial infections of fish are (Reyer, 1991):

- Columnaris (*Flexibacter columnaris*)
- Furunculosis (*Aeromonas salmonicida*)
- Piscine tuberculosis (*Mycobacterium marinum*)
- Vibriosis (*Vibrio sp*)

Fish parasites can be inserted in the classification of fish infectious disease and are often accompanied by secondary bacterial or fungal infections (Huntingford and Kadri, 2008). They attack and stay in the gills, skin, gut, and muscle tissue, causing irritation, impaired function, weight loss, and eventually death (Figure 28).

FIGURE 28 - SKIN ULCERS FOUND ON THE FISH'S SKIN

Fungal and viral diseases can also affect fishes, although usually, they are not as common as those mentioned previously. According to the Organisation for Animal Health (OIE), some popular fish infections caused by the virus are:

- Infection with koi herpesvirus
- Infection with red sea bream iridovirus
- Infection with salmonid alphavirus
- Infection with viral haemorrhagic septicaemia virus

As stated before, the killing process is also a concern regarding fish welfare and, according to European regulation – General Farming Directive (95/58) –, the practices and techniques used should cause the minimal amount of discomfort and pain to the animals. For this to be achievable different methods are available, that may or not include stunning.

Electrical stunning (in or out the water), as well as percussion, is used before the slaughter process and are more humane to include in the killing process. Other methods such as live chilling with CO_2, asphyxia in air or ice, salt, beheading, and spiking are also possibilities acceptable by European policy, although some of them might conflict with fish welfare conduct.

Nevertheless, these practices are acceptable in Europe most probably, as exposed before, since large-scale business sometimes leave fish welfare concerns aside, as a production it is the principal objective. However, the OIE advises to always use stunning methods before any killing takes place, to avoid inflicting pain and to maintain it as quick as possible.

In an informative manner only, the killing processes and respective description, according to the Regulation EU2016/429 (Animal Health Law), are described in the next table. All the processes are included in table 17, even though according to the OIE, the same may be considered less humane since they can lead to a slow death and even inflicting pain to the fishes.

TABLE 17 - SLAUGHTERING AND STUNNING TECHNIQUES AND THEIR DESCRIPTIONS

<table>
<tr><th colspan="2">Technique</th><th colspan="2">Description</th></tr>
<tr><td rowspan="3">**Stunning methods**</td><td rowspan="2">Electrical</td><td>In water</td><td>Commonly used for smaller fishes such as rainbow trout. An electrical field created by two electric plates inside the tank will cover all the group and after will shock the fishes.</td></tr>
<tr><td>Out water</td><td>This method is frequently used in Norway for Atlantic salmon. For this, there is a special device and for this method to be successful avoid injuries, fishes must be put head-first to the stunning device. Some stress can be caused to the animals during the process.</td></tr>
<tr><td>Percussion</td><td colspan="2">This technique is using force and lead fish to unconscious before the killing process. Most used in large fish such as Atlantic salmon but also possible in smaller fishes such as rainbow trout and even carp. It is crucial to have skilled personal whenever using manual percussion since whenever badly applied this method can lead to high levels of pain for fishes.</td></tr>
<tr><td>**Non-stunning methods**</td><td>Live to chill with CO2</td><td colspan="2">It is important to highlight that this process might be not protected fish welfare since it might not perform unconsciousness to fish. Water should be between 0,5 – 3ºC while adding CO_2 to the water. Usually used for salmon.</td></tr>
</table>

	Asphyxia	Ice it is added to the tank or container so water can be between 0 – 2ºC. Some fishes can suffer from stress since they suffocate to death. Commonly used for seabass, seabream, and rainbow trout.
	Beheading	This technique does not meet the Organisation for Animal Health (OIE) guidelines still there are some records of carp producers using this method.
	Salt	Animals such as example, eels can be put in a salt bath before stunning. This might cause stress and discomfort to the animals.
	Spiking	A small spike is inserted in the fish brain, cancelling all nervous communications. This is considered by OIE the most humane way of killing fishes although it can be tricky when performed by unskilled staff.

According to Council Regulation (EC), No 1099/2009 (Slaughter Regulation) animal welfare, including fishes, should be always protected, even at the time of the killing. In the above-mentioned document regarding fishes, it can be also found that “shall be spared any avoidable pain, distressed or suffering during their killing and related operations”.

Although this is stated, there are no detailed conditions as there are for other groups of animals, thus leaving space for various and different interpretations. That is why biosafety control, as well as previous risk analyses, should be run before starting any kind of productions concerning all the mentioned above.

According to all that was exposed and for demonstration purposes, I developed a risk analysis simulation (Figure 29) that includes all the information, concerns and topics exposed previously, adding up to personal experience obtained in the internship at BIGH.

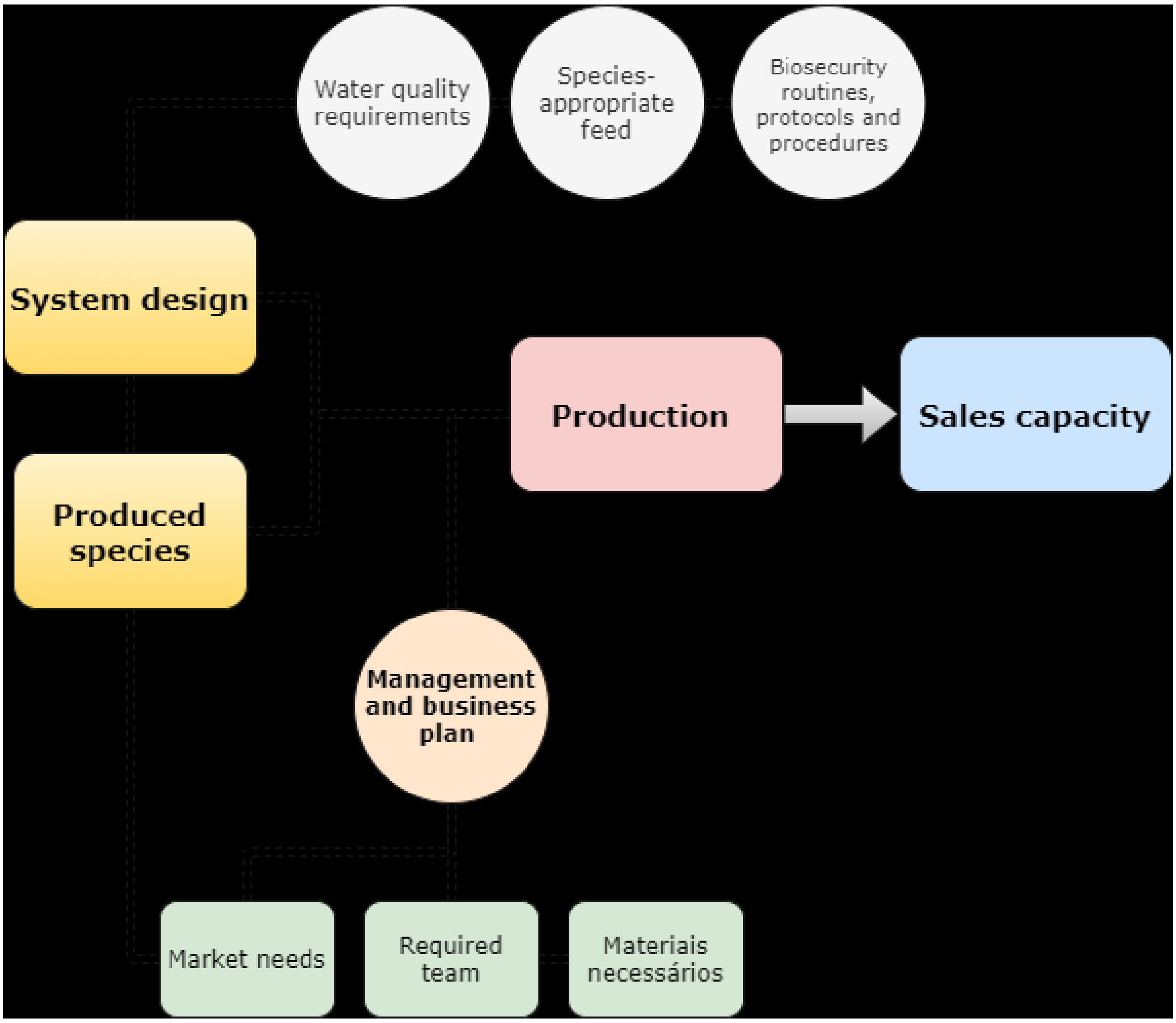


FIGURE 29 - RISK ANALYSES SIMULATION FOR BIG'S PISCICULTURE

A risk assessment applied to aquaculture involves several factors. A prior market study should be carried out making it possible to gather information and reveal which species will be the best to produce according to the market needs, location and even population preference. It is important to have in mind the human resources available and all the safety and personal material as well. These combined can be put together in the business plan.

