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Modeling Carbon Dioxide Dynamics In A Land Based Fish Farm

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Contents

1. Abstract

The demand for fish is estimated to rise in the future due to the growing population, increased income, and the health benefits connected with fish intake. Fisheries and aquaculture products market are big business in Europe, one of the world's most important seafood marketplaces, with consumption steadily rising over the last few decades. Aquaculture being one of the fastest growing sectors of the global food industry, and playing an increasingly significant role in global food production and economic expansion. Aquaculture appears to be the most reasonable option to supply the EU's seafood demand. In the EU and Italy, rainbow trout (*Oncorhynchus mykiss*) is one of the most widely cultivated fish species. Italy accounted for 21% of the total EU output in 2018.

Carbon dioxide has both direct and indirect physiological impacts on fish, as well as indirect effects on water chemistry through the modification of pH. This thesis presents the development of a dynamic model for CO_2 concentration within a raceway of rainbow trout (*Oncorhynchus mykiss*) farm as a step toward the implementation of a management system based on Precision Fish Farming.

The model was applied to a data set collected during a 55-day survey in July and August 2020 on a trout raceway farm in Trentino-Alto Adige, Northern Italy. The survey was carried out in the frame work of the H2020 project GAIN- Green Aquaculture Intensification in Europe. Water temperature, pH, and dissolved oxygen concentration were measured every 20 minutes in the influent and effluent. Fish biomass and size distribution was also estimated with a daily frequency using a non-invasive monitoring devise. The model parameters were estimated based on the literature. The results indicate that the model could be utilized to develop a cost-effective automatic carbon dioxide concentration control system.

2. Introduction

According to FAO, demand for fish is predicted to increase in the future due to the growing population, increased income, and the health benefits connected with fish intake. Between 1961 and 2017, worldwide edible fish consumption increased at a 3.1 percent yearly pace, about double the rate of yearly global population increase (1.6 percent) and more than all other animal protein sources combined, which increased by 2.1% per year (The EU Aquaculture Sector – Economic report 2020). There is a large variety of fast-growing fish species with high economic value that have the potential to meet food, income, and seed supply requirements. As a result, if capture fisheries and aquaculture are combined, the impact will be enormous. Inland aquaculture is regarded as a "close friend" of inland fisheries (Ma'ruf et al., 2021). Besides, the scientific advances over the past half-century, have resulted in a significantly improved understanding of the features of aquatic ecosystems, as well as the importance of managing aquatic ecosystems in a sustainable way. Finally, SDG 14 - Preserve and sustainably manage the oceans, seas, and marine resources for long-term development – as well as other SDGs relevant to fisheries and aquaculture were stablished in an unified and coordinated manner (FAO,2020).

Aquaculture refers to the raising of aquatic organisms including fish, mollusks, crabs and plants: it is among the most rapidly expanding food producing industries and is becoming an increasingly significant source of food and economic growth throughout the world. Furthermore, at the global scale, more than 90 percent of fish populations being monitored by the FAO have been depleted or overexploited, where appropriate, aquaculture is therefore mentioned as a possible option for addressing food insecurity. According to (Okeke-Ogbuafor et al., 2021), aquaculture can provide up to six times as much food as the ocean, as farming involves intervening in the rearing process to make it more productive, for example by stocking, feeding, protecting against predators, and so on. In 2018, Global fisheries production (capture & aquaculture) reached a record 178.5 million tonnes, of which 82.1 million tonnes came from aquaculture. Human consumption accounted for around 88 percent (156 million tonnes) of global fish production. The remaining 12 percent (22 million tonnes) was diverted to non-food uses, with 82 percent (or 18 million tonnes) going to the manufacturing of fishmeal and fish oil (FAO,2020). Approximately 46 percent of overall production was derived from aquaculture, and 52 percent of fish consumed by humans came from this source (The EU Aquaculture Sector – Economic report 2020). According to FAO, a contribution made by the EU to world aquaculture productivity (including aquatic plants) has decreased significantly over time in both volume and value terms, accounting for only 1.0 and 1.5 percent of global production in 2018 (FAO 2020).

Aquaculture is becoming an increasingly significant source of seafood production, and as space for coastal cages becomes increasingly limited, there is a growing proportion of fish being cultivated in land-based aquaculture systems that recycle the majority of the system water, called RAS (Recirculating Aquaculture System). In recent years, land-based aquaculture has grown, however, fish farmed in such system may be exposed to far higher levels of carbon dioxide than naturally occurring fish (Stiller et al., 2015). In the literature on fisheries in developing country, inland aquaculture is frequently mentioned as a potentially valuable source of income growth in terms of revenue generation from export items. Inland water resources are under stress as a result of growth in both the economy and the population. This pressure is exacerbated by overfishing, contamination of the waters, destruction of habitat, invasive species, and changes in water flows. The declining water quality has several consequences, including a decrease in the number and size of fish caught, as well as the scarcity of some fish species. Some effort must be taken in order to boost the community's fishing profits and, land base aquaculture could be one of the solutions (Okeke-Ogbuafor et al., 2021). Nonetheless, this activity is regarded high risk, not only because of the difficult-to-control environmental component, but also because improper aquaculture may result in environmental degradation and disruption of the current aquatic ecosystem in which the aquaculture is performed (Ma’ruf et al., 2021). Temperature, salinity, dissolved oxygen, carbon dioxide, un-ionized ammonia, and pH are all essential water quality characteristics in aquaculture systems. It may also be necessary to consider total dissolved gas pressure and suspended particles when using particular water sources (Colt et al., 2009). In this system, carbon dioxide is released by fish via their gills as a byproduct of aerobic respiration.

Under normal settings (20 °C, 10^5 pas), the concentration of carbon dioxide ($\mathrm{CO_2}$) in fresh water is around $0.59\ \mathrm{mg/L}$, but in intensive aquaculture systems, $\mathrm{CO_2}$ concentrations can exceed 20 to 100 times that value (Stiller et al., 2015). Carbon dioxide accumulates in these systems due to fish and microbial respiration, and while degassing devices are employed to remove as much $\mathrm{CO_2}$ as possible, the concentration of dissolved carbon dioxide in these systems could be significantly higher than the ambient $\mathrm{CO_2}$ level (Stiller et al., 2015). Due to the low stocking density in the traditional aquaculture system, $\mathrm{CO_2}$ does not accumulate significantly, and is not harmful for fish. The stocking density increases and the water exchange rate decreases (by around 10%) in a recirculating aquaculture system. As a result, high concentrations of dissolved carbon dioxide will significantly limit the productivity. The acceptable operating levels of carbon dioxide depends on the species, stage of growth, and general water quality: in general the $\mathrm{CO_2}$ concentration should not exceed 10 mg/L (Hu et al., 2011).

Aquaculture's growth in the future must rely on more eco sustainable farming methods, utilizing the latest and best techniques to minimize these systems' environmental consequences. A framework called Precision Fish Farming (PFF) was recently developed, which was adapted from the Precision Livestock Farming concept that has been implemented in terrestrial livestock production since the turn of the millennium. This novel approach aims at: i) increasing the precision, accuracy, and reproducibility of farming operations; ii) enabling more flexibility and continuous monitoring; iii) to give more reliable decision support; and iv) to reduce dependency on manual labor and subjective assessment. Precision Fish Farming approaches, as a result, involve transitioning the aquaculture sector from a system built on farmer experience to one built upon scientific knowledge. This results in a reduction in the environmental impact as well as an enhance these systems' profitability and a more accurate assessment of the welfare of the fish. This strategy can be implemented as real-time data concerning environmental conditions and fish functional responses are available, as well as the affordable availability of new technologies that perform better than the instruments used in previous decades (Føre et al., 2018).

2.1 Trout Farming in Italy

Europe is the world's second largest dealer of seafood products and one of the world's major markets for seafood, with consumption steadily rising over the last few decades (The EU Aquaculture Sector – Economic report 2020). The rising demand represents a great opportunity for the EU to expand its aquaculture production.

Rainbow trout, is a member of the Salmonidae family and the genus *Oncorhynchus*. Rainbow trout catches account for a negligible portion of global catches. The EU accounted for 75 percent of global rainbow trout catches. Between 2008 and 2018, global rainbow trout (*Oncorhynchus mykiss*) production grew from 518 thousand tonnes valued at €1,952 million in 2008 to 848 thousand tonnes valued at €2,608 million in 2018(STECF - Scientific Technical and Economic Committee for Fisheries, 2020). In 2018, Iran and Turkey are the leading producers, both of which have expanded their output greatly in the last 10 years. The EU produced 20% of the world's farmed rainbow trout which, Denmark, Italy, and France are the top three EU producers, accounting for 25 percent, 21 percent, and 20 percent of total volume, respectively, according to the European Commission. Aquaculture is an activity that has been practiced in Italy for a long time. It was discovered that the nobles used to have tanks in which they stored fish in the excavation houses of Pompeii (Naples), and that they practiced fish farming(The EU Aquaculture Sector – Economic report 2020). Italy is one of the EU's greatest producers of trout, thanks to a combination of tradition and specialization in freshwater aquaculture. From the 1960s to the 1990s, rainbow trout production in Italy grew steadily, reaching a peak of over 50,000 tonnes in 1997. The market became saturated, resulting in a depreciation of the products, and this upward trend was followed by a drastic decline. Farmers began selling processed fish (such as smoked fillets, hamburgers, and fish skewers) to cope with the financial crisis and thereby boost the value of the commodity. As a result, only small-size fish (under 500 g) are sold as head-on-gutted trout presently, whereas larger fish (500 g to 12 kg) are produced mainly to be prepared into processed items (Maiolo et al., 2021).

The optimal environmental conditions are characterized by temperatures below 21 degrees Celsius in fast-moving, oxygenated water, but, this species can adapt to a wide variety of environmental situations and is found in headwaters, lakes, and even seas (Maiolo et al., 2021). Trout farms are usually land-based, often located in mountain areas, near rivers. In Italy, trouts are farmed in raceways, where the water flows supply continuously from the inlet to the outlet. Trout requires high-quality water with dissolved oxygen concentrations near saturation, which is why they are typically, farmed in raceways with fast-flowing water. In Italy, these characteristics are typical farmed of mountainous watercourses in the Alps and Apennines, as well as karst springs (springs at the end of water filled cave systems), which are abundant in the northern Plain of the Po (Maiolo et al., 2021). As a result, 78 percent of trout farms are located in Northern Italy, specifically in Veneto, Friuli-Venezia Giulia, and Trentino-Alto Adige.

2.2 Thesis Goals and Structure

There is a wealth of knowledge on the effects of carbon dioxide on fish, particularly from short-term freshwater fish trials (Fivelstad, 2013). Assessing the capacity of aquatic species to adapt to environmental stresses will become increasingly relevant in the future due to anthropogenic-driven environmental concerns such as global climate change and deteriorating water quality (Dennis et al., 2014). Increased dissolved carbon dioxide concentrations in water will trigger the primary stress responses in fish, generating severe physiological disruptions that might result in decreased development, poor feed conversion, nephrocalcinosis, or hypercalcinosis (Pfeiffer et al., 2011)

Carbon dioxide monitoring has become a significant concern, as aquaculture operations have increased in scale (Summerfelt et al., 2000). Given the abundance of sources for aquatic hypercarbic environments, several researches have been conducted to determine the impact of increased $\mathrm{CO_2}$ on marine and freshwater fish species. These studies showed a range of physiological effects on fishes. A high $\mathrm{CO_2}$ level is detrimental to fish in aquaculture systems. When Carbon dioxide concentrations exceed the safe level (is defined based on fish species, water temperature and so on) the quantity of oxygen that fish's blood hemoglobin can transport is greatly decreased, and respiratory distress can develop, even with high dissolved oxygen concentrations in the water (Hu et al., 2011). Consequently, the pH of the entire system rapidly drops, as a result of high carbon dioxide concentration.

