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# A Model for Predicting Alkalinity, pH, and $CO_2$ Levels in a Flow-thru or Recirculation Aquaculture System Independent of Salinity

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# A MODEL FOR PREDICTING ALKALINITY, pH, AND $CO_2$ LEVELS IN A FLOW-THRU OR RECIRCULATION AQUACULTURE SYSTEM INDEPENDENT OF SALINITY

By

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# SUMMARY

Carbon dioxide is one of the limiting factors for construction of recirculation aquaculture system in the fish farming today. The level of carbon dioxide in the water heavily depends on fish metabolism and water chemical composition. Carbonate chemistry itself is complicated because carbon dioxide exists in different forms in the water. Alkalinity and pH are the two main factors that are affecting the total available carbon dioxide and its form, which was described by Kenneth S. Deffeyes in the mid-1960s.

Carbon dioxide affects the fish health, behavior and welfare in recirculation aquaculture systems, and it is important to know in every production step how much is produced and how much is spent. The developed model uses fish metabolism contribution and Deffeyes diagram chemistry explanation, to predict levels of carbon dioxide in different components of the RAS system. The model is designed for simple use in a user-friendly Excel sheet and for mobile phone application. The users are not required to understand the complex mathematics and chemistry behind the model to use it effectively. The users need to put values obtained from different measurements on the fish farm into the dedicated boxes in the program.

The model is still in its developing stage, meaning that it needs further testing and more results from fish farms to confirm efficiency and robustness of the model. There are a number of other possibilities for developing and the model can include for example heavy metal precipitations, medicals precipitation or particle removal. With different add-ins, farmers or engineers can predict the maximal capacity of the farm with the inlet values that they get from the water quality, fish size, and the type of the diet for the specific fish category.

The Excel sheet and an online mobile application allow users to get accurate predictions with a single click, without fear that it will affect the fish health in the running system. The results obtained by the application simulation can then be used for fine-tuning of the equipment installed on the fish farm and to optimise regarding fish performance and the used feed.

*This thesis is dedicated to my wife and daughter, my parents, siblings and friends for all support and love they gave me during my master studies in Norway*

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# 1. INTRODUCTION

Fishes are vertebrates specially adapted to live in the water. As a part of a vertebrate taxon, fishes are the largest and most diverse group. Despite that fact, most of the fish species are using specialised breathing organ called gills. Some of the species adapted to specific conditions without enough oxygen and can use swim bladder or lungs as a secondary breathing organ.

Gills are highly vascularized organs with a high surface area of epithelium which provides a thin layer of defence between blood and aquatic environment. Besides breathing, gills are regulating excretion of nitrogenous products made during the metabolism and the salt level in seawater species (Evans et al., 2005; Roberts, 2012). The thickness of the epithelium is minimal to enable the fast gas exchange and products of metabolism. The thin epithelium is very vulnerable to outside pathogens. A small change in its composition can lead to disease or osmoregulation problems (Roberts, 2012).

The respiratory function of the gills is to obtain and meet the requirements of oxygen for cells and to remove produced carbon dioxide. The necessity of fish organism and metabolism is to react and reply fast to the changes in the environment or in the cell itself. Chemoreceptors, which are by abundant evidence situated in the gills, can register the changes of $CO_2$, $O_2$, and pH in the environment and adjust cardiorespiratory rhythm to maintain the physiological state inside the organism (Hara & Zielinski, 2007).

Research and history of fish ventilation begin in the early 1700s and develops until today with the most significant contribution of George M. Hughes, who is known as a scientist who started a new era in the fish respiratory science. A particularly important year was 1960 when Hughes presented a biomechanical model of fish breathing (Figure 2-5). Hughes also made a significant work in presenting gill morphometrics in a relation of a ventilatory stream, and a diffusion role of oxygen in gas exchange (Wegner & Graham, 2010).

"*Hughes legacy is thus as a leading figure in comparative animal respiration who brought considerable insight into the comparative mechanics of fish ventilation, the relationship between*

*gill morphometrics and natural history, and the modelling of respiratory gas transfer*"(Wegner & Graham, 2010).

Fish in a closed recirculation aquaculture system lives in an insufficient amount of space. Every factor must be controlled because of a high degree of recirculation which can go up to 99%. The amount of space and high degree of recirculation are affecting the fish health. It is important to understand the fish biology, anatomy, and fish environmental demands to construct healthy environment for living. In the nature, fishes have physiological mechanisms to avoid places with high concentrations of carbon dioxide (Clingerman et al., 2007). In the RAS the carbon dioxide level must be regulated to meet the certain level. Amount of carbon dioxide is set to 15mg/l by Norwegian authorities – Mattilsynet. However, for water with low alkalinity some authors propose even lower level of 10 mg/l (Fivelstad, 2013).