After choosing the species, it is possible to obtain the most efficient design of the system and estimate the biological requirements – the amount of water, temperature, pH, photoperiod and even the feed. All this information will indicate the maintenance needed.

All the mentioned factors will influence the production, that is intrinsically linked to sales and the ability to profit. Any problem found in one of the sectors can, and most likely will, influence final sales. So, having all the sectors in harmony is important for business success.

3.1 DISEASE FOUND AT BIGH'S SYSTEM

In *Morone* case, most of the diseases appear as a response to external stressors, which is when the fish appears to be more susceptible to infection (R. M Harrell, 2016). Therefore, it is highly recommended to avoid stress when producing this species. In theory, this might seem easy but the correct management on early stages of new and distinctive projects such as BIGH can take some time to achieve since some mishaps may happen. Not only that but establishing suitable and proper protocols to the needs of fish farming can also be challenging when starting a new pisciculture.

Although The Food and Agriculture Organization (FAO) considers that stress is the main inductor for the most common diseases of *Morone*, it is important to understand that the diseases have different agents such as bacteria, virus, fungus or even parasites like protozoan, metazoan, crustaceans and even annelids.

Since the beginning of the internship, fishes appeared to be sick. This was confirmed after a veterinarian analysis that detected the presence of *Streptococcus iniae* and *Aeromonas hydrophila* in the water. The presence of these two types of bacteria resulted in many losses of fish, affecting the production as well as the company's profit. *Aeromonas* involves a diversity of bacteria such as *Aeromonas hydrophila* (gram-negative), capable of growing and living in both marine and freshwater, although they lead to more serious diseases when cultured in freshwater. Some of the symptoms associated with this bacteria are cellulitis, muscle necrosis or septicaemia (Lehane and Rawlln, 2000). Leigh Lehane and Grant T Rawlin (2000), described that the first-ever infection of fish with *Streptococcus iniae* was recognised in 1958. Some of the symptoms described where septicaemia with endocarditis, meningitis, and arthritis but according to the New South Wales Research and Government other indicators can also be ulcers, cellulitis, and even lymphadenitis.

3.2 APPROACHES WHEN FACING A DISEASE IN AQUACULTURE

3.2.1 VACCINATION

The advancement of knowledge shows that many different types of pathogens can enter an aquaculture system, leading to losses and production failure. This way, biosecurity plans are essential to prevent and combat diseases and infections.

According to Jean de Barbeyrac in "*Disinfection: the critical step in aquaculture and intensive farming"* (2015), there are different types of microorganisms: bacteria (gram-positive and negative), viruses (naked and enveloped), fungi and parasites. Some of the most common pathogens associated to aquaculture are *Aeromonas* and *Vibrio* species, which are bacteria that prefer highly organic environments as, for example, when uneaten feed is found in tanks or system, besides infecting and living within fish, sometimes without causing any visible symptoms. Not so frequently, but still possible, *Streptococcus* strains can also infect and spread in aquaculture systems and are particularly difficult to eliminate once established. Mycobacteria can also live inside the biofilms that coat the tanks, filters and pipes (Yanong and Erlacher-Reid, 2012).

Parasites can also be established in the system and some more common examples are *Trichodinids*, *Tetrahymena* and *Uronema (*Halver, 1954*)*.

High densities, biofilms and sediments can help concentrate the microorganisms (Maeda et al., 1997). When already settled on the system there are two types of reservoirs where these microorganisms can be found: non-living reservoirs such as water, system components, equipment, floors and walls and even feed; or living reservoirs which include living animals and even plants (Kennedy et al., 2016) .

Contamination should be avoided if possible and, for this, a routine diagnosis should be implemented, allied to correct disease control. This starts with the correct cleaning and disinfection of materials, facilities, and systems. Also, particularly important is the correct and regulated people management, including visitors. If there is a contamination, antibiotics and medicine can be used but it is not ideal to expose the animals to long-term use of this kind of substances (Smith, 1998).

Vaccination is a prophylactic strategy that appears in the response of drug resistance issues and safety concerns related to antibiotics and other chemotherapeutics that may be implemented for disease treatment in aquaculture. Vaccination is considered by experts an effective method of preventing a wide range of bacterial and viral diseases. Since the first report in the 1940s of fish vaccination for disease prevention, several vaccines were produced leading to a decline of the bacterial and some viral diseases impacts in fish (Ma et al., 2019).

For a better understanding of this topic is crucial to explain what the desired outcomes of fish vaccines are.

Vaccines usually include or trigger the production of a substance that serves as an antigen that will stimulate an innate and/or adaptive immune response within the fish against a certain pathogen (P.Anderson, 1997).

Moreover, it is important to explain the different approaches in fish vaccination. There are three methods to obtain a vaccine: with a virulent disease-causing agent, also known as an inactivated vaccine; live vaccines that are prepared from one or more viruses or vaccines that use part of a bacteria displaying attenuated virulence or natural low virulence toward the target fish species. This type of vaccine can enter and proliferate in the host and stimulate greater cellular responses when comparing to the inactivated vaccine (Kurath, 2005).

Vaccines can be administered in three different ways: injection, immersion or oral administration and normally the first two methods are the most common due to their high viability (Halimi et al., 2019b). Nowadays other tactics are being developed for example administration of vaccines via the mucosal including skin, gills, gut, and nasal mucosae which in fish represent a big surface area. This route can be more practical and affordable for some sectors than injection (Adams, 2019).

Bacterial vaccines have been successfully produced on a commercial level and, among diseases caused by Gram-positive bacteria, *streptococcus* appears to be the next best nominee for a successful vaccine (Aruety et al., 2016). Recent studies showed that an oral vaccine for *Streptococcus iniae* and *Lactococcus garvieae* in rainbow trout revealed to be efficient with high survival rate and immune-related gene expression showing that the fish became immunized by the administered vaccine (Halimi et al., 2019a).

Although the efficiency of vaccines for fish bacterial diseases is well established, there is a long way to go since for other pathogens, such as viruses, there are not many commercial vaccine options. Also, although antigens produced by several viruses administered by injection or immersion have shown to provoke protective immunity, for commercial purposes these viral vaccines don’t guarantee enough protection to be industrially produced (Adams, 2019; Gudding et al., 1999). Nowadays there are still few vaccine options in the context of aquaculture. Nevertheless, at BIGH, vaccination was not implemented and that is why the company had to take another route when there were detected microorganism in the system.

3.2.2 Dealing with mortality

Copping with mortality is just the approach of not administering any treatment and just removing the dead (Figure 30). This is not ideal as well as common, and it is important to highlight that this approach should not be extended for a long period since this could lead to serious risks in terms of production. Since fish were produced to human consumption, biosecurity laws and standards should be respected. The presence of a pathogen infecting the fish can represent a hazard to human health.