Despite a lot of technological advancements over the years, quantifying $\mathrm{CO_2}$ in water, particularly in seawater, remains a challenging task. The issue is that $\mathrm{CO_2}$ is highly soluble in water, and the total $\mathrm{CO_2}$ level varies significantly depending on the water chemistry. Total $\mathrm{CO_2}$ in the water can differ due to factors other than respiratory gas exchange [e.g. acid–base imbalance], and measurement methods can be affected by variations in water pH or organic compounds. Mathematical modeling is a method for encapsulating all of the available knowledge about a biological process in a concise and rigorous manner and is currently widely used in a lot of fields including biology and ecology (Villaverde et al., 2022). Perhaps more crucially, it is a tool for comprehending, analyzing, and forecasting the behavior of a complicated system in the absence of experimental evidence. Since measuring $[\mathrm{CO_2}]$ in water is more difficult than measuring $[\mathrm{O_2}]$, the rate of $\mathrm{O_2}$ consumption has been utilized in most analyses on fish for estimating the carbon dioxide concentration within the water (Nelson, 2016). As a result, in order to predict the concentrations of $\mathrm{CO_2}$, we will use a mathematical modelling, which serves as a convenient and efficient method compared to real time analysis.

A dynamic model for carbon dioxide concentration within a raceway of the rainbow trout (*Oncorhynchus mykiss*) farm is presented in this thesis as a step towards the implementation of Precision Fish Farming. Field data were collected in the EU H2020 project GAIN - Green Aquaculture INtensification in Europe, which was coordinated by Ca' Focsari University of Venice. The inlet water quality data, will be used as an input for estimating fish respiration and $\mathrm{CO_2}$ concentration in the influent water. The exchange rate will be computed based on air-water partial pressure gradient and transfer velocity. On the other side, based on outlet data, the $[\mathrm{CO_2}]$ in the effluent will be estimated, in order to evaluate the performance of the model. In the next step, an attempt will be made for assessing the effect of transfer velocity on the carbon dioxide concentration. Finally, is evaluated whether the predicted $\mathrm{CO_2}$ within the safe level for fish welfare. The safe level is defined based on literature review.

The framework of the model is detailed in the following chapter (Chapter 3), which defines the state equation, the functional expressions and parameters used to model the dynamic of $\mathrm{CO_2}$ concentration. These parameters were estimated based on the literature. The results are presented in Chapter 4, while in Chapter 5, the assumptions and analysis of the model are discussed. The later part also includes a validation of the model, based on reference data and a qualitative approach to a simulation under actual water temperature circumstances. Concluding remarks are given in Chapter 6.

3. Methods

Quantifying correlations between the properties of the environment and those of animals, is an essential component of the mathematical model that converts these variables into current and future descriptions. Water temperature is one the most critical forcing factor for various processes, including respiratory rate, metabolic rate, and oxygen saturation rate of fish. Other common environmental factors include oxygen and other chemical species concentrations, as well as pH (Lima et al., 2022).

Water quality in aquaculture systems is affected by several processes, some of which are discussed below (Colt et al., 2009). Fig.3.1, illustrates the physical and biological processes that impact water quality in aquaculture systems.

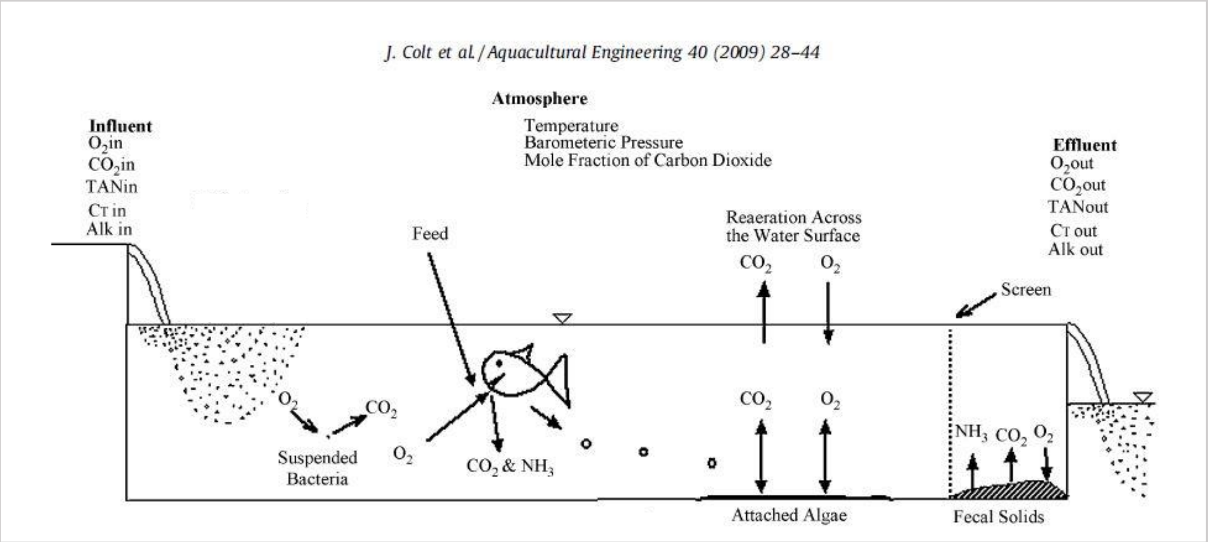

Figure 3.1: Physical and biological processes that impact water quality in aquaculture systems.(source: (Colt et al., 2009)

3.1 Carbon Dioxide

The carbon dioxide levels in low - intensity aquaculture systems are controlled at acceptable levels via water exchange and/or aeration. Opposite to other gases such as oxygen and nitrogen, the equilibrium carbon dioxide content in water does not depend only on a gas–liquid partitioning equilibrium interaction but also on CO_2 chemical reactivity (Summerfelt et al., 2000). The gas–liquid equilibrium has an effect on the exchange of carbon dioxide between atmosphere and water. On the other hand, chemical forms of dissolved inorganic carbon, DIC, in water are determined by acid–base equilibria: therefore, carbon dioxide concentration is determined by the total quantity of DIC (e.g. the sum of carbon dioxide, carbonic acid, bicarbonate ions, and carbonate ions) and by pH. As a result, carbon dioxide concentration can also be controlled by both gas exchange or chemical treatments (Summerfelt et al., 2000).

The amount of dissolved carbon dioxide that is considered safe for a given fish species, depends on alkalinity, pH, and dissolved oxygen levels. For example, salmonids are affected by dissolved carbon dioxide at concentrations of around 20 mg/L, whereas tilapia and catfish can withstand far higher level of dissolved carbon dioxide (Summerfelt et al., 2000). Nephrocalcinosis, or the accumulation of calcareous deposits in the kidneys, can also be caused by long-term exposure to high amounts of carbon dioxide (Colt et al., 2009).

Furthermore, if dissolved oxygen concentrations in the water are around or higher than saturation, the safe level of carbon dioxide rises. It is difficult to set strict thresholds due to the general uncertainty surrounding safe carbon dioxide levels. However, dissolved carbon dioxide levels should be kept as low as feasible, both technologically and economically.

In this context, a dynamic mechanistic model of carbon dioxide concentration was developed that can be applied to raceways. The dynamic model forecasts how animal variables will change dynamically in response to environmental conditions (Royer et al., 2021). The model is based on real-time processing of water temperature, pH and DO data derived from non-invasive fish size distribution monitoring.

3.2 Carbon Dioxide Dynamic Model

Dynamic models have been used in bio- and process technology to develop and improve biotechnological processes. Ordinary differential equation models are increasingly commonly employed to describe biological processes and their temporal evolution mechanistically (Villaverde et al., 2022). Differential equations are those in which the unknown function and its derivative are included in an equation. A dynamic model predicts how individual variables respond to environmental changes in a dynamic way (Royer et al., 2021). This dynamic model uses the differential equation to simulate the carbon dioxide content in rainbow trout fish farming.

The model equations were derived from existing deterministic and chemical literature as well as earlier publications (Mook, 2001,Lazzarino et al., 2009). In order to simulate carbon dioxide dynamic within the raceway the following assumption were made:

1. The raceway's photosynthetic activity was not taken into account;
2. The constant flow rates in both the influent and effluent of raceway;
3. The water in the raceway is well mixed: in this case the system dynamic can be modelled using the system of Ordinary Differential Equation "ODE."

To simplify the dynamic model, we quantified the three main processes which contribute to the CO_2 budget, namely:

1. The carbon dioxide released by fish respiration (R_{CO2});
2. The carbon dioxide supplies from the river's influent water (x_{in}) :
3. The exchange between water and atmosphere (F_u).

The dynamic model equation reads as:

$$\frac{dx}{dt} = \frac{Q}{V}(x_{in} - x) + R_{CO2} + F_u \tag{3.1}$$

In which:

- x is the state variable of the equation and it represents the concentration of CO_2 in the raceway at the time t;
- R_{CO2} is the carbon dioxide excretion by fish respiration; expressed in $\frac{mg}{L.h}$
- x_{in} is the carbon dioxide concentration estimated from inlet water quality parameters in $\frac{mg}{L}$
- F_u is the term referring to exchange rate between the atmosphere and water body in $\frac{mg}{L.h}$

Carbon dioxide excretion by fish, R_{CO2}, carbon dioxide concentration in the water, x_{in}, and carbon dioxide exchange with the atmosphere, F_u , were evaluated using some functional expressions to estimate the CO_2 concentration in relation to the so-called "driving functions"(water temperature, pH, alkalinity and feed composition)which was provided by a farmer. The formulations used to estimate the terms of Equation (3.1) are described in details below.

3.3 Carbon Dioxide Excretion by Fish Respiration

At the conditions of temperature and pressure usually experienced by fish, both oxygen (O_2) and carbon dioxide (CO_2) are gases, and their stoichiometric intake (O_2) or production (CO_2) can be measured to determine the rate of this reaction (Equation (3.2)).

$$nO_2 + (CH_2)_2 \Rightarrow nCO_2 + nH_2O \tag{3.2}$$

The carbon dioxide excretion by fish respiration, can be predicted based on fish oxygen consumption rates and respiratory quotient (RQ.=moles CO_2 excreted / moles O_2 consumed), where 1.375 is a factor that converts R_{CO2} and R_{O2} from $(\frac{\mathrm{moL}}{\mathrm{g_{fish}.h}})$ to $(\frac{\mathrm{mg}}{\mathrm{g_{fish}.h}})$ in the below equation

(Sanni & Forsberg, 1996b).The molecular mass of carbon dioxide and molecular mass of oxygen are (44.01 $\mathrm{g/moL}$) and (32 $\mathrm{g/moL}$).

$$R_{CO2} = 1.375 * R_{O2} * RQ. \tag{3.3}$$

3.3.1 Fish Respiration

Aerobic and anaerobic respiration are the two most important operational elements in cell respiration, with aerobic respiration necessitating the presence of oxygen while anaerobic respiration does not. The aerobic respiration process converts oxygen and glucose into carbon dioxide, water, and energy, i.e. cells can synthesize and store ATP during these cycles, and carbon dioxide is released as a by-product.

In both fresh water and sea water, as well as in fish and human blood plasma, the carbonate system is the most effective buffer system. Fish have acquired skills for coping with the acute consequences of high tissue $\mathrm{CO_2}$ (called hypercapnia) associated with vigorous activity, and they can quickly modify blood pH and transport across the gills to limit the acidification caused by carbonic acid production (Stiller et al., 2015).There is a variety of information available about the effects of carbon dioxide on fish, notably from short-term freshwater fish studies (Fivelstad, 2013). In general, as ambient $\mathrm{P_{CO_2}}$ levels rise, plasma $\mathrm{P_{CO_2}}$ rises as well. The acid base reaction to a rise $\mathrm{P_{CO_2}}$in fish plasma involves first a drop in a pH plasma, which inhibits oxygen transport considerably. After a few hours, higher bicarbonate concentrations compensate for this. Long-term carbon dioxide exposure in freshwater causes decreased growth, lower feed conversion efficiency, and nephrocalcinosis in rainbow trout (Fivelstad, 2013; SMART et al., 1979). Oxygen, carbon dioxide, pH, alkalinity, and hardness are all factors that affect water quality (Fivelstad, 2013). The current study will focus on carbon dioxide, pH, and alkalinity since they are interconnected as part of the carbonate system.