Carbon dioxide is considered as the limiting factor in present projecting of the complete RAS systems (Gaumet, 2018). Control and measurement of carbon dioxide are challenging. Carbon dioxide exists in different forms in the water and forms are determined with water alkalinity, pH, and temperature. Fish contributes to the carbon dioxide production by its activity inside the tank, and this can be calculated using different mathematical models (Forsberg, 1993; Sanni & Forsberg, 1996; Sanni, 1993). The amount of produced carbon dioxide can be predicted by the amount of oxygen spent in fish metabolism (Timmons, 2010). Biological filter contributes to the spending of alkalinity (Ebeling et al., 2006) and 5% degassing of the carbon dioxide because of the enormous surface area (Gaumet, 2018). At the end is the carbon dioxide stripper which takes one part of the carbon dioxide out and the whole process begins again.

The first goal of this master thesis is to explain the way of carbon dioxide production in fish and its behaviour in water. Second is to develop a well-organised and straightforward tool which can predict the amounts of carbon dioxide produced by fish and predict the carbon dioxide form and quantity in the water according to alkalinity and pH.

# 2. LITERATURE REVIEW

## 2.1. Biology and anatomy of the teleost fish gills

The teleost fish group is the most diverse and the most advanced of the bony fishes. Teleosts include virtually all sport and commercially important fishes. Teleost fish gills consist of four respective pairs of gill arches, which are forming the sides of the larynx (throat). From gill arches, many gill filaments are rising, and every arch has two branches of filaments. Filaments are projecting from a posterior edge of the gill arch in such a way that the free tips are almost touching themselves. On the filaments, secondary structures called lamella are positioned, where the gas exchange takes place. The lamella is placed on both sides of the filament, making the surface area bigger for gas exchange. Water is forced to go thru the lamellar system (Figure 2-1) because of its specific positioning (Evans et al., 2013; Roberts, 2012).

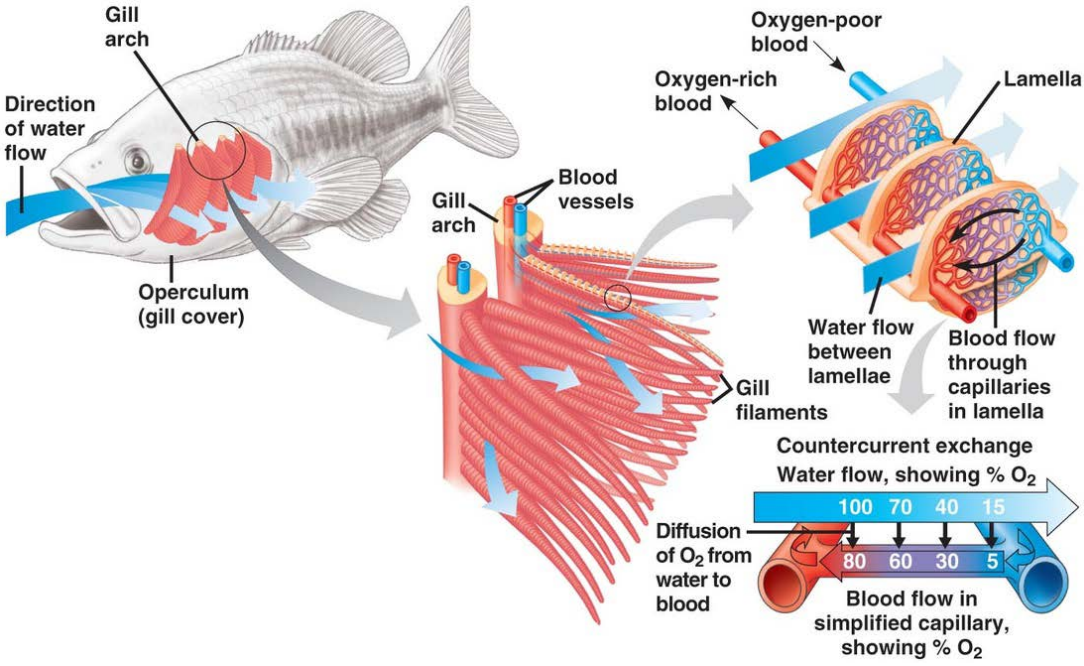



*Figure 2-1 Schematic gills of teleost fish(Campbell & Reece, 2002)*

Filaments and lamella are covered with very thin epithelium which consists of several layers of cell types making a membrane that separates intracellular space from extracellular fluids (Evans et al., 2013).