Figure 30 - Technique used to collect fish carcases

This approach was used at BIGH for a short period before starting to administrate any treatment and only while waiting for lab analysis. This approach led to a decrease in the stock and therefore also the profit reduced with that.

As the name suggests, this approach can be simply explained by handling with high mortality rates during these periods, increasing the control of daily mortalities. In BIGH's case, operators should be alert and monitored more often the tanks, having to remove the dead fishes three or even four times a day.

3.2.3 Antibiotics

Antibiotics, also denominated as antimicrobial drugs, include all the medicine that has as focus fighting infections originated by bacteria. These drugs are non-toxic to the host and can be either of natural or synthetic origin (Serrano, 2005). The main concerns linked to the use of antibiotics in aquaculture are resistance transference, understood as the ability to transfer a certain gene between bacteria, as well as allergy and possible toxic residues. Besides human health issues, there are also environmental risks that also represent a concern related to the use of drugs for fish farming nowadays, since effects on soil microns, wildlife, algae and aquatic organism might be observed (Alderman and Hastings, 1998).

According to OIE, both bacteria strains found in BIGH required treatment using antibiotics. *Trimazin*® (30 %) and *Nuflor*® are ideally targeted to use in bovine and pig farming, although they can also be administered in fish farming always having the correct dosage in mind (Table 18). They are both suitable in treatments for pathogens that can be hazardous to fish such as *Streptococcus.* The first treatment was *Trimazin®* due to the fact of the low cost associated. As this was a powder, it was necessary to use vegetable oil to incorporate the treatment into the feed. Most of the times fishes would accept the feed but this method was used to ensure that there was good incorporation of the powder in the feed. Soon this was replaced by *Nuflor®* since this was an injectable suspension. Although more expensive, this option was more suitable since the administration was easier.

TABLE 18 - DOSAGE AND REACTION TIME OF ANTIBIOTICS ADMINISTERED IN THE COMPANY

Medication	Dosage	Days	Principal component
Nuflor®	10 mL/kg feed	5 days	Florfenicol
Trimazin® (30%)	120 mg/kg	3 days	Trimethoprim (5%) and Sulfadiazine (25%)
	60 mg/kg	7 days	

When using these antibiotics incorporated on the feed, there was a reduction of feed, so the amount distributed was just enough for all batch while guaranteeing that the antibiotic was successfully administered to all. During the internship, the use of antibiotics was strictly used for the first two months, November, and December. After that period, the number of sick animals decreased. Since BIGH is an eco-friendly company which prefers to minimize the use of antibiotics, these were only given for small periods and whenever strongly needed. During the periods when antibiotics were administered, according to each lot daily feed, the amount of feed would be reduced to half. This tactic used at BIGH of reducing the feed given was aimed at reducing the rejection of feed by the fish. Serving as an example, if the tank 4 needed 13 kilos of feed for all fishes, then only 6,5 kilos would be used. The antibiotic was incorporated in the feed and the 6,5 kilos were distributed during all day and always according to the response and appetite of the fish.

Whenever antibiotics are used it is extremely necessary to always respect the exposure times because whenever the animals are fed antibiotics for long periods, residue bioaccumulation or chronic toxicity can be detected. These substances may have toxic or allergenic properties (Alderman and Hastings, 1998) although most of the antibiotics administered in the therapeutic form to animals are also approved for human use (Lulijwa et al., 2019).

3.3 CASE STUDY ON A SYSTEM SHUTDOWN

FAO describes biosecurity as a strategic and integrated approach to analyse and manage risks in feed safety, animal and plant life and health, and biosafety.

It provides policy and a regulatory framework to improve coordination and take advantage of the synergies that exist across sectors, helping to enhance protection of human, animal and plant life and health, and facilitate trade.

The veterinary analyses conducted by *VetEau®* and the lab report confirmed that there were *Streptococcus iniae* and *Aeromonas hydrophila* in the system. This was the major cause of the high mortality rates observed.

The presence of these pathogens proved to be more hazardous to younger batches since juvenile fishes were more susceptible to bacteria.

After selling off all fish lots, the tanks were emptied and the conditions for shutdown were reunited. Disassembling all the pipes, inlets and outlets and pieces was crucial for the beginning of the cleaning and disinfection process. Not only that but since some structural changes were planned some parts needed to be replaced or changed.

The tasks referred in this report focus mainly in the two first phases since the last one was only conducted after the internship had already ended.

The first phase was mainly preparation for the actual shutdown, during which by request of BIGH, research and developing of a biosecurity protocol was made, focused on the system cleaning as well as the biosecurity measures needed at the quarantine and fish farm after the shutdown. Some changes on the routine for after the shutdown were also suggested. The entire process of disassembly, cleaning and modifications to the system were included on the second stage of the shutdown, which means that the last step was the resumption of production and reception of juveniles.

3.3.1 Problems found on the system before the shutdown

The shutdown was the solution found more appropriate to rectify some of the flaws that existed in the system, that appeared over time, such as specific materials or parts that were broken. More importantly, this was the opportunity to do some improvements on the system and to modify the species that were producing. The option for an alternate species to the hybrid striped bass was the rainbow trout (*Oncorhynchus mykiss*).

For a better understanding of the changes submitted to the system and the quarantine, it is essential to mention the obstacles found in the initial system. During the internship, as mentioned before, the water quality monitoring and management were one of the responsibilities attributed. Throughout this period the carbon dioxide (CO_2) represented a special concern for BIGH. The levels were not satisfying and there was a preoccupation about the high levels of CO_2 when the system reached their full capacity. The lack of biosecurity measurements and the fact that the old quarantine was in the same room of the rest of the tanks also was a motivation for the modifications planned at BIGH.

Regarding the quarantine, for BIGH this facility should not be in the same room for biosecurity reasons and to guarantee the correct fish health conditions. The main purpose of quarantine is to create an isolated environment for the animals newly arrived. This way preventing the exposure of the animals to an infectious or contagious disease. At the same time, if the newly fishes are sick, the quarantine will also prevent them to be a pathogenic vector for the fish found in the system. Furthermore, BIGH's quarantine was not the only place that had some flaws. The main system in the fish farm also had some issues that over time have diminished the efficiency of the entire system. For example, some hydraulic problems existed, not only due to the fact of the water supply channel being too wide (which reduced water speed) but also because there was a higher current after the biological filter becoming weaker along the canal. This represented trouble since it led to a gradual accumulation of organic matter at the end of the waterway. Inside the biological filter, over the years since BIGH is functioning, it was found that the position of the water outlets from the drum filter was aligned with the pipes that took the water to the canal, which means that some water was injected directly to the water supply channel. The lack of water level control in the MBBR was also an issue since the computer was originally programmed by *Spranger®* to only use one pump each time.

3.3.2 PISCICULTURE FACILITIES MODIFICATIONS

The change of BIGH's quarantine to a separate division was expected to be the most time-consuming operation of all the transformations that took place in that part of the infrastructure. Four tanks with 2 m^3 were built and those were supposedly prepared to receive 12 kg/day of feed. The figure 31 represents the new disposition of the quarantine room. According to it, all the water came out from outlets to a pipe and then enter the drum filter (1) with 2 m^3 of water volume and flow rate of 0,02 m^3/H. The main pump in the system was installed underneath a water tank (2). This tank was designed to slow down the water before entering a station equipped with a UV light (3). This UV light promoted water disinfection initially and before the water entered the MBBR (4). A CO_2 stripper (5) was built together inside a tower. These structures had a way to work, the water entered the structure from the top and gradually the tank would be filled. After reaching the top, the water would overflow and enter the second division, the CO_2 stripper. The water passed through a grate and after reaching the deposit it was ready to be distributed to all the four tanks. Regarding the feeding method, BIGH believed that belt feeders were the best option for the quarantine. The pipeline system would have two directions: tank outlet pipes (purple) and water inlet pipes (blue).