It was discovered that linear correlations existed between feed ration and metabolic rate. Temperature, fish size, as well as feed ration, swimming speed, and salinity, all influence fish oxygen consumption (Forsberg, 1997). According to research, energy expenditure associated with feeding digestion and transportation may be the most important factor in determining total oxygen consumption in fish. In farming production with little swimming activity, the relation between feed intake and metabolic rate in fish is typically a linear function. Metabolite production such as nitrogen excretion and carbon dioxide emission are proportional to feed intake (Forsberg, 1997).

3.3.2 Respiratory Quotient (RQ.)

The respiratory quotient (RQ.), generally known as the respiratory ratio (RQ.), is defined as the volume of carbon dioxide emitted divided by the volume of oxygen taken during breathing (Sanni

& Forsberg, 1996b). When feed was restricted, the respiratory quotient data clearly demonstrated that fish became more reliant on lipid oxidation as an energy source (Forsberg, 1997). In this study, during the monitoring period fish were not feed for a few days, after vaccination. Therefore, two different values of RQ. were used for feeding and fasting days.

RQ. is computed by dividing the amount of carbon dioxide produced by the body by the amount of oxygen used by the body. For species in metabolic balance, respiratory coefficients typically range from 1.0 (reflecting the value estimated for the oxidation of pure carbohydrate) to 0.7 (the anticipated value for pure lipid oxidation).

$$RQ. = \frac{0.7E_l + 0.9E_p + 1.0E_c}{E_l + E_p + E_c} \tag{3.4}$$

in which E_l, E_p, and E_c are the metabolizable feed energy (KJ/g) from lipid, protein, and carbohydrate, respectively.

3.3.3 Dissolved Oxygen (DO)

Dissolved oxygen (DO) is an essential factor in intensive aquaculture; its concentration impacts fish metabolic rate and can connect with other mechanisms, including bacterial activity and plankton metabolism, all of which impact its control. The amount of oxygen dissolved in water is expressed in milligrams per liter or as a percentage of the air saturation value. The concentration of dissolved oxygen (DO) in the water is a significant limiting factor in the survival and growth of fish, with tolerance varied from species to species (Vaage & Myrick, 2022). Salmonids as well as trout have the most stringent oxygen requirements in water; their optimum concentration is 8–10 mg per liter, and if it drops below 3 mg per liter, they start to suffocate. Specific species have different growth limitations in terms of dissolved oxygen content (DO) and are controlled by a variety of parameters, with guidelines varying by system type but requiring at least 5 mg/L is often regarded as an excellent aquaculture guideline (Vaage & Myrick, 2022), with the exception of some warm water fish, for which 3–3.5 mg/L is adequate (Colt et al., 2009). Presence of dissolved oxygen concentrations lower than these values will result in decreased growth as well as higher mortality (Colt et al., 2009). DO concentrations lower than 5 mg/L have a significant impact on growth and at 2 mg/L dissolved oxygen, fish can no longer survive (Khater et al., 2021). The oxygen requirements of fish are also influenced by several other parameters, such as the water's temperature, pH, and carbon dioxide level, as well as the fish's metabolic rate.

In most cases, when stocking densities are lower than 30 to 60 kg/m^3, standard aeration systems could be sufficient in removing CO_2 from the water by transporting oxygen into the water through the use of air stones, surface movement and water falls. However, with the increase in fish density to 100 kg/m3 or more, in order to increase the productivity of the aquaculture system, oxygenating system have become a popular aerobic approach for fulfilling the demand fish (Hu et al., 2011).

Liquid oxygen is used in intensive fish farming to increase the amount of fish that can be stocked in a certain volume. This means that oxygen is no longer limiting the carrying capacity (Summerfelt et al., 2000). Since the 1970s, pure oxygen gas has been utilized to achieve supersaturated levels of dissolved oxygen and increase fish productivity in all types of aquaculture systems. Most land-based systems, including raceways, rely on oxygenation (Royer et al., 2021).

Oxygen requirements increase with increasing temperature (e.g., a 10°C increase in water temperature at least twice the oxygen demand); a greater overall weight of fish per unit of water volume might result in higher activity and hence increased respiration as a result of overpopulation. High $\mathrm{CO_2}$ concentrations are often linked with low dissolved oxygen concentrations (high respiration); oxygenation used to increase dissolved oxygen concentrations will help to lessen excess $\mathrm{CO_2}$ by enhancing $\mathrm{CO_2}$ diffusion back into the atmosphere. (Wurts & Durborow, 1992). There were several interactions to consider for estimating the oxygen consumption via respiration mechanisms. On the other hand, we have to consider that the oxygen generator and flow aeration add oxygen to the water.

Fish oxygen consumption rate was computed as follows (Vaage & Myrick, 2022; Khater et al., 2021; Sanni & Forsberg, 1996a):

$$R_{O2} = (DO_{in} - DO_{out}) * Q * B^{-1} \tag{3.5}$$

Where

- DO_{in} , and DO_{out} are the dissolved oxygen concentrations (mg/ L) in the inlet and outlet water;
- Q is the water flow rate (L /h);
- B is the fish biomass (g) in the raceway.

3.4 Concentration of Carbon Dioxide in The Inlet Water

The water quality in aquaculture systems, is affected by two factors: the quality and flow rate of the influent and the quality changes caused by the feeding and biological process of the animals and plants within the system (Colt et al., 2009).

Previous research, shows that net heterotrophy, or the dominance of respiration within the rivers and lakes, is the principal mechanism responsible for the commonly observed super saturation of $\mathrm{CO_2}$ in inland waters (Sobek et al., 2005). Even while direct $\mathrm{CO_2}$ inputs from the terrestrial environment may have a little effect on the $\mathrm{P_{CO_2}}$ of water body, the data shows that the majority of the $\mathrm{CO_2}$ in water body is produced by internal metabolic activities rather than external inputs.

The estimation of carbon dioxide is based on equations that relate to the ionization equilibria of carbonates and water. There are a variety of formulae available for determining the total dissolved carbon dioxide concentration, based on given data (e.g. alkalinity, DIC and pH) (Summerfelt et al., 2000). In this study the $\mathrm{CO_2}$ concentration was calculated using the water temperature, pH, and total alkalinity (Pfeiffer et al., 2011). Because it is difficult to discriminate between the two ions, $\mathrm{CO_{2(aq)}}$ and $\mathrm{H_2CO_3}$ are combined together and represent as the cumulative concentration of $\mathrm{CO_{2(aq)}}$. The model is used to describe the interaction between water temperature, pH, alkalinity and $\mathrm{CO_2}$ concentration in fresh water fish farming (Mook, 2001).

3.4.1 Characteristic of Natural Water Quality

Physical and chemical changes in the aquatic environment are the most commonly reported causes of fish damage in fish production sites. Oxygen, carbon dioxide, pH, alkalinity, and hardness are all factors that affect water quality (Fivelstad, 2013). The current study will focus on carbon dioxide, pH, and alkalinity they are all interconnected as part of the carbonate system.

3.4.1.1 Water temperature

Fish are poikilothermic, which means that their body temperature is the same as, or 0.5 to 1°C up or down than, the water temperature inside which they inhabit (Svobodová et al., 1993). The metabolic rate of fish is directly proportional the temperature of the water; the greater the temperature of the water (i.e., closer to the optimal values within the normal range), the greater the metabolic activity. This generalization is especially true for warm-water fish. Cold-water fish, such as salmonids, and trout, have a unique metabolism: their metabolic rate can continue at relatively low temperatures, even though at high water temperatures, often above 20°C, they become less energetic and consume lesser food. Water temperature also has a significant impact on the onset and progression of various fish illnesses. The majority of fish species' immune systems operate best in water temperatures around 15°C. If fish are fed and then moved to water that is 8°C or colder, their digestive processes will stall or stop. The food will stay undigested or partially digested in the digestive system, causing the fish to get bloated, lose balance, and eventually die from the gases generated (Al, 1993).

3.4.1.2 Water pH

pH is a measurement that determines whether water is acidic or basic. The molar hydrogen ion concentration (-log [H+]) is used to calculate pH and indicates the hydrogen ion concentration in water. The concentration of hydrogen ($\mathrm{H^+}$) or hydroxyl ($\mathrm{OH^-}$) ions in the water are reflected in the water's pH. The amounts of $\mathrm{H^+}$ and $\mathrm{OH^-}$, are then stated to be equivalent in neutral water (pH = 7.0). Water is acidic (pH <7.0) if it contains more $\mathrm{H^+}$ ions than $\mathrm{OH^-}$ ions. Otherwise, it will be alkaline (pH more than 7.0) (Mustapha & Scientific, 2020). The pH is affected by alkalinity and carbon dioxide levels in the environment (Colt et al., 2009). The majority of pH readings encountered are in the range of 0 to 14 (Wurts & Durborow, 1992).For fish, a pH range of 6.5 to

8.5 is ideal (Figure 3.2). Rainbow trout can be damaged and killed by pH values above 9.2 and acidity below 4.8. Fish can create more mucus on their skin and the inside of their gill covers as a defense against the effects of low or high water pH.

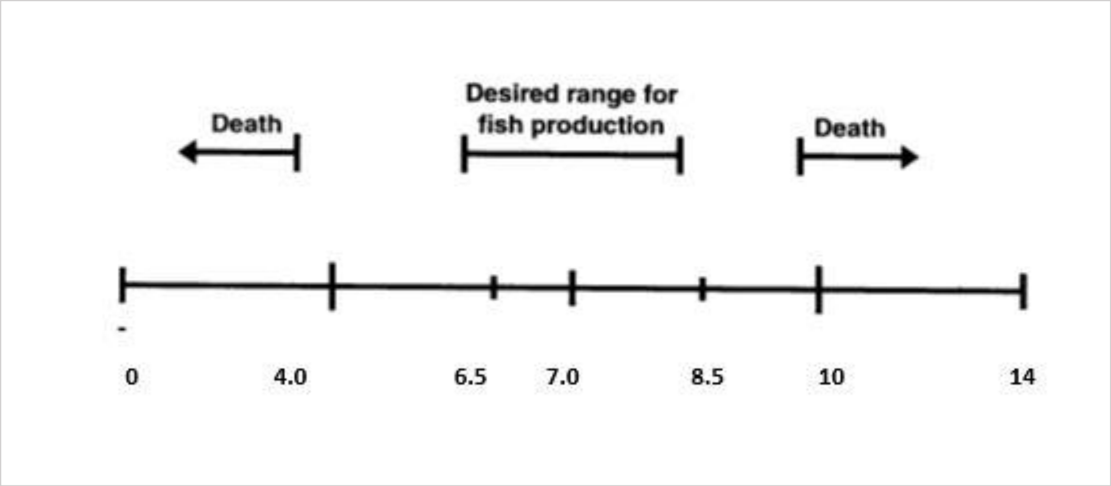

Figure 3.2: pH scale showing recommended ranges (Wurts & Durborow, 1992)

Besides, the level of total ammonia in toxic (NH_3) or non-toxic (NH_4^+) forms is influenced by the pH of the water. The ammonium ion (NH_4^+) is converted to ammonia gas (NH_3) as pH rises, increasing the amount of poisonous ammonia in the water. As a result, the pH has a role in the risk of ammonia poisoning in fish (Mustapha & Scientific, 2020).