Gill arch of teleost fishes is a curved structure from which the filaments extend with structural osseous support. Ventral aorta, main blood supplier of the gills, divides into many fine branches when it enters the lamella to extend the contact area with the bloodstream. The afferent arteries are continuing along the opercular edge of the filament, and they go downstream of the water flow. Blood from the filament is entering to lamella by small afferent lamellar arteries. In lamella, the blood then flows in the opposite way of the water flow and creates the counter-current exchange between water and blood (Figure 2-1). The counter-current exchange is a very efficient system which enables the oxygen exchange rate to be from 60-80% of the total oxygen in the water transferred to the blood. Oxygenated blood goes then to the efferent lamellar arteries and dorsal aorta, and then distributes to the body tissues. In lamella, there is a shunt to take some of the oxygenated blood to the nutritive circuit of the gills itself (Roberts, 2012).

The lamella (Figure 2-2) is the primary place and most important for gas exchange. Each lamella consists of two epithelial layers separated by pillar cells (Figure 2-3). Pillar cells are keeping the shape of the lamella, together with collagen fibres (Hughes, 1980).

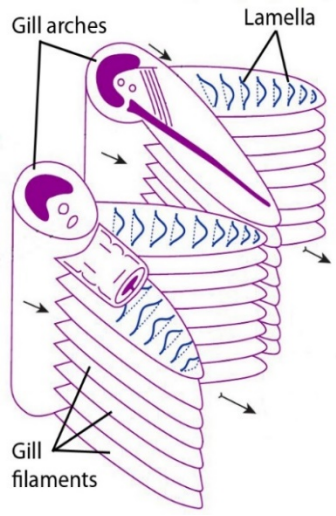



*Figure 2-2 Macroscopic schematic view gill filaments with lamella – arrows on the schema are presenting the water flow from (Roberts, 2012)*

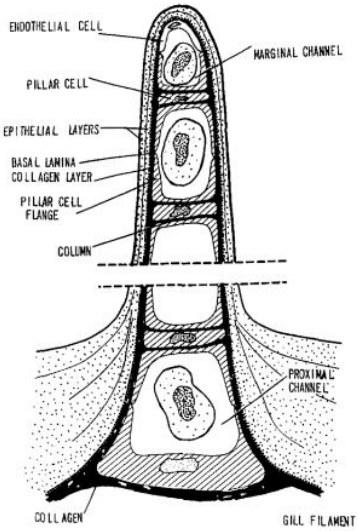



*Figure 2-3 Microscopic schematic view – Transverse section view through the lamella of teleost showing the basic organization of layers; adapted from (Hughes, 1980)*

Teleost specific epidermis tissue covers the gill arch. The lamella surface has a very thick layer of epithelium, enriched with mucous cells. Under the layer of epithelium, the layer of lymphocytes is a part of the defending (immunological) system. Saltwater species in lamella have the chlorine cells which are essential for life in the saltwater conditions (Evans et al., 2013; Roberts, 2012).

In the lamella, the bloodstream is passing through channels filled with red blood cells. The number and the shape of the lamella itself vary among fish species, and it is dependent on fish behavioural habits (Evans et al., 2013; Hoar & Randall, 1984; Hughes, 1966).

## 2.2.Respiration path from mouth to opercula

### 2.2.1. Gill ventilation – ventilatory mechanics

Gill ventilation defined as ventilatory mechanics is the best way to describe the path of water entering the mouth and exiting in the gill opercula.

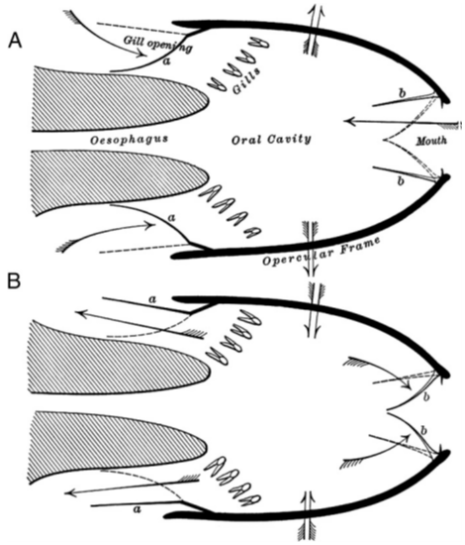



*Figure 2-4 Schematic picture of fish gill ventilation - picture shows inspiratory (A) and expiratory phase (B)(Dahlgren, 1898). Looking from above, the double arrows show the movement of the opercular frame. Single arrows are the water movements in the cavities. Opercula and mouth are presented as valves (a) and (b)(Wegner & Graham, 2010)*

According to Wegner and Graham (2010), an essential year for a fish respiratory explanation was 1960 when Hughes showed the present used biomechanical model driving fish ventilation, and showed that both bony and cartilaginous fishes breathe through the use of a dual-pump mechanism:

1) a buccal or orobranchial pressure pump to force water over the gills and
2) an opercular or parabrachial suction pump to pull water through the branchial chambers'' (Wegner & Graham, 2010).