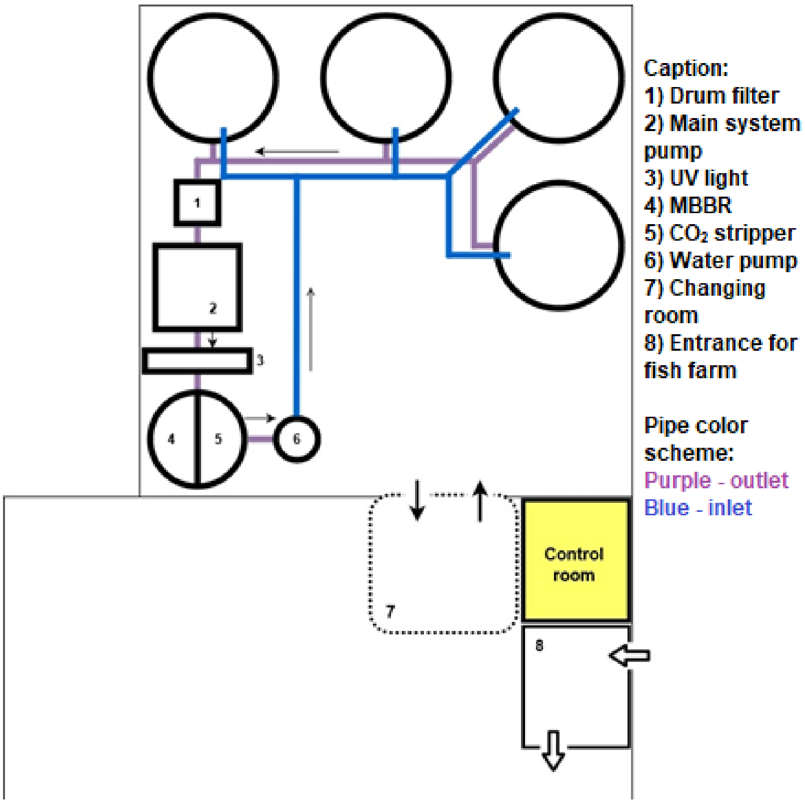


FIGURE 31 - BIGH'S QUARANTINE PROJECT

According to the production aim and goals, BIGH's quarantine would receive four to six batches of juveniles and, between them, thorough cleaning and disinfection were demanded. After the arrival of new fishes, they would stay in this room for three weeks to one-month (maximum) meaning that this room would not be operating all year long.

For the transfers between quarantine and the fish farm, a new entrance was created (7). A changing room equipped with all the individual equipment, used strictly for quarantine, was also built. Inside this room, equipment such as boots, laboratory coats or coveralls and gloves were available. Any staff member should change to the correct outfit before entering the quarantine. Not only those supplies were exclusive, but inside the quarantine kits which included pH, conductivity, temperature and O_2 probes were also found and used rigorously for quarantine only.

In addition to all these adjustments, there were also minor modifications, but still essential, on the processing room (Figure 32) such as a new floor and the installation of a new cooling system.

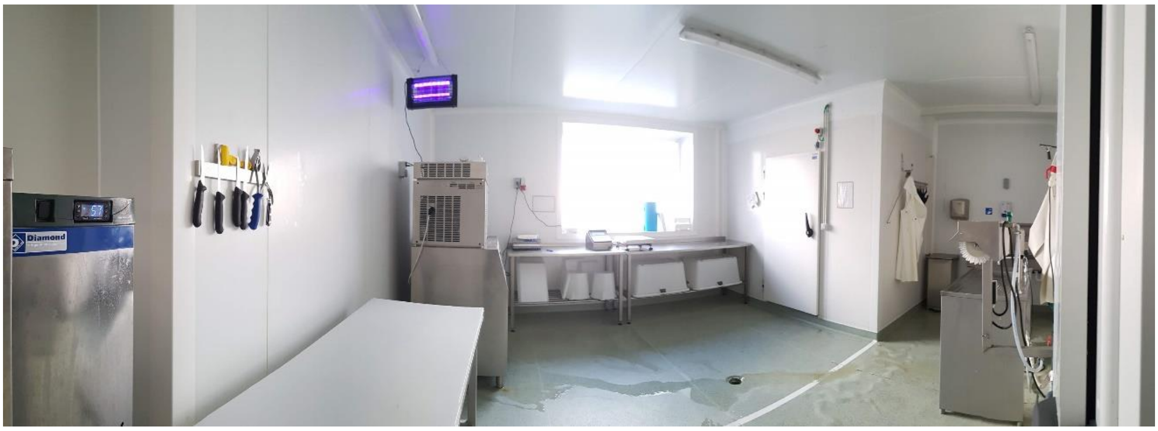


FIGURE 32 - BIGH'S PROCESSING ROOM BEFORE THE SHUTDOWN

For the main system, there were major changes programmed to improve the efficiency of production and the biosafety inside the facility. For this last topic, as seen in the figure 31 a new entrance was made (8).

Regarding the changes related to the tanks, although the structure itself was the same, their inlets were changed. In the tanks with 5 m^3 new water entrances were installed. With these modifications, those tanks could also be used as purging tanks if needed. For the oxygenators, there were also some changes made since the inside structure was removed so the less organic matter could get caught inside the O_2 column. Other big changes were in the waterways, both upper and system water outlets, as at the end of the system, the connection between the two of them was cut off. The water supply channel became a closed pipe with outlets equipped with valves so there was better control of the water flow to each tank. For the system's water outlet , new barriers were built inside the canal to increase the water velocity and at the same time to reduce the sedimentation of the organic matter from the tanks. These barriers were built in the main pipeline and would decrease the area available for water passage. This would raise the pressure and consequently increase the water speed.

Also, BIGH chose to change the media in the MBBR to the biomedia K5 from *Krüger Kaldnes®*. To improve the control of overflows a new automatic control system was installed. In the case of water overflowing, a buoy would emit an alert and the system would decrease the pumps flow automatically. To better understand the following description, attention should be paid to the figure 32. Still in the MBBR, there was an augmentation of the area where the media were found (9). This avoided the entrance of biomedia into the new system of pipes. This new system directed the water with more precision of water speed (180 m^3/h) to the installed CO_2 strippers (12). Although there were two CO_2 columns, only one was installed to work full time and the other one would have the function of a protein skimmer.

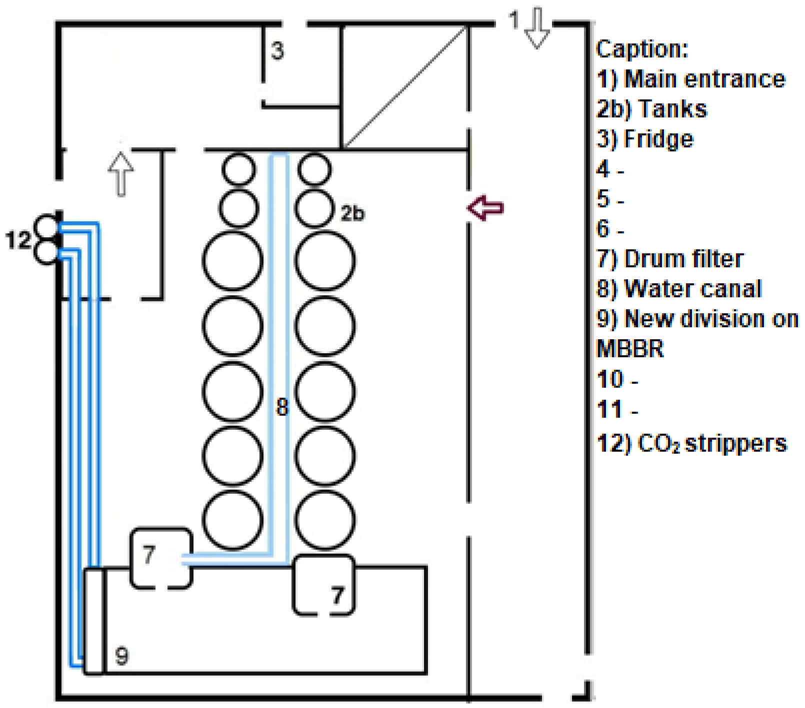


FIGURE 33 - BIGH'S PISCICULTURE PROJECT FOR AFTER THE SHUTDOWN

Subsequently, changes were made in the drum filter (7). More precisely, the mesh inside was changed from a combination of 150 µm (75%) and 65µm (25%) to a uniform mesh of

85 µm. This was mainly for biosecurity reasons since this mesh can retain some life stages of microorganisms, possibly avoiding the proliferation of some pathogens in the system.