3.4.1.3 Alkalinity

The capacity of water to neutralize acid is known as alkalinity (Fivelstad, 2013).Total alkalinity refers to the amount of base in a given volume of water. Carbonates and bicarbonates are the most prevalent and essential components of alkalinity. Alkalinity is measured in Milligrams per liter (mg/L) or parts per million calcium carbonate (ppm CaCO3). For fish cultivation, a total alkalinity range of 75 to 200 mg/L CaCO3 is ideal (Wurts & Durborow, 1992). For freshwater aquaculture single pass systems, Alkalinity can be estimated as (Fivelstad, 2013);

$$\text{Alkalinity} = [\mathrm{H}CO_3^-] + 2[CO_3^{2-}] + [OH^-] - [H^+] \tag{3.6}$$

3.4.1.4 Carbonic acid equilibrium

To improve theoretical knowledge of CO_2 dissolving kinetics in water and the chemical processes that follow, we must first analyze the chemical reaction of carbon dioxide in the aquatic system (Mitchell et al., 2010).

Gaseous CO_2 dissolves quickly in water, resulting in a hydrated aqueous state in reaction(3.7) (Bong et al., 2001)

$$CO_{2(g)} = CO_{2(aq)} \tag{3.7}$$

The gaseous and dissolved phases are represented by g and aq, respectively. This is a quick reaction. After that, the aqueous CO_2 reacts with the water to generate carbonic acid, Equation(3.8). The hydrogen ions in carbonic acid breakdown to form bicarbonate, Equation(3.9) and ultimately a carbonate ion, Equation(3.10)(Mitchell et al., 2010 ; Fivelstad, 2013). Only a small portion of $CO_{2(aq)}$ is converted to H_2CO_3 , Equation(3.8). As it is difficult to distinguish between the two ions, in this study, $CO_{2(aq)}$ and H_2CO_3 are added together and represented as the cumulative concentration of $CO_{2(aq)}$.

$$CO_{2(aq)} + H_2O \leftrightarrow H_2CO_3 \tag{3.8}$$

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+ \tag{3.9}$$

$$HCO_3^- \leftrightarrow H^+ + CO_3^{2-} \tag{3.10}$$

We develop a mathematical model for the dynamics of this system and using the equilibrium constants in R programming environment. The dissociation of acidity constants quantifies the equilibrium conditions (Diem et al., 2008). For pH levels lower than 9, according to Neal et al. (1998), the error of CO_2 concentration calculations using temperature and pH is less than 2%. Equations in (Diem et al., 2008; Mook, 2001) were used to calculate equilibrium constants for carbonic acid (K_1) and bicarbonates (K_2).

$$K_1 = \frac{[H^+][HCO_3^-]}{[H_2CO_3]} \tag{3.11}$$

$$K_2 = \frac{[H^+][CO_3^{2-}]}{[HCO_3^-]} \tag{3.12}$$

Combining the above equations yields an analytical solution in which carbon dioxide in the inlet water can be calculated using pH, temperature, and carbonate alkalinity. We estimated the equilibrium constants (K_1 and K_2) as a function of water temperature, Equations (3.15) (3.16). The Equation (3.13) calculate the carbon dioxide concentration in terms of mg/L, as a function of temperature in the inflowing water from the river in which $\dot{M}$ is a molecular weight of CO_2.

$$x_{in} = \left[CO_{2(in)}\right] = \left[\frac{Ac*[H]^2}{([H]*K_1)+(2*K_1*K_2)}\right] * \dot{\text{M}} \tag{3.13}$$

$$[H] = 10^{-pH} \tag{3.14}$$

$$K_1 = 10^{\left(\frac{3404.71}{(T_w+273.15)} + 0.032786*(T_w+273.15) - 14.8435\right)} \tag{3.15}$$

$$K_2 = 10^{\left(\frac{2902.39}{(T_w+273.15)} + 0.02379*(T_w+273.15) - 6.4980\right)} \tag{3.16}$$

3.5 Exchange of carbon dioxide between atmosphere and water

As most world's inland waters, e.g. rivers and lakes are supersaturated in CO_2, a CO_2 export from inland waters to the surrounding atmosphere takes place (Ran et al., 2017; Sobek et al., 2005). Carbon dioxide emissions to the atmosphere from natural water ecosystems and reservoirs can significantly affect climate change(Ran et al., 2017; Wen et al., 2017). Fish, plants, and other organisms' respiration lead to carbon dioxide emissions. Carbon dioxide is not considered a major issue in most fishponds and is actually necessary for photosynthesis in a water system containing plants. On the other side, high levels can be harmful because carbon dioxide limits fish capacity to transfer oxygen through its blood.

The most significant factor in the flow equation is water P_{CO_2} . Atmospheric P_{CO_2} varies seasonally and diurnally, although to a considerably smaller amount. As a result, P_{CO_2} in a water body is the most important element in defining the amplitude and direction of the gas gradient (Sobek et al., 2005). Shortly, water and air concentration gradients and the gas exchange coefficient k_e for a specific gas are the two most important parameters in determining the flux(Cole & Caraco, 1998).

The magnitude and direction of carbon dioxide flow between air and water are determined by the difference between the saturation CO_2 concentration and the present CO_2 water concentration. Carbon dioxide is transmitted from the bulk liquid to the surrounding atmosphere due to the fact that the water concentration of carbon dioxide is larger than the saturation carbon dioxide concentration in intensively cultivation systems (Colt et al., 2009). Carbon dioxide solubility in water follows Henry's law, which states that the gas solubility in water is proportional to the gas partial pressure on the liquid surface at a given temperature (Hu et al., 2011).

In this study, we are dealing with an open system that is in contact with the atmosphere (CO_2 exchange).The CO_2 partial pressure in the atmosphere, P_{CO_2} is assumed to be constant (Mook, 2001). The concentration of CO_2 in the raceway in equilibrium with air is expressed as follows (Mook, 2001; Wang et al., 2022):

$$[CO2]_{air} = K_0 . P_{CO_2} \tag{3.17}$$

In which the partial pressure of CO_2 in the atmosphere, P_{CO_2} , is expressed in (μatm) and , K_0 is molar solubility (Henry's low) in ($\frac{mol}{L.atm}$) was determined as a function of the water temperature (Mook, 2001);

$$K_0 \ = \ 10^{-(-\frac{2622.38}{T} - \ 0.0178471 * T \ + \ 15.5873)} \tag{3.18}$$

Where T is the water temperature in Kelvin;

$$T = T(°C) + 273.15 \tag{3.19}$$

The flux of slightly soluble nonreactive gases over the air-water interface, Fu, can be calculated by multiplying the gas transfer velocity, by the concentration difference between the top and bottom of the liquid boundary layer (the difference in gas concentration between air and surface water) (Sobek et al., 2005; Diem et al., 2008; Wanninkhof et al., 2009; Yan et al., 2021);

$$Fu = k_e * c * ([CO_2]_{water} - [CO_2]_{air}) \tag{3.20}$$

In which Fu, is the rate of CO_2 movement across the air–water interface in ($\frac{mg}{L\ h}$), and c, conversion factor contributing to length, time, and concentration units. The transfer velocity in $cm/\ h$ is denoted by k_e and, $[CO_2]_{air}$ is the concentration of CO_2 in the raceway in mg/L in equilibrium with air at a partial pressure of 400 µatm, and $[CO_2]_{water}$ is the concentration of free CO_2 in the water in mg/L .

k_e is the CO_2 transfer velocity (cm/h), calculated using K_{600} (Diem et al., 2008; Yan et al., 2021).

For $U_{10} < 3.7\ m/s$; $K_{600} = 0.72\ U_{10}$ (3.21)

For $U_{10} > 3.7\ m/s$; $K_{600} = 4.33\ U_{10} - 13.3$ (3.22)

where K_{600} indicates the transfer velocity for the Schmidt number S_c=600 (Asher, 2014) and U_{10} refers the wind speed ten meters above ground. We used K_{600} to calculate the actual transfer velocity k_e of the gas.

$$k_e \ = \ K_{600} \, (\frac{S_c}{600})^{n} \tag{3.23}$$

S_c indicates the Schmidt number of the greenhouse gas (CO_2) at the water's surface temperature (Diem et al., 2008; Yan et al., 2021) and

For $U_{10} < 3.7\ m/s$; $n = \ -2/3$ (3.24)

For $U_{10} < 3.7\,m/s$; $n = -1/2$ (3.25)

Given that the monthly average wind speeds were < 3 .7 m/s , n was -2/3. For fresh water, S_c of carbon dioxide equal by 600, therefor $k_e = K_{600}$. Therefore we used the Equation (3.21) for estimating the k_e (Lazzarino et al., 2009) .

$$k_e = 0.72\,U_{10} \tag{3.26}$$

The functional expressions needed to characterize the dynamic model are summarized in Table 1

Table 1. Functional Expressions and parameters

Functional expressions	Number of equation
$R_{CO2} = 1.375\,R_{O2}.\,RQ$	(3.3)
$RQ. = \frac{0.7E_l + 0.9E_p + 1.0E_c}{E_l + E_p + E_c}$	(3.4)
$x_{in} = \left[CO_{2(in)}\right] = \left[\frac{Ac*[H]^2}{([H]*K_1) + (2*K_1*K_2)}\right] * \dot{M}$	(3.13)
$[H] = 10^{-pH}$	(3.14)
$K_1 = 10^{(\frac{3404.71}{(T_w+273.15)} + 0.032786*(T_w+273.15) - 14.8435)}$	(3.15)
$K_2 = 10^{(\frac{2902.39}{(T_w+273.15)} + 0.02379*(T_w+273.15) - 6.4980)}$	(3.16)
$[CO2]_{air} = K_0.P_{CO_2}$	(3.17)
$K_0 = 10^{-(-\frac{2622.38}{T} - 0.0178471*T + 15.5873)}$	(3.18)
$Fu = k_e.c([CO_2]_{water} - [CO_2]_{air})$	(3.20)
$k_e = K_{600}\,(\frac{S_c}{600})^n$	(3.23)
$U_{10} < 3.7\,m/s$; $K_{600} = 0.72\,U_{10}$	(3.21)
$U_{10} > 3.7\,m/s$; $K_{600} = 4.33\,U_{10} - 13.3$	(3.22)
$U_{10} < 3.7\,m/s$; $n = -2/3$	(3.24)
$U_{10} < 3.7\,m/s$; $n = -1/2$	(3.25)
$k_e = 0.72\,U_{10}$	(3.26)

3.6 Model Parameters

The model requires the estimation of several parameters, provided by farmer and estimated based on data. Table 2, provides the model parameters, as well as their units and sources.

Table 2. List of parameters used in the model

Parameters	Description	Value	Unit	Source
Q	Volumetric flow rate	1584000	$^{L}/_{h}$	Provided by farmer
V	Raceway Volume	1280000	L	Provided by farmer
E_p	Energy content of protein	23.6	$\frac{KJ}{g_{prot}}$	Provided by farmer
E_l	Energy content of lipid	36.2	$\frac{KJ}{g_{prot}}$	Provided by farmer
E_c	Energy content of carbohydrate	17.2	$\frac{KJ}{g_{prot}}$	Provided by farmer
$\dot{\mathrm{M}}$	Molecular weight of CO2	44.01	g/mol	
$RQ.$	Respiration quotient	0.82	$\frac{mol\ CO_2 \text{excreted}}{mol\ O_2\ consumed}$	Estimated in this study
k_e	Transfer velocity	0.66	$\frac{cm}{h}$	Estimated in this study
$\mathrm{P}_{\mathrm{CO}_2}$	Partial pressure of atmosphere	400	µatm	

The model performance was assessed by plotting the carbon dioxide concentration in the effluent, computed using Equation (3.13) versus the predicted values. From this plot, we applied linear regression method in R and based on R-squared, determined how well our model fits to the data.