The ventilatory mechanism consists of four stages (Hughes, 1960b). The original experiment of Hughes (Hughes, 1960a) showed that the pressure in the buccal cavity is always higher than the one in the opercular cavity which provides the water flow from higher to lower pressure (Hughes, 1960b; Wegner & Graham, 2010). Hughes was using the schema (Figure 2-4) from Dahlgren, as a beginning point to understand how water flows thru cavities. Using approaches from Dahlgren, Hughes gave a more detailed explanation of breathing chambers and constructed a model which is used today to explain the four stages of the ventilation process (Figure 2-5). This process is known is a dual – pump (Hughes, 1960b).

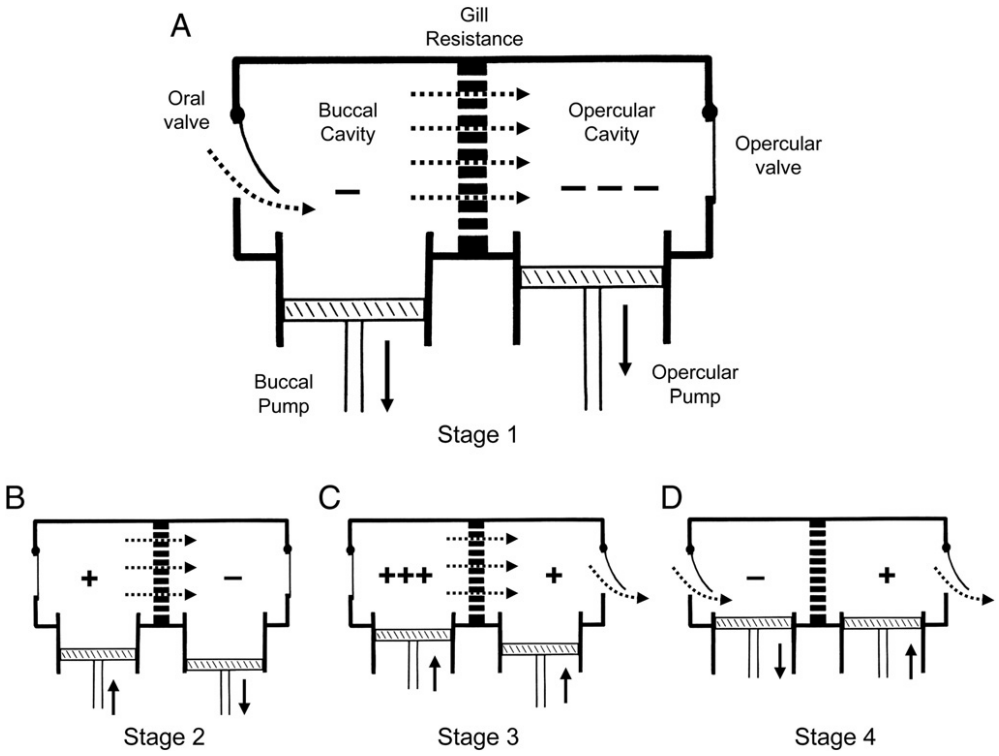



*Figure 2-5 Simplified schematic of dual – pump described and illustrated by Hughes (1960a). The picture presents positive and negative pressures and the water flow from mouth thru gills and out thru opercula. Water flow in the 4th stage is not given because it can vary in some fish species*

In the first step, water is entering the buccal cavity by the suction. The opercular valve closes, and water is flowing from buccal cavity thru gills and to the opercular cavity. In the second stage, the mouth closes, the buccal pump is changing from suction to force pump which increases the pressure and moves water again towards the opercular cavity. The third stage has both force pumps, and the opercula opened. The fourth step is starting with both valves, mouth and opercula, opened, and in this stage in some fish species, the flow can go backward, like in Largemouth bass, *Micropterus salmoides* (Lauder, 1984), or the Corkwing wrasse, *Crenilabrus melops* (Hughes, 1960a).

In Figure 2-5, in the 4$^{th}$ stage, the water flow is not presented because it can be specific for some fish species. From a general point of view, the water flow is in most fishes shown as a continuous flow (Hughes, 1960b). However, some fish species are using so-called RAM ventilation. During ram ventilation, both valves, mouth, and opercula, are opened. By moving thru the water, when the fish gain enough speed, the pressure in the buccal cavity raises, and it does not need a buccal and opercular pump to work (Hughes, 1960a). This moving water thru the gills occurs