Lastly, smaller changes such as the installation of automatic feeders on each tank (2b) and a UV lamp in the water capture point (8) were added as a biosecurity measure and were also very important to improve the effectiveness of the overall system in the fish farm. The change of the routines was also a goal for BIGH, and the company stipulated that all the routine and tasks should be started from the quarantine when this was operating and afterwards the staff would work in the fish farm.

3.3.3 BIGH'S SHUTDOWN

For this case study, an entire system shutdown has been scheduled since the presence of some diseases had been detected for a few months in the system. Adding to this, a new quarantine and some system design changes are also implemented.

Firstly, after stopping the production and shutting down all the mechanical equipment, the water should be drained of the system. This must be done after all the livestock is sold. After this, cleaning must take place.

Cleaning involves removing all the organic material resorting to firmly scrubbing. Brushes of different sizes should be used according to each surface and area. Pressurized water should be used for better results. Water and detergent are highly recommended for this step. *Biosolve*® is a cleaning agent, commonly known as detergent and should be used for this purpose.

After this first step, sterilization should happen. For this, we need to use chemical products. There are different kinds of chemicals for this stage and each one should be used according to the desired objective.

According to the OIE - Aquatic Animal Health Code - 29/08/2019 the chemical list can be divided into these categories:

A. Oxidising agents include chlorine compounds, chloramine T, iodophors, peroxygen compounds, chlorine dioxide and ozone

B. Alkalis and acids also identified as pH modifiers. These are ideal for areas where the application of other effective disinfectants is not possible, such as in pipes or on biofilter surfaces

C. Aldehydes - formaldehyde and glutaraldehyde. They are highly effective against a wide range of organisms but require long exposure times. Considered only mildly corrosive.

Glutaraldehyde is used in the liquid form as a cold sterilant, particularly for heat-sensitive equipment.

Formaldehyde may be used as a mist or a gas for fumigation for general materials, tanks, containers, and facilities.

D. Biguanides - non-corrosive and relatively safe, usually used for the disinfection of skin surfaces and delicate equipment. Chlorhexidine is the most common example.

E. Quaternary ammonium compounds (QACs) - effective against some vegetative bacteria and some fungi, but not all viruses. They are most active against gram-positive bacteria but not spores. They are non-corrosive, but they might be toxic to aquatic animals and should be well removed from surfaces.

In “Disinfection: the critical step in aquaculture and intensive farming” by Jean de Barbeyrac (2015), *Pseudomonas sp.* are known to develop resistance to disinfectants, particularly with some quaternary ammonium compounds, meaning that chemicals with ammonia complexes may not be a good option for the sanitation of BIGH’s system. In BIGH’s case, the process of disinfection should be done with calcium hypochlorite ($Ca(ClO)_2$), never forgetting that after any use it is necessary to neutralize it with sodium thiosulphate ($Na_2S_2O_3$).

Although remarkably effective, this chemical must be left for several days to obtain successful results. Alternatively, since the entire system will be shut down (meaning that no live animals will be in the fish farm), another possible substance is sodium hypochlorite ($NaClO$).

3.3.4 Protocol proposed for BIGH

MATERIALS THAT CAN BE CLEANED

Containers, tanks and transfer containers, hoses, PVC pipelines and tubes, quarantine equipment and nets.

Using all the information from existing bibliography and World Organisation for Animal Health, it was possible to develop a protocol aimed for BIGH. Having in mind BIGH's system design, all the characteristics and correct measurements, a protocol proposal was developed for the company. The information tables 19,20 and 21 gather all the details concerning the disinfection process, depending on each type of surface or material.

Table 19 - Information regarding the reaction time and dosage depending on the material used

Type of material	Chemical amount and exposure time			
	NaClO	Exposure time	$Na_2S_2O^3$	Exposure time
Tanks	0,4 mL/L	10 min	0,6 g/L	30 sec [2]
Filters	1 mL/L	24 H [1]	140 mg/L	At least 12H
Surfaces and nets	12,5 mL/L	24 H	-	Air dry
Containers (50L)	200 mL/L	10 min	-	Air dry

[1] in case of emergency never less than 1 H

[2] Let it air dry afterwards

Table 20 - Estimated chemicals dosage and reaction time for BIGH's tanks

BIGH'S tanks	Capacity	Amount	NaClO needed	$Na_2S_2O_3$ needed
Purging	3,3 m^3	2	1,320 mL	1,980 g
Small	5,0 m^3	2	2 mL	3 g
Big	10 m^3	10	4 mL	6 g
Total	116,6 m^3 = 116,650L	14	46,64 mL	69,96 g

TABLE 21 - ESTIMATED CHEMICALS DOSAGE AND REACTION TIME FOR BIGH'S FILTRATION SYSTEM

BIGH'S filters	Capacity	Amount	NaClO when spraying	$Na_2S_2O_3$ needed	NaClO when foaming	$Na_2S_2O_3$ needed
Drum filter (2580 mm length ; 1690 mm height ; 1580 mm weight)	6,889 m^3	2	172,22 mL	**NO NEED. AIR DRY**	13778 mL	1929,2 mg
MBBR	5,0 m^3	1	687,50 mL		55000 mL	77000 mg
Total	18,778 m^3	3	859,72 mL = 0,859 L		68778 mL = 68,778 L	78 929,2 mg = 78,929 g

In BIGH's case, the suggested protocol can be summarized in seven steps and each one had some notes and important remarks.

1. Manually remove dirt and organic matter

   The process starts with the disassembling of all the detachable parts, such as pipes and tubes, creating space and allowing for the staff to have good conditions for scrubbing and physically removing organic matter. This should be done with simple tools such as brushes, water pressure machine and sponges if needed.

2. Rinse with water pressure machine
   Rinsing after the cleaning process and drying afterwards is advised, to prepare all surfaces for the disinfecting process.
3. Add the sodium hypochlorite (NaClO). Beware to the strong smell.
4. Leave the chemical to react for the time needed (according to table 11,12 and 13)
5. Add sodium thiosulphate ($Na_2S_2O_3$) and let it activate for the correspondent time.
   According to the veterinary report, the presence of *Streptococcus iniae* and *Aeromonas hydrophila* was detected. For their disinfection, sodium hypochlorite (13%), with approximately 0.3% of active chlorine, can be the more affordable and

effective option (always leave the solution on a dry place away from direct solar exposure).

A different option can be sodium hypochlorite. On daily products, this compound can be found on bleach, which is why this can be also a good option. When using this chemical, leave to inactivate for several days or to avoid the waiting time, it can be neutralised with sodium thiosulphate afterwards for at least 3 hours.

6. Rinse with water
7. For tanks, let the system run with water for at least 24 hours (control physical and chemical water parameters).

3.3.4.1 BIOFILTER MATURATION

Starting a biofilter involves managing and controlling the “seeding” of nitrifying bacteria cells in a biological filter. It is important to highlight that this procedure has to be done before the inclusion of any fish in the system (Y. Yang and Allen, 1994).