3.7 Case Study and Monitoring Strategy

The pilot site is a trout farm in Northern Italy's Trentino-Alto Adige area. Around 80% of the rainbow trout farms are located in Northern Italy, accounting for about 75% of the Italian trout, due to the abundance of freshwater suited for trout wellbeing, low temperatures, and high oxygen concentration. Trout farming is a well-established, traditional activity in Northern Italy, and the farming environment is often made up of small, family-run businesses where technological progress is hampered by a lack of financial resources.

In seven 200-meter-long and 8-meter-wide raceway basins, fish grow from fingerlings to market weight. The water enters the raceways from the neighboring Sarca river and gets a direct supply of oxygen, meeting the oxygen need of fish respiration while also monitoring quality of water in the raceways and discharges to the Sarca river (Royer et al., 2021). Liquid oxygen is stored in a steel tank and transported to each raceway through a pipelines. The sensors are recorded DO in the inlet water after adding liquid oxygen to the raceway. When oxygen comes into contact with ambient temperature, it changes from the liquid state to the gaseous state, with an efficiency of around 90%. Currently, the oxygen flow to each raceway is controlled by manual valves. The feed is manually launched from a gantry, which is put in motion along the raceways' lateral direction during feeding. Fig.3.3, depicts the raceway tanks, the liquid oxygen tank (the white tank on the left), and the gantry that includes the adjustable catwalk that bridges the raceways. The farm is outfitted with sensors that measure water temperature and dissolved oxygen in real-time. These data were gathered in the pilot raceway from two identical multi-parametric automated EXO2 sensors placed near the raceway's input and output ends. Data is managed and visualized using specialized software; currently, operators use this information to determine when to start the oxygen supply. In case of a low DO concentration, an alert mechanism is activated.

Figure 3.3: Raceway tanks, the liquid oxygen tank (white tank in the left) and the gantry (including a mobile catwalk across the raceways) (Lima et al., 2022)

Testing non-invasive technologies for animal variable monitoring and developing novel modeling methodologies for the PFF implementation on a various types of aquaculture, such as rainbow trout raceways, was one of the goals of the H2020 project GAIN - Green Aquaculture Intensification in Europe (Royer et al., 2021).

Direct fish surveys include periodic weighting of fishes and daily recording of mortalities. Biomass Daily (BD), an 80 x 80 cm submerged frame equipped with an infrared sensor, that detects a signal whenever a fish species passes across the frame, was used in a study for the measurement of fish weight (Lima et al., 2022; Royer et al., 2021). Temperature and DO sensors are permanently installed, usually at the raceway input and output, and data is recorded at regular intervals, usually every 15 minutes to 1 hour. The water temperature, pH, fish respiration rate, and DO content at the water source are all assumed to be hourly values in this model.

The initial fish number, N = 38842, was determined using a fish-counting instrument manufactured by Calitri Technology. The number of fish decreased during the monitoring down to 29396 on August 31. The average fish weight at the start was 308.18 g, for a total estimated initial biomass of 11,970 kg. The diet used was a commercial diet of Aller Aqua's 6 mm meal pellets, which contained 44 percent protein and 26 percent lipids. Every day at 9 a.m., the fish were fed. Feed was manually distributed using a mobile gantry that moved from one side to the other at a speed of 10 meters per second, taking roughly 20 minutes (Royer et al., 2021).

3.8 Description of The Programming Environment

The model was written in R, a programming language and free software created in 1993. This programming language was named R, based on the first letter of the first name of the two R authors (Robert Gentleman and Ross Ihaka). R is an extensible, open-source language and computing environment for Windows, Macintosh, UNIX, and Linux that serves as an alternative to traditional statistical packages such as SPSS, SAS, and Stata. R is trusted not only by academics, but also by many large corporations such as Uber, Google, Airbnb and Facebook, to mention but a few.
R is a statistical computing and graphics language and the environment, offers a wide range of statistical and graphical techniques (linear and nonlinear modeling, classical statistical tests, time-series analysis, classification, clustering, etc.).

The original window of R studio is divided into four distinct panels:

1. Script area, in which it is possible to create and save your scripts;
2. Console, in which lines of code are executed;
3. Workspace, in which the list of the all created objects appears, such as: functions, arrays, matrixes, variables;
4. Visualization area, in which the lists of downloaded packages and plotted graphs are shown.

4. Results

As was mentioned in the case study section, water temperature, T_w, dissolved oxygen concentration, DO and pH were recorded every 20 minutes in both inlet and outlet water while all the input data (inlet and outlet) were assumed to be hourly values in this model.

DO data in the inlet includes both water oxygen concentration and liquid oxygen added. The model and parameter estimation procedure were written in R Studio. The deSolve R package was used to solve the differential equation. Carbon dioxide concentrations estimated in the effluent at the start of the period were used as the starting point for solving the model equation. Water temperature, DO, pH and alkalinity were used to calculation the carbon dioxide concentration in the inlet and outlet water (Equation (3.13)). The carbon dioxide concentration in the inlet was used as an input for our dynamic model, combined with the carbon dioxide released by fish and the carbon dioxide exchange rate, while the estimated carbon dioxide equilibrium concentration in the effluent compared with the output of the dynamic model.

4.1 External Forcings and State Variable

Data collection started on July 8, 2020 and ended on August 31, 2020. As water from the Sarca river is withdrawn, the quality of the influent varies over time. The influent flow rate was 1584 m^3/h and the volume of the raceway was 1280 m^3.
Figures (3.2), (3.4),(3.5), illustrate the time series of water temperature, pH and DO measured in the influent and effluent, whereas Fig.4.6 shows the inlet and outlet DO differences. Table 3 presents descriptive data such as range, mean and standard deviation.

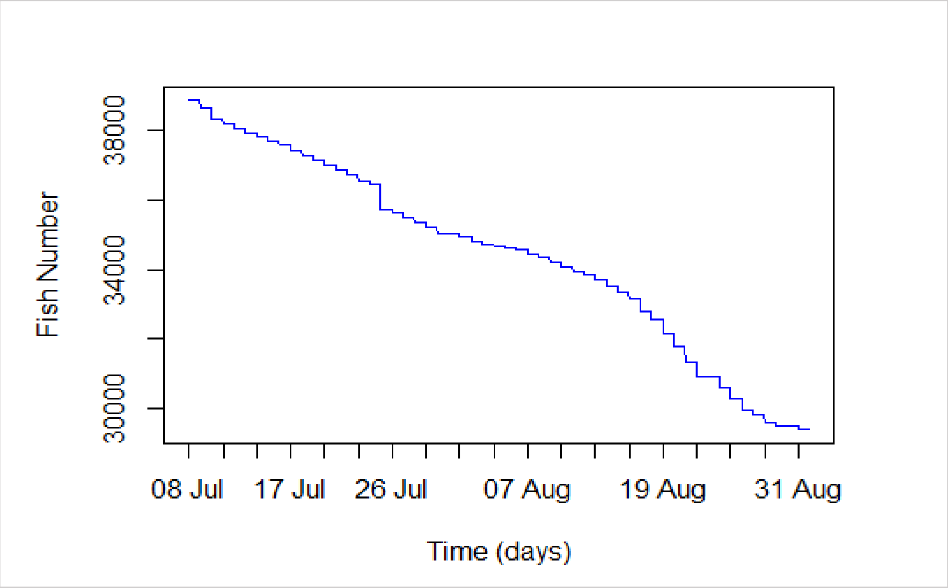

Figure 4.1: Time series of fish number

According to the Fig.4.1, during monitoring, the number of fish exhibits a decreasing trend, due t o mortality during the 55 days.

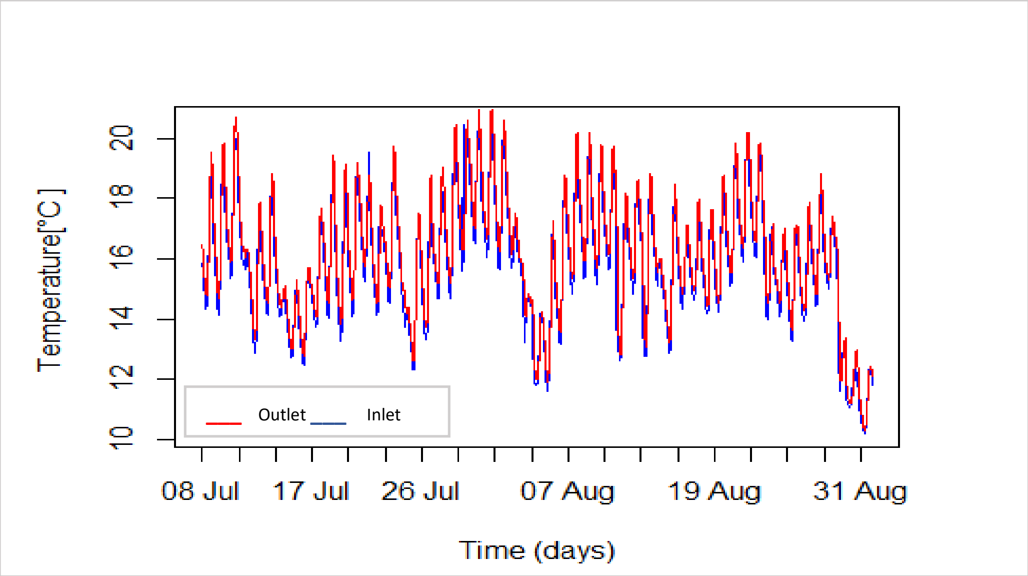

Figure 4.2: Time series of water temperature in the influent and effluent

As it can be seen from Fig.4.2 the water temperature represents a daily pattern. The water temperature ranged approximately between 10.6 ℃ and 20.16 ℃ . The difference between water temperature in the outlet and inlet was marginally positive, most likely due to the conversion of solar radiation energy into heat within the water within the quite long raceway. Based on the flow rate of 1540 m3/h, the residence time was 48 minutes, or 0.8 hours.

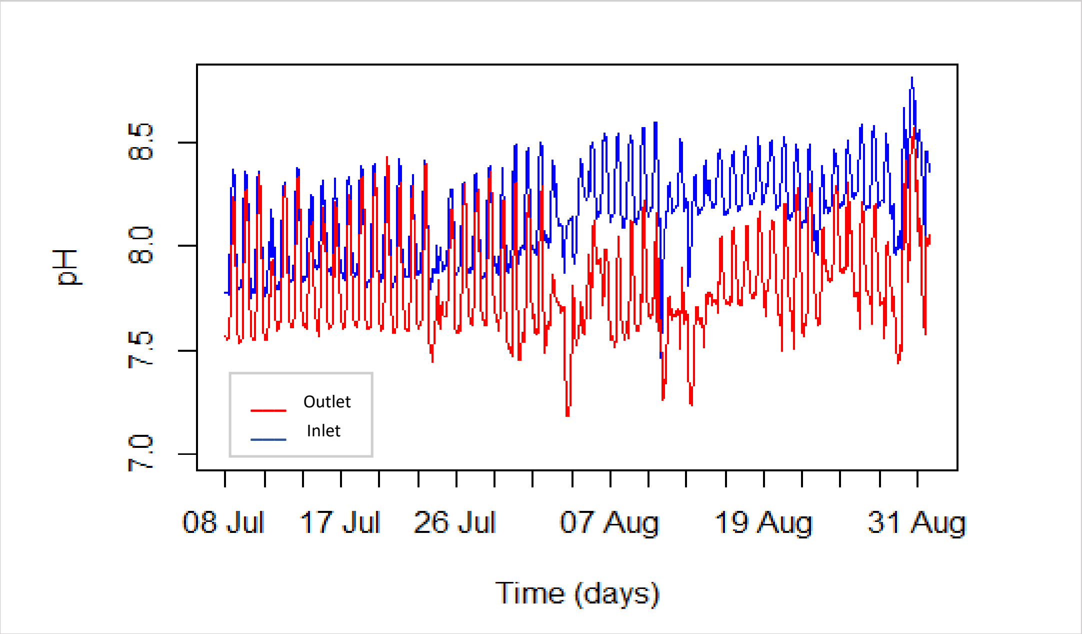

Figure 4.3: Time series of water pH in the influent and effluent

The time series of pH, see Fig 4.3, also shows a daily pattern. In both the influent and effluent, from morning to midafternoon (2:00 PM), the rise in pH maybe due to the activity of microalgae which extract carbon dioxide from the water during photosynthesis. The daily pattern of the pH in the inlet water is shown in Fig 4.3.a. The reduction in CO_2 concentration during the day drops the concentration of H^+ ions while increasing the concentration of OH^- ions, causing the water to be more alkaline. On the other hand, the temperature considerably impacts pH levels.