To make biofilters more efficient the surface area is one of the most important features to have in mind. The unit of measure used to describe this parameter is called specific surface area (SSA). After choosing the material, its shape and size for the system the process of maturation can be started.

Once again it is important not to forget that most waste is produced (if not all of it) by the fishes as a result of the feed they take. These animals expel ammonia (NH_3), mostly from their gills (Kardong, 2012) and urine, dissolving in the water afterwards. This waste product is toxic to the fish and is an environmental stressor that causes reduced appetite, reduced growth rate, and death at high concentrations.

Fortunately, bacteria that can oxidize the ammonia can also be found growing in the water, converting ammonia to nitrite (NO_2^-) (L. Yang et al., 2001).

As said before, *Nitrosomonas* are responsible for this aerobic process (Tidwell and Bright, 2019). Nitrite produced by these bacteria can be also toxic to the overall aquatic organisms. That is why *Nitrobacter* are the next group of bacteria of the nitrogen cycle and are responsible for the oxidation to a less toxic form of nitrogen. These bacteria metabolize and oxidize the nitrite (NO_2^-) produced by *Nitrosomonas* and convert it to nitrate (NO_3^-). Nitrate it is the last product on the chain of events of nitrification and it is non-toxic if not higher than 200 mg/L (DeLong and Losordo, 2012).

Therefore, the first thing to take into attention is water chemistry, setting parameters such as pH, salinity, alkalinity, and water hardness following the incoming stock needs. The temperature should be settled as well but according to the Southern Regional Aquaculture Center, it can be settled a little bit higher than the anticipated culture temperature to increase the rate of growth. The system should be operating with water passing through the biofilter and free of residual chlorine that may have been used for disinfection or pathogen control. Since carbonate (CO_3^{-2}) and bicarbonate (HCO_3^-) ions are carbon sources, sodium bicarbonate or baking soda for daily use can be used to increase alkalinity. When restarting the system, it is recommended by the Southern Regional Aquaculture Center to increase the alkalinity to proximally 150 mg/L.

Nitrobacter also can grow in higher alkalinity environments with 200 to 250 mg/L alkalinity. To raise the alkalinity by 10 mg/L, it is advised to add 53 grams of sodium bicarbonate for every 4 litres of system water.
For carbon dioxide, controlling water circulation and aeration or degassing are common methods used in fish farms.
At the same time, pH should be controlled. The ideal range for both *Nitrosomonas and Nitrobacter* is 6.8 to 7.2 (DeLong and Losordo, 2012).

Other water parameters that needed to be verified are ammonia and nitrite. For these, adding ammonium hydroxide, or household ammonia used for cleaning are good solutions. Ammonium chloride and ammonium nitrite are the other two available options (Rogers and Klemetson, 1985). Generally, 60 ml of clear household ammonia (10% ammonia) will raise the ammonia concentration of 3,8 litres of system water by about 1.6 mg/L. The ideal levels of total ammonia should be between 3 and 5 mg/L. Nitrifying bacteria can be added when this value is achieved (DeLong and Losordo, 2012). After this step, water quality must be tracked regularly and be checked to avoid peaks of ammonia or nitrite. Reaching a maximum value for nitrite means that *Nitrobacter* bacteria have developed and started to oxidize nitrite decreasing the nitrite concentration. As more bacteria become established on the media surfaces, as evidenced by daily decreases in ammonia and nitrite concentration, aeration and agitation can be gradually increased (DeLong and Losordo, 2012). After this, animal stock can be added to the system.

This way, in short, the plan for biofilter maturation can be:

1. Stripping all the chlorine that might be in the system and the biofilter
2. Rinsing with water

3. Controlling temperature
4. Controlling salinity (>15 psu)
5. Controlling alkalinity
6. Controlling pH
7. Controlling Ammonia and nitrite
8. Carry out regular parameter's checks
9. Introduction of livestock –3 to 5 days after the stabilization.

3.3.4.2 BIOSAFETY AND ROUTINE CHANGES AFTER THE SHUTDOWN

The revision of the routines and the procedures on the farm was a particularly important step to improve biosafety as well as biosecurity in the fish farm. For starters, it is important to distinguish both.

The containment principles, technologies and laboratory practices that are implemented to prevent unintentional exposure to pathogens, or their accidental release are all included in the biosafety category (Bakanidze et al., 2010).

For instance, biosafety includes personal measures designed to prevent the loss, theft, misuse, or intentional release of pathogens that can lead to unwanted diseases (Shahi and Nadershahi, 2009). Measures such as staff equipment or mandatory cleaning can be included in this category as well as product availability for the correct staff sanitation.

Additionally, biosecurity in aquaculture consists of practices that minimize the risk of entering an infectious disease and spreading it to the animals at a facility. Biosecurity should also focus on minimizing the risk that diseased animals or infectious agents will leave a facility and spread to other sites and other susceptible species. Practices that focus on reducing stress to the animals, thus making them less susceptible to disease (Smith, 1998) can also be integrated on biosecurity category.

For example, changing the feed method, daily feed distribution or just the simple choice of increasing the number of times per week tanks are cleaned are included on this topic.

The improvement of these processes would hopefully lead to gradual improvement of the fish welfare, BIGH's production and sales, decreasing minor errors and pathogen entranceways. Adding to this, the right feed can be a vital way for optimal growth rate in fish farms.

Starting with the feed storage and having in mind that the feed can be a vector for a pathogen to enter the system, it is crucial to maintain ideal conditions to store it. Some factors such as humidity, pests and even temperature can affect and, with time, change feed quality. According to the composition of the feed (percentage of lipids and vitamins), temperature, moisture and even oxygen can influence and slowly lead to early degradation if the storage conditions are inadequate.

Insufficient aeration, temperatures between 25 °C – 35 °C, humidity levels greater than 62 % and moisture levels above 14 % prevalent in the feed storage, predispose the feed to microbial spoilage (Cochrane, 2002).

Adding to this, being established in an urban centre sometimes might also lead to the appearance of pests. In BIGH's case, there were occasionally some problems with attic mice (*Apodemus sylvaticus)*, and this leads to a biosafety problem since they usually find a way to enter the feed storage. Also, they can be considered a potential health hazard to workers that handle the feed.
Pests, not only mice or rats but also insects should be avoided at all cost. For the insets, the majority of the species grow very well between 26 ºC – 37 ºC (Gilbert and Raworth, 1996) so to control this kind of pests a good aeration system and temperature management should be guaranteed. Mice and rats can usually establish their nests in the storage or simply consume some amount of the feed. In addition to this, they can cause a large number of feed losses through packaging damage resulting in feed spillage or exposure to insects or moulding environments.
Therefore, since there was a lack of procedures concerning feed storage management, cleaning, and feed transportation, it was necessary to develop them. New protocols were also created for the routine after the shutdown, aiming to improve BIGH's production to maintain fishes as much healthy as possible.

Although in BIGH's case the feed storage was a shipping container used exclusively for that effect and normally these kinds of storage are quite safe and waterproof still they are not completely sealed leaving small entrances for mice or insects.
Having these kinds of containers excludes problems with rain and sun exposure, but it should have a ventilation system to control temperature and moisture inside it. Although at BIGH this might be rare to happen, since Brussels can be considered a cold country, with global warming, in the summer, heatwaves have been experienced.

3.3.4.3 FEED STORAGE CLEANING AND MAINTENANCE PROCEDURES

Due to the fact of an early presence of mice in the storage, during the shutdown, all the feed should be taken so the container could be sanitized. After emptying this storage, hydrogen peroxide it is the best option for disinfection. A solution of ¼ H_2O_2 for ¾ of water should be enough for the desired effect. After the disinfection process, it is necessary to let all the room dry and let it ventilate so chemical smell does not stay inside the storage.

Further cleaning should be made weekly or every two weeks. To speed up the cleaning routine the feed should be stored above ground and the wood racks should be changed to roller carts. Bleach products are a good option for the cleaning process of the storage. Special attention should be given to possible signs of pests before every cleaning routine.

For stacking, there should also be an organization so the handling of the feed could be as fast as possible, minimizing the chances of changing the parameters inside the facility (Figure 34) and possible entrances for pests. This way, when new batches of feed arrive, they should be put behind the old batches and stacks must be limited in size and depth. Stacking must be done above the ground to avoid spoiling and should not be packed against the wall of the storage facility. There should always be a space between the stacks and the wall.

FIGURE 34 - INTERIOR OF BIGH'S FEED STORAGE FACILITIES

It should be noted that in BIGH's case feed should be stored not more than four to five bags high to maintain air circulation and to make the withdrawal process easy and safe to workers.

Besides this, another particularly important topic is the correct handling of the feed. Firstly, all workers should handwash before entering the fish farm and most important before operating any feed, being advised to wear gloves.

At BIGH before the shutdown, each feed batch weighted 25kg and the staff only took feed sacks when inside the feed farm the feed station containers were empty. Depending on the growth-out situation, the amount of feed taken to the feed station inside the fish farm ranged from 30 kg to 80 kg. No feed sack should be opened inside the storage. All the containers used for the daily feeding should be cleaned and washed with *Virkon*® or *BioSolve*® after every feeding as well as the feed containers in the feed station inside the fish farm that is advised to be cleaned every two weeks.

Furthermore, it is also essential to guarantee the cleanliness outside of the feed storage since the main entrance leads to a passageway. This is a transit area for both customers and staff. For aesthetic reasons but mainly because it is a place of passage, it must be clean, avoiding that people become possible agents of transmission of pathogens to other production sites of the company.

Traps can be set outside the storage but inside this should be avoided since it could take to some work-related accidents. Natural traps such as bait stations should be enough to the type of mice found.

3.3.4.4 DAILY ROUTINES MODIFICATIONS

First of all, after analysing the changes expected for the fish farm, it is important to have in mind that the modifications proposed for the routines should always avoid unnecessary entrances and exits between the two spaces – fish farm and quarantine – because this can lead to unwanted contaminations or a possible entry for dangerous microorganisms.

For this reason, and assuming that the fish farm had the conditions and all the water quality parameters stable, as well as the correct maintenance of equipment and machines, it should be better to start the routines from the quarantine to the fish farm.

As it happens the quarantines routine depend on several factors such as fish species, life cycle stage and even their origin.

Reports show that 80% of public aquariums and zoos chose to use hypo and hypersalinity treatments when starting quarantine procedures. Usually, for most of the freshwater animals, 3-5 psu long-term baths are the most common choice. Salinity changes are highly effective for parasite control (Hadfield and Clayton, 2009) and should be the first way of avoiding the use of chemicals. When this is not an option there are some other prophylactic treatments involving medications.

In closed systems, formalin and chloroquine are also commonly used for this. For long-term baths, doses ranging from 12,5 mg/L to 25 mg/L are recommended. At the same time, doses of 125 mg/L to 250 mg/L can be used for 30 - 60 min baths. Medicated feeds containing fenbendazole and praziquantel can also be administrated and are often used routinely (Hadfield and Clayton, 2009).

Due to BIGH's quarantine dimension and since the company prefers to maintain the task of being eco-friendly and biologically responsible, the prophylactic treatments involving medications should be avoided. So, the option for prophylactic treatments should be hypo and hypersalinity therapies. According to FAO, logbooks are recommended for the better tracking of diseases, treatments, operations, and entries.

These kinds of records should have essential information's such as:

- Supplier and country of origin
- Date of arrival
- Total number of animals in the original shipment
- The initial number of animals stocked in each tank
- Details of any clinical signs of disease and number of affected individuals, by tank
- Mortality rate, by tank
- Details of any diagnostic tests and examinations

Besides all what has mentioned, it is important to know that the effluent and wastes generated by the quarantine facility should be treated in a way that effectively destroys all pathogens. For this, active disinfectants can be used as they do not represent a major hazard to the environment.

Regarding the cleaning and maintenance for the quarantine, the facility and tanks should always be kept clean. If there is no possible way to have individual material for each tank, at a minimum, all nets should be disinfected with *Virkon*® before being moved between tanks and at the end of the routine.

Relating to staff safety, all equipment such as gumboots and protective clothing used in the quarantine area should stay in a restricted site. In BIGH's case, a changing room with a footbath containing disinfectant placed at the entrance door to the quarantine was planned. *Virkon*® could be used for this purpose and the solution inside the footbath should be exchanged every 5 days. Before protective footwear or clothing were removed from the quarantine area, they should be cleaned using an approved disinfectant such as *Betadine*® (5 % solution).

After exiting the quarantine, the staff should add to a registry all the information such as date and time of entry, reason and take note of any irregularities. It is important to wash and clean the hands after leaving the quarantine and before starting any other task in the fish farm.

It is also important not to forget to create conditions to receive visitors. For this purpose, supplies such as foot covers and gloves are good options. These should be given before entering the fish farm. Guests should be asked not to touch anything, and it is highly advised asking the visitors to disinfect their hands before entering the facility.

Likewise, for the fish farm, some improvements can be done. During the internship, the entry logbook was added as an improvement for the fish farm, controlling the staff and visitor entries.

Cleaning of the fish waste refrigerator was carried out during the shutdown. The incorporation of a monthly routine fish fridge cleaning with *Gamaclean®* could be beneficial to guarantee the refrigerator always clean.

Subsequently and whenever there are no orders, deep disinfection with *Virkon®* should be performed. If possible, let the reagent sit for 24 hours and rinse after that period so all microorganism can be removed.

3.3.4.5 FISH HANDLING AND TRANSPORTATION

For transportation, the most important factor to retain is that these should be as fast as possible, minimizing the risks and stressors that can lead to premature or sudden death. The number of operators for the system that is established at BIGH should not exceed three workers, because more can make the process of transfer difficult.

For this to be possible some measures should be considered, such as:

- Checking the water temperature inside the final tank
- Activating O_2 supply in the tank
- Whenever possible, using dark containers to avoid light exposure
- Avoiding air exposure, since this can lead to stress for the fish
- Only using nets and choosing the appropriated mesh size according to fish size
- Keeping track of health state after the first 72 hours

Evidently, the registry of the transfers should be done, together with the identity of the staff who performed it and the number of deaths associated with each transfer.

Strict measures should be taken regarding pest control. During the shutdown, it is advised to close all possible entries such as cracks, crevices and openings with gun foam or copper mesh. Adding to this, mice traps can be set, never rodenticide, since this can lead to decomposition of the mouse body on hidden places. Multiple mice trap also known as "curiosity traps" can gather multiple animals. These kind of traps are more humane since they trap the animals inside with a trap door that closes after entering. Other kinds of traps possible in this scenario are the natural traps such as water buckets with “bridges” or even the interaction of natural predators such as ferrets or weasels trained to detect mice (but these animals only can be used on rare occasions and for a brief period always with supervision). To avoid attracting mice, it is necessary to maintain all the facility clean. After the daily feeding, it is necessary to brush all the feed that might fall to the floor and all the feed containers should be well closed.

Finally, the feed containers found in the feed station should be made of metal and always with a lid to cover (Figure 35).

FIGURE 35 - EXAMPLES OF RESISTANT CONTAINERS

3.3.4.6 EMERGENCY PLAN

A plan involving procedures and emergency contacts should be developed, to use in case of technical problems or power failure. All machinery and spare parts for the water pumps should have backups. Some materials such as lids, silicone or expanding foam could be vital in case of an unexpected problem such as water leakage.