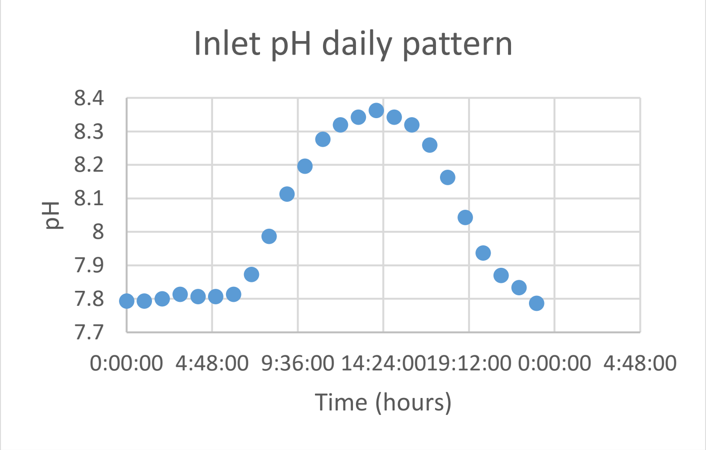

Figure 4.4.a: Daily pattern of water pH in the influent

Based on Fig 4.3, the pH of the outlet decreases significantly on August 7; this could be due to sensors malfunctioning (e.g., modifying the battery or sensor settings) or other unanticipated phenomena; however, anomalous mortality should be reported if the pH abruptly decreases. After August 7, the difference between inlet and outlet pH increased, maybe as a result of respiration and photosynthesis process. Dissolved oxygen (DO) concentrations decrease after sunset since photosynthesis halts and all fish utilize oxygen (for respiration).

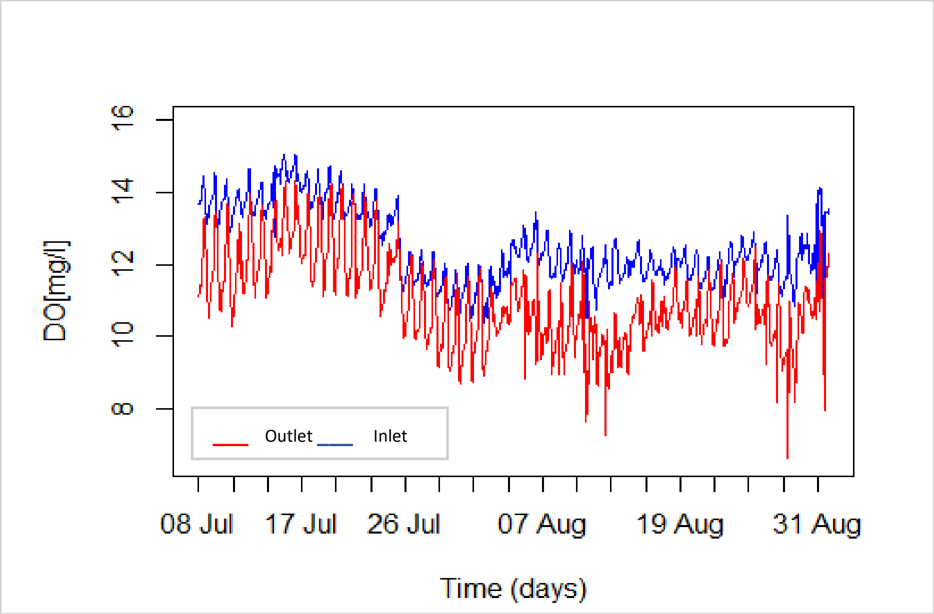

Figure 4.5: Time series of DO concentration in the influent and effluent

The time series of inlet and outlet dissolved oxygen (DO) was shown in Fig.4.4, which presents a less predictable pattern, which could be influenced by the feeding regimen. The DO sensor was placed after adding liquid oxygen, Fig.4.5, as the oxygen supplied during a day in the inlet water, the DO recorded in the input water should be higher than in the outlet. The other explanation can be related to fish respiration, since the fish consume oxygen for respiration the DO concentration dropped in the outlet water therefore, it is understandable why the concentration of DO in the outlet is so low.

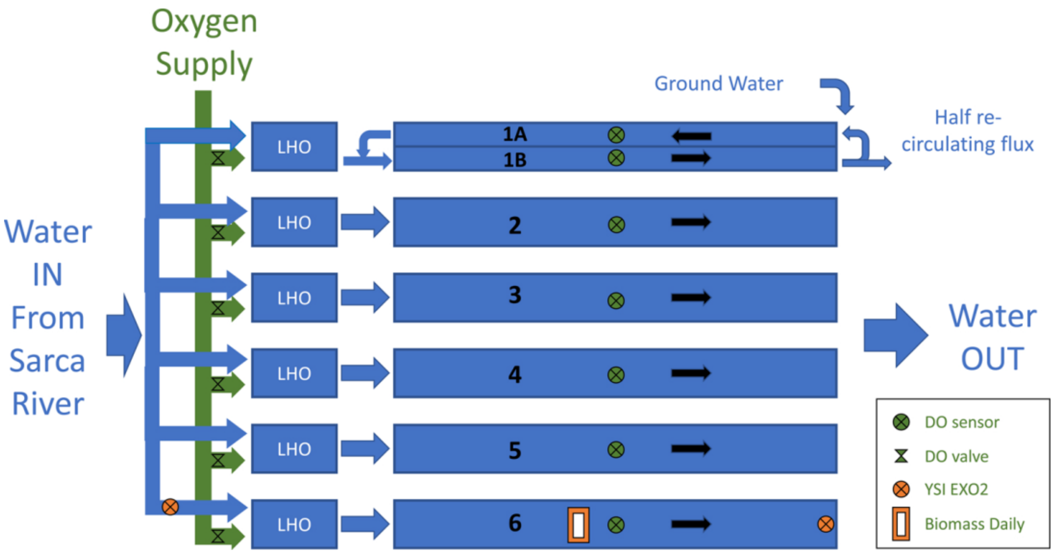

Figure 4.6: Trout farm raceways and monitoring system overview. Source: (Royer et al., 2021)

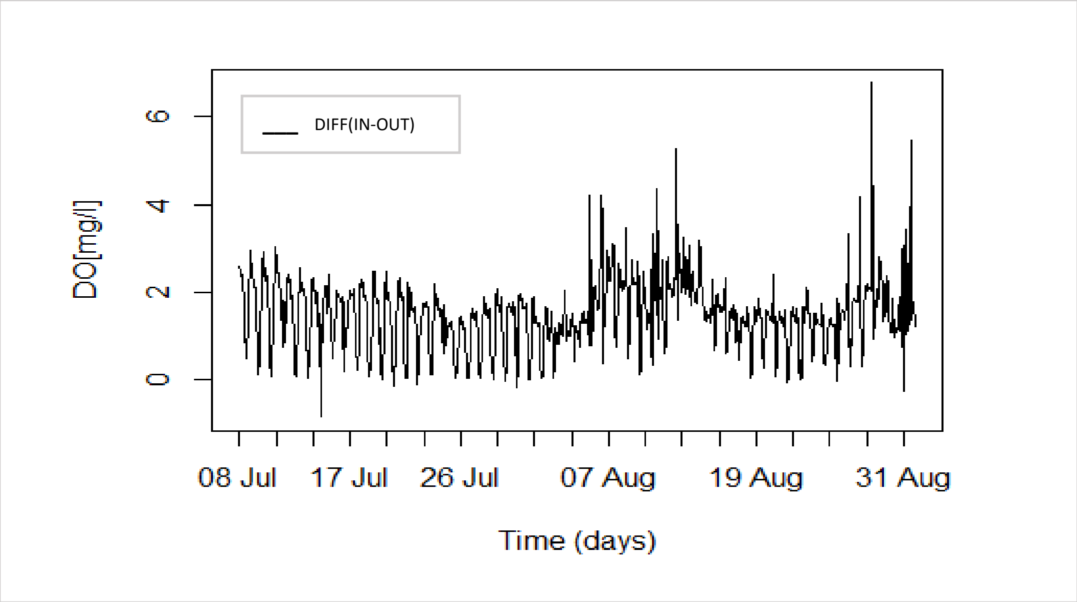

Figure 4.7: Time series of DO concentration differences between the influent and effluent

The difference between DO in the inlet and outlet of raceway is showed in Fig.4.7. As it can be seen the DO in the inlet was higher than in the outlet, as a result the differences were marginally positive. Concerning this figure, on July 18 and August 29, the DO in the outlet is greater than in the inlet, which might be due to instrument malfunction or measurement inaccuracy. Other possibility can be related to photosynthesis process by bacterial and microalgae existence in the water which produce more oxygen.

Table 3. Descriptive statistics of the environmental variables

Statics	Temperature [◦C]		pH		DO [mg /L]	
	Influent	Effluent	Influent	Effluent	Influent	Effluent
Maximum	20.6	20.9	8.8	8.6	15.0	14.3
Mean	15.8	16.1	8.2	7.8	12.5	11.0
Minimum	10.1	10.2	7.5	7.0	8.2	6.6
SD	2.0 1	2.0 4	0.21	0.26	1.05	1.24

As was noted in methodology, the dynamic model was defined in three main processes, including functions and parameters. In this chapter, the findings are given.

4.2 Fish Respiration and Carbon Dioxide

The oxygen consumption was estimated in order to estimate the carbon dioxide excretion by fish respiration. It was assumed that the difference between DO in the outlet and inlet water was due to the fish consumption for respiration. This estimated carbon dioxide was used as an input for our dynamic model.

In this study, the trout diet, the E_l, E_p, and E_c in feed was reported, 6.2, 23.6 and 17.2 (KJ/g) respectively then, the RQ. was calculated as 0.82 based on the feed composition, Equation(3.4).