4 FINAL EVALUATION OF THE INTERNSHIP

4.1 WORKING CONDITIONS

The management team has always been receptive to any suggestion regarding materials, however, and mainly due to the fact of how the system was set up, there were some difficulties, such as accessibility problems, for example, to clean the tanks and the waterways.

Closer to the end of the internship, there was a lack of materials such as some chemicals solutions in the kits used for water parameters control and backup material. There were also some problems with the probes and equipment used for the water daily evaluation that could lead to some unclear results.

There was some disorganization regarding the customers' requests which sometimes led to situations that became a bit stressful. Despite everything, when this happened the supervisors would recruit other staff of BIGH to help the pisciculture team.

There was always a good atmosphere in the company and all the team was very understanding and nice. This atmosphere led to the feeling of being useful and when requested the team always heard personal opinions and suggestions.

4.2 ATTAINMENTS / ACHIEVEMENTS

Being able to watch how to plan a shutdown was very enriching since made possible to understand that in a fish farm there is always room to improve efficiency. Production and profit ratio should be taken very seriously and need to be very well evaluated and studied because competing with big companies, already well established, is a very challenging task and this could be even more complicated if the aim is to produce biologically and organically.

For companies such as BIGH, it could be beneficial to focus on educational purposes besides profit, providing workshops to the population and schools. In terms of sales, these should be directed to local restaurants. It could be more beneficial to focus on the quality of fish produced and let the customers see and choose their final product.

For the entire period of the internship, it became clear that disorganization can lead to a decrease in the biosecurity conditions, that are central to the proper functioning of fish farming.

4.3 SUGGESTIONS / PROSPECTS

The first thing that could be advantageous is to change the feeding method. On the last weeks of internship, there were some trials made and the results showed that feeding the animals three times a day – 9H in the morning, at mid-day and 15H30 in the afternoon was more beneficial.

Still concerning the feed, maybe changing to slow sinking pellets would be more beneficial due to seabass being more receptive to feed that stays in the water column when comparing to feed at the surface or from the bottom (Wilson, 2017). Another aspect to be considered could be the use of immunostimulants such as chitin and chitosan, vitamin C and E that play an important role in several physiological functions including growth, resistance to infections, wound healing and response to stressors (Barman et al., 2013).

In the system, some slight modifications could be helpful, not only to increase the production but also to save time in some tasks in the fish farm routine. For example, changing the colour of the tanks because this reduces the possibility to visualize the dead while at the same time makes it more difficult the observation of animal behaviour and external wounds. Another thing that could be controlled is the water flow in the tanks because this highly influences the sinking speed of the pellets.

Moreover, equally important should be the introduction of control of phosphorus to the daily routine and it could be interesting to control the salinity levels in the system. Phosphorus can be beneficial to the plant development, being an aquaponic system controlling this parameter can reveal to be advantageous.

For the alkalinity, this parameter could be safely controlled only two times each week since all the other parameters are taken daily. The light and darkness cycle could be changed to a seasonal light cycle since the one established may cause some stress in the animals.

To avoid entrance of fishes into the drum filter, some nets could be set up in its entrance. This could be important to avoid fishes entering and eventually dying inside of it, leading to an increase of organic matter.

Subsequently, concerning the organization, creating a rule for high quantity orders (for example between 100 kg to 200 kg or 80 to 100 filets) could be very advantageous due to the small team working in the fish farm. Avoidable situations such as having low quantities of ice or cleaning the processing room more than two times a day would be minimized if large orders only were accepted if made 24 hours in advance.

Lastly, the changes made in the drum filter and the mesh, changing from a mixture of 150 µm (75 %) and 65 µm (25 %) to a mesh of 85 µm, may not be as beneficial as initially thought since, not only suspended organic matter and solids can have various sizes, but also microorganisms can have varying ranges of size. So, this change could let some particles and even different stages of the microorganism pass and to stay inside the system.

4.4 CONCLUSIONS

Initially, the main goal of the internship was to carry out tests related to feed efficiency, nevertheless, as it was also necessary to respond to customers' requests, the fulfilment of this objective could be jeopardized. In other words, the need to carry out customer requests would affect the consistency of the tests as it would not be possible to guarantee accurate feeding at a fixed time under constant conditions.

Despite that, a small experiment was carried out focusing on testing what would be the best approach to feed distribution.

That being said, and since water quality control is the basis of an optimal pisciculture production or aquarium, focusing on this topic appeared to be interesting. At the same time, as it was clear from all that was presented throughout the report, good water quality and the entire water maintenance process is the key to the success of aquaculture and aquaponics.

After the confirmation of pathogens inside BIGH's system, it became evident that this could be another noteworthy topic. To understand and to learn how to deal cope with biosafety and biosecurity issues. These two can easily affect the water quality and therefore the establishment of protocols regarding all the biosafety and security measures was considered extremely important.

These measurements should primarily focus on the prevention and simple steps as a stabilised and organized cleaning routine, maintaining animals healthy, providing equipment to the staff and monitor water periodically are vital and can minimize entry of harmful microorganisms.

It is important to have in mind that it will not be possible to avoid permanently the entrance of pathogens, but this can be reduced in many and diverse ways. Even so, knowing that there may still be a possibility of contamination, companies must be prepared to face such situations. Having prior knowledge of common pathogens that can harm the selected species as well as emergency plans regarding how to act and lists of possible treatments can help to diminish all the losses.

Adding to all, the mentioned above will inherently affect the health of the animals. Although these are produced for human consumption, maintaining conditions for these animals to grow in the healthiest and most beneficial environment is also important.

Overall, the topics referred in this report are vital for the correct functioning so that a fish production, inserted in an aquaponic system or not, is fully functional.

In this matter, is also important to highlight that this internship provided the conditions to developed new skills while gaining independence and confidence on the personal abilities while in a professional environment.

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6 APPENDIX

CALCULATIONS CARRIED OUT

$$Average\ Daily\ Growth\ (ADG) = \frac{Mean\ Weight\ Gain}{Days\ of\ Trial}$$

$$Biomass = Number\ of\ Fishes * Average\ Fish\ Weight$$

$$Feed\ Convertion\ Ratio\ (FCR) = \frac{Feed\ given}{Animal\ Weight\ Gain}$$

$$Feed\ Eficiency = \frac{Fish\ Gain\ Weight}{Total\ amount\ of\ feed} * 100$$

$$Feed\ Input = FCR * (\ Weight\ Gain\ per\ day * Number\ of\ Total\ Fish\ )$$

$$Hydroponics\ Unit\ \left(\frac{kg}{day}\right) = \frac{Fish\ feed * Fish\ Sludge}{Total\ solids\ sludge}$$

$$Mean\ Weight\ Gain\ (MWG) = Final\ Weight - Initial\ Weight$$

$$Mortality\ Rate(\%) = \frac{Number\ of\ Dead\ Fishes}{Initial\ Number\ of\ Fishes} * 100$$

$$Percentage\ Weight\ Increase\ (PWI) = \frac{Mean\ Weight\ Gain}{Initial\ Weight} * 100$$

$$Specific\ Growth\ Rate\ (SGR) = \frac{Ln\ Final\ Mean\ Weight - Ln\ Initial\ Mean\ Weight}{Days\ of\ trial}$$

$$Survival\ rate\ (\%) = \frac{Number\ of\ Fishes\ at\ the\ end}{Initial\ Number\ of\ Fishes} * 100$$

$$Total\ Ammonia - Nitrogen\ (TAN) = NH_3 + NH_4^+$$

$$Active\ chlorine = \frac{\%\ concentration\ powder * 1\ 000\ mg/g * Grams\ power\ added}{Amount\ of\ water\ to\ be\ treated\ (L)}$$

$$Thiosulphate\ (neutralizing) = \frac{\%\ concentration\ solution * Litres\ solution\ added}{Amount\ of\ water\ to\ be\ treated\ (L)}$$