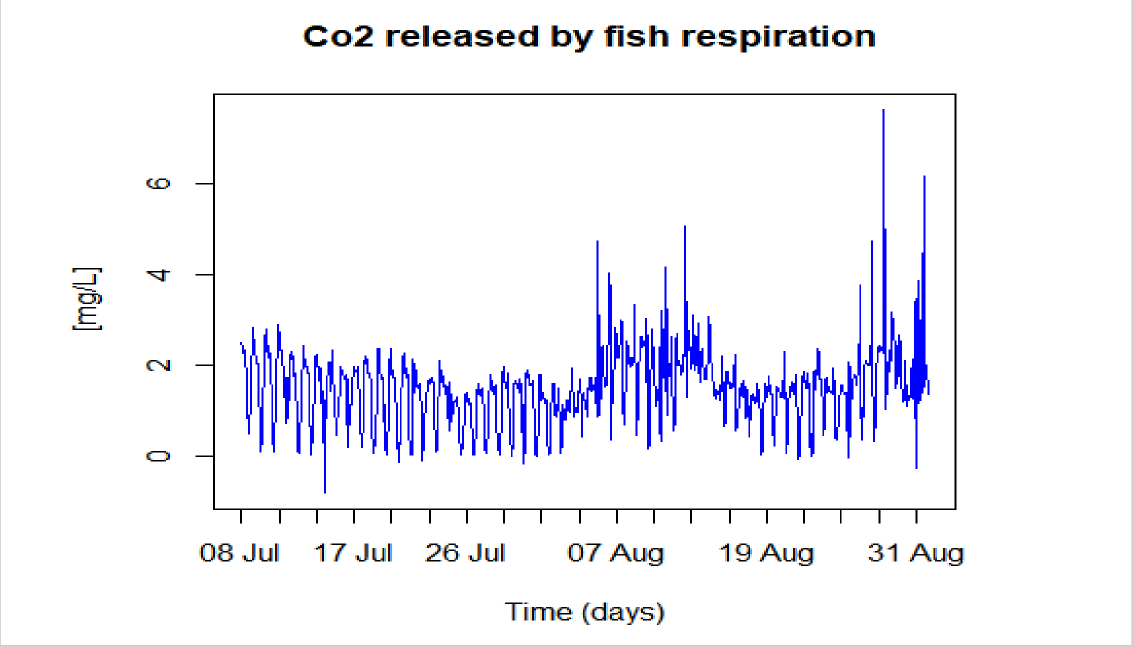

Figure 4.8: Carbon dioxide concentration excreted by fish respiration

The Fig.4.8, illustrates the carbon dioxide produced by fish respiration, estimated from Equation (3.3), by substituting measured fish oxygen consumption rates and estimated RQ. values. Regarding the figure the maximum $[CO_2]$ released by fish respiration is 7.6 mg/L. The carbon dioxide excreted by fish shows a daily pattern, it is clear that with increasing the oxygen consumption rate, the carbon dioxide released by fish increase due to the increase catabolism rate in the fish body.

4.3 Carbon Dioxide Input from The Influent

As mentioned prior, the raceway water supply by the Sarca river. The inflowing water contains temperature, pH and alkalinity. Regarding to these data the carbon dioxide in the inlet of raceway was estimated by Equation(3.13). The alkalinity was 1.5 $mmol/L$, was estimated based on 24-hour observation for two different days. This carbon dioxide concentration is the second input for the dynamic model.

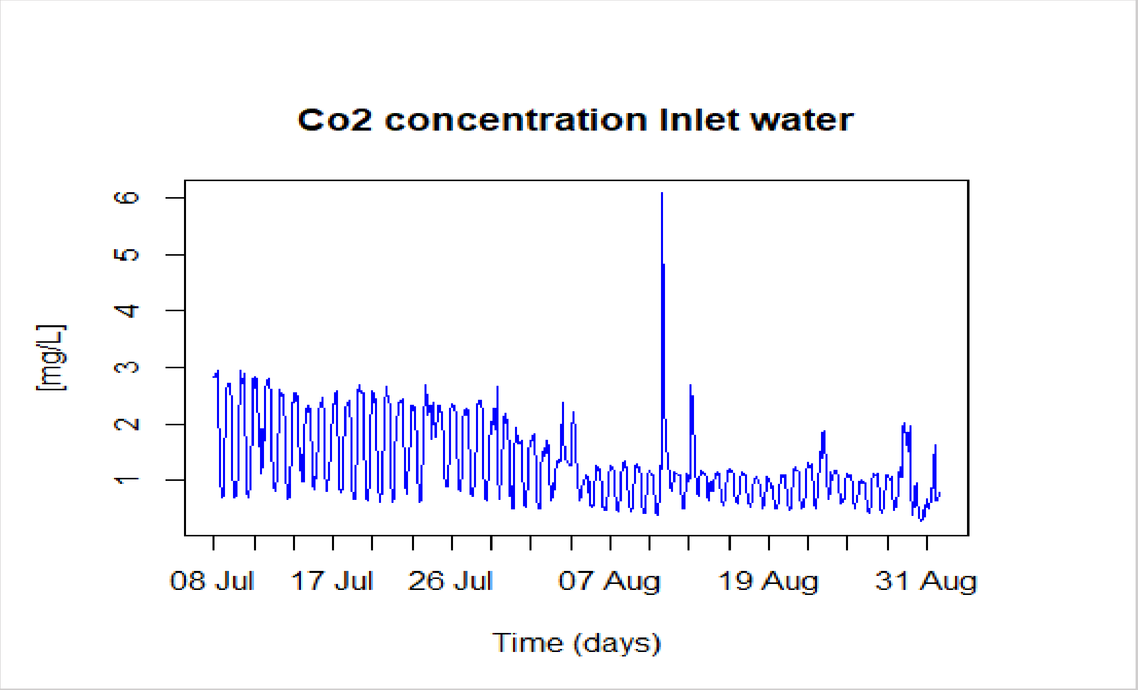

Figure 4.9: Carbon dioxide concentration in the inlet water

The Fig.4.9, shows the $[CO_2]$ estimated using the pH, total alkalinity, and temperature values of water determined in situ. In accordance with several research, the safe limit of carbon dioxide content is less than 10 mg/l (Davidson et al., 2011).

4.4 The Air-Water Exchange of Carbon Dioxide Within The Raceway

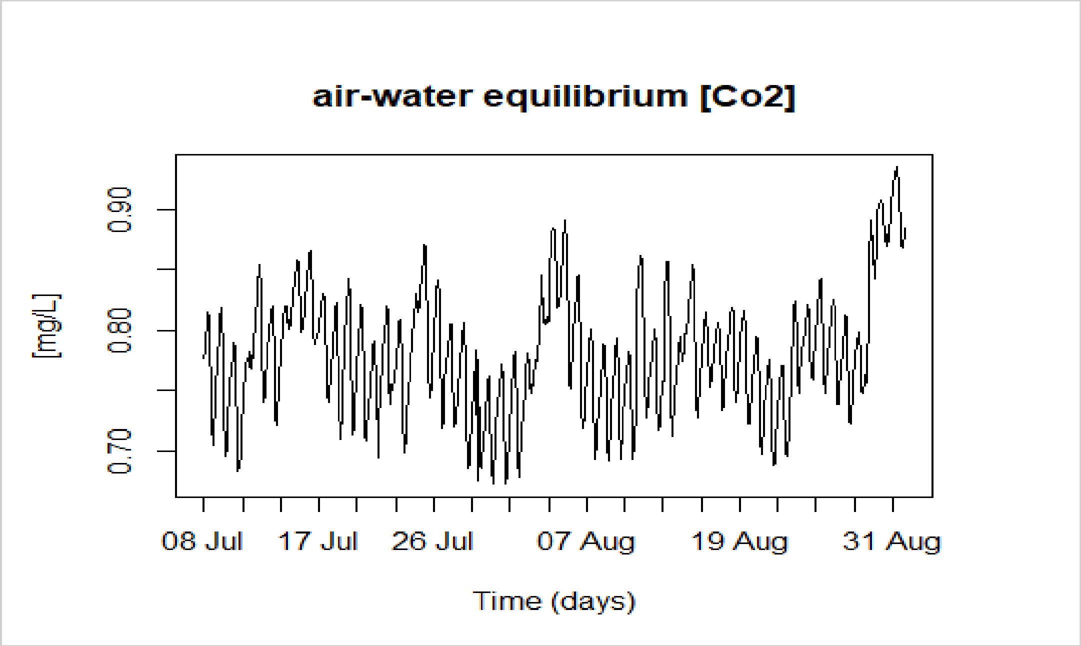

Figure 4.10: The equilibrium concentration of Carbon dioxide

The equilibrium concentration of carbon dioxide is shown in Figure 4.10. These values(Table.4) were used as an input ($[\mathrm{CO_2}]_{\mathrm{air}}$) in Equation(3.20) for evaluating the exchange rate of carbon dioxide between air and waterbody in the fish farm.

Table 4. Water temperature, molar solubility and equilibrium concentration of carbon dioxide

$Temperature$ (°C)	K_0 $(mol\ L^{-1}\ atm^{-1})$	$[CO2]_{air}$ (mgL^{-1})
15.8	0.044	0.776
15.6	0.044	0.782
15.4	0.044	0.787
15.2	0.045	0.791
15.0	0.045	0.796
14.8	0.045	0.801

Regarding to meteorological data around the fish farm, the average wind speed was 0.86 m/s which was less than 3.7 m/s, therefore the Equation (3.21) was used to estimate the gas's transfer velocity, k_e= 0.62 $\left(\frac{cm}{h}\right)$.

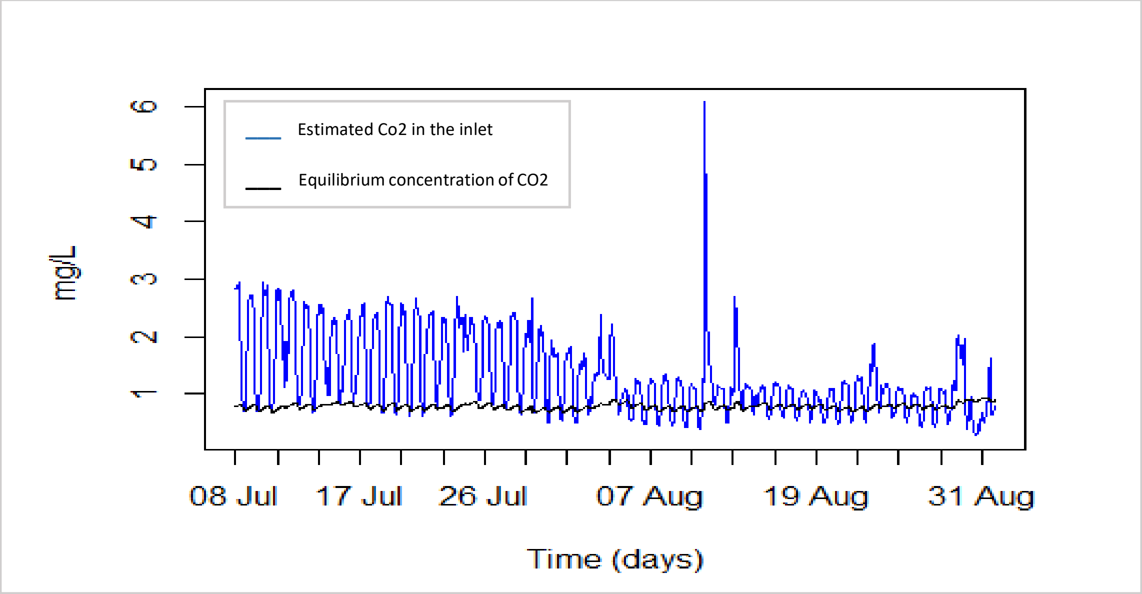

Figure 4.11: Comparison between equilibrium concentration vs inlet water concentration of carbon dioxide

Fig.4.11, compares the $[CO_2]$ concentration in the influent with the equilibrium concentration. In general, the influent was oversaturated, this is in line with the results of Cole et al. (1994), who looked at lakes all around the globe and found that the majority of them (87 percent) were supersaturated, with an average concentration of nearly three times the equilibrium concentration. Furthermore, according to Ran et al. (2017), river waters were supersaturated with dissolved CO2, typically 2 to 20 times the atmospheric equilibrium (i.e. 400 µatm), and this is clearly visible since the water is thrown from the river for fish farming.

4.5 Simulation of Carbon Dioxide Concentration within The Raceway

The carbon dioxide concentration predicted by the dynamic model are compared with the carbon dioxide concentration calculated in the effluent water, assuming that thermodynamic equilibrium is achieved.

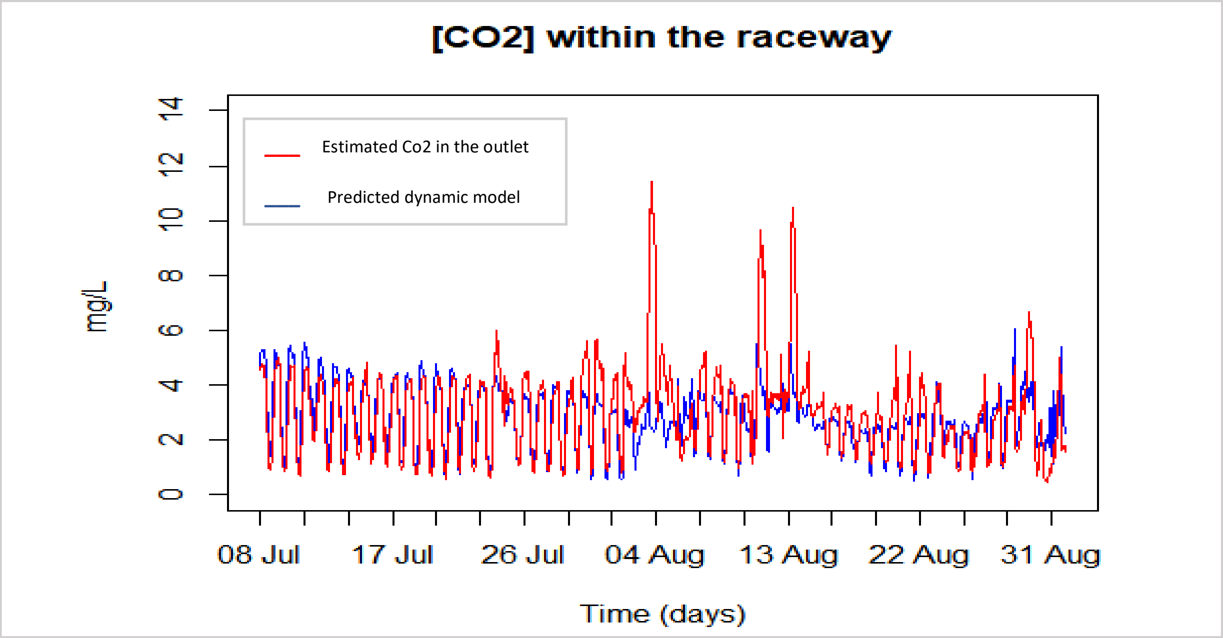

Figure 4.12: Carbon dioxide concentration predicted form model vs estimated from outlet's data

The final results are shown in Fig.4.12. The model output is compared with carbon dioxide estimates based on the outlet's collected data (temperature and pH). A visual comparison demonstrates that the model approximately simulates the daily pattern of carbon dioxide concentrations within the raceway. The R-squared value was 0.60 which meant that the model was able to explain 60% of data.

The points in the predictive dynamic model that could not fit the data perfectly, around August 7, represent periods in which the effluent pH drastically and suddenly dropped. Due to the sudden and unexpected drop in the pH, it resulted in the increase of $[CO_2]$ as well, preventing the model from efficiently fitting the data. It is possible to explain why the output of the dynamic model is lower than the effluent concentration by considering the fish farm's geographical position and, possibly, the unpredictability of the weather. According to Fig.4.8, fish respiration reduced at the same period, possibly due to poor weather conditions, and this decline was represented in the dynamic model's output. Regarding the dynamic model, the maximum concentration of carbon dioxide was estimated at 8.96 mg/L.

5. Discussion

The 55-daytime series of water temperature, pH and DO, data collected during this study enabled the identification of a dynamic CO_2 model based on three main processes (fish respiration, river source water, air-water exchange) and the estimation of the daily pattern of rainbow touts' carbon dioxide production.

5.1 Performance of The Model

The results indicate that the model adequately reflects the system throughout the period. On the other hand, it is possible that the performance of the model was impacted by some limitations and uncertainties, which will be discussed later. In this section, we will go over some of the findings of previous studies.

Khater et al., 2021, found that the use of oxygen by fish respiration increases as the water temperature rises. Temperature increases result in an increase in the rate of metabolic function in the majority of fish species. When fish have a faster metabolism, their respiration rate and requirement for dissolved oxygen (DO) rise, and their eating and digestion habits may change as a result. In this study, for instance, the results indicated that when the temperature increase from 14.46 to 16.65 ^{o}C the oxygen consumed by fish changed from 1.52 to 2.54 mg/L respectively. As expected, when the oxygen consumption rate increases, the metabolism of the fish increases, result in increasing the amount of carbon dioxide released by the fish. According to (Hu et al., 2011), in general, fish gills excrete around 13–14 mg/L of CO_2 for every 10 mg/L of oxygen absorbed.

In the chapter 3, some assumptions were made, to simplify the dynamic model. These assumptions are addressed in below (see Dynamic model)

1. The raceway's photosynthetic activity was not taken into account. Generally, fish are thought to be the dominant organism in raceway systems that require oxygen for respiration. Additional organisms and plants that may have an effect on oxygen and carbon dioxide concentration including: 1) Bacteria that are suspended in water; 2) Bacteria adhering to the surfaces of the raceways and fecal particles in the raceways; 3) Floating and growing algae in the raceway. The edges and bottoms of breeding raceways can be coated with adhering algae and, in some cases, submerged aquatic plants, according to Colt et al., 2009. The rapid growth of attached algae at higher elevations (1000–2000 m) may be due to strong light exposure and highly transparent spring water at these altitudes. Because the rate of bacterial activity in warm water systems is growing, it is expected that respiration from floating bacteria will become increasingly important in warm water systems. Photosynthesis rate is dependent on raceway direction, solar exposure (time of day and seasonality), extinction coefficient and depth, species of algae, and algal covering

of the raceway bottom and sides. we have to take in account the algal and bacterial photosynthetic oxygen generation and respiration rates. The effect of attached algae on oxygen and carbon dioxide levels should be computed separately for day and night conditions. A significant amount of microalgae is unlikely to grow because to the low water temperatures and short water detention periods in typical trout raceway systems. At greater water temperatures and longer detention durations, a considerable quantity of microalgae may occur. In the absence of actual raceway data, microalgae's potential influence was not evaluated in this study. Photosynthesis has a substantial effect on pH during the day. This is related to the carbon dioxide consumed in the process of producing oxygen. During the day, photosynthesis creates a large amount of oxygen. Most serious oxygen shortages occur at night when connected algae are not producing oxygen. The differences between DO in the inlet and outlet in our dataset showed negative values at some points (around July 17 and August) and this means DO in the outlet is higher than in the inlet. It means the photosynthesis process occurred within the raceway and produced extra oxygen or there was some inaccuracy in the recorded DO data.

2. The constant flow rates in both the influent and effluent of raceway; carbon dioxide concentration can be affected by water flow. In this dynamic model, when the flow rate fluctuated between half to the double of its current value, the carbon dioxide concentration and the R-squared value fluctuated within ±0.32 $\mathrm{mg/L}$ and ±0.03% respectively. We can conclude, this assumption was correct.

5.2 Parameters Estimation

The model parameters can have a significant impact on the predicted accuracy of the oxygen and carbon dioxide balances. Three different empirical formulas were used to figure out the value of transfer velocity based on the average wind speed to evaluate the sensibility of the model in this parameter. Transfer velocity was used to estimate the carbon dioxide exchange rate with atmosphere (Equation (3.20)). Table.5, shows the formula and the value of k_e.

Table 5. Estimation of transfer velocity

Formulate	$k_e\ (\frac{cm}{h})$
$2.07 + 0.215\ U_{10}{}^{1.7}$	2.24
$0.72\ U_{10}$	0.62
$0.168 + 0.228\ U^{2.2}$	0.33

The tested results indicated that the changes in transfer velocity may change the carbon dioxide concentration within the raceway by the means of ± 0.01 and has no effect on R-squared value. As a result, the effect of transfer velocity on the dynamic model output is negligible.

5.3 Carbon Dioxide Concentration Range

Increased amounts of $\mathrm{CO_2}$ in the water can reduce fish's ability to excrete $\mathrm{CO_2}$, leading to respiratory acidosis. According to Danley et al. (2005), dissolved $\mathrm{CO_2}$ concentrations of 34.5 ±3.8 mg/L reduced rainbow trout growth compared to 22.1 ± 2.8 mg/L of $\mathrm{CO_2}$. Research shows that freshwater fish can tolerate greater $\mathrm{CO_2}$ concentrations of 100–200 mg/L for a short time, but, the long term consequences of increased $\mathrm{CO_2}$ concentrations on freshwater fish still need to be investigated. Even though, warm water freshwater fish, as opposed to cold water freshwater or marine species, could be more tolerant of elevated $\mathrm{CO_2}$ concentrations (Stiller et al., 2015). According to Loyd and Jordan (1964), around 20 mg/L carbon dioxide proved lethal to rainbow trout (*Oncorhynchus mykiss*), but trout survived up to a threefold quantity in neutral water (Fivelstad, 2013). Additionally, the Norwegian production of Atlantic salmon adheres to a safe carbon dioxide level of 15 mg/L. In this study, the maximum carbon dioxide concentration in the raceway was estimated at 8.9 mg/L which is in the safe range for fish welfare. Also, Stiller et al., 2015, was discovered that carbon dioxide treatment had an influence on the mean individual fish length, breadth, and weight during a short term study, so that, fish maintained at the highest $\mathrm{CO_2}$ concentration (42 mg/L) weighed 38% less than fish maintained at the lowest concentration (5 mg/L).

Moreover, the alkalinity in the inlet and outlet water was constant and equal to 1.5 mmol/L. The studies indicated in the water with high alkalinity (0.6–4 mmol alkalinity), carbon dioxide may have a minor effect on growth of rainbow trout (Smart et al., 1979; Fivelstad et al., 2003b; Good et al., 2010).

6. Conclusion

The identification of a dynamic CO_2 model based on three main processes (fish respiration, influent carbon dioxide concentration, and exchange rate) were accomplished by the 55-day time series of water temperature, DO, and pH data collected during this study. In fact, there were two feeding regimes (feeding and fasting), the transition from a feeding to fasting, altered RQ. value, from 0.82 to 0.7(pure lipid oxidation), which was applied to the model. Another limitation of model can be related to calculation partial pressure in the water body based on temperature and as a key parameter for estimating CO_2 exchange, accurate water P_{CO_2} is required to quantify CO_2 exchange rate with atmosphere. In this case, finding of Ran et al. (2017), indicates that the conventional P_{CO_2} calculation from water characteristic has been criticized for introducing biases when compared to direct measurement. Furthermore, Sobek et al. (2005), investigated the concentration of dissolved organic carbon (DOC) in inland waters predict P_{CO_2} much better than temperature.

As was discussed in the results chapter, the assumption which was applied to the model was confirmed by the test on the model. The water flow rate and transfer velocity had a negligible effect on the raceway's carbon dioxide concentration in this study. The impact of microalgae photosynthesis may have been considered in future studies. In addition, the sensitivity of the model to transfer velocity was evaluated, and the results suggested that this parameter had a negligible impact on the model's output. It seems carbon dioxide has a negligible effect on rainbow trout in high alkalinity water; on the other hand, in aquaculture, even though the estimated carbon dioxide concentration by the model is at a safe level. The maximum carbon dioxide concentration form estimated dynamic model (Fig.4.12) was 8.9 mg/L which is lower than the threshold for rainbow trout welfare (lower than 20 mg/L) (Fivelstad, 2013) and even lower than 10 mg/L (Davidson et al., 2011) . In addition, we used DO data to estimate the carbon dioxide released by fish respiration; consequently, the predicted CO_2 is dependent on DO accuracy and the assumption that only fish use oxygen within the raceway.

In this study, a good agreement was observed between the dynamic carbon dioxide model and the carbon dioxide estimated from output data and the dynamic model produced an appropriate forecast of the daily carbon dioxide oscillation pattern within the raceway. We hope that this experiment will shed new light on the measurement of carbon dioxide concentration and its process in freshwater fish farming, as well as provide the basis for building more precise dynamic models to forecast the oscillation of gases inside the raceway.

7. References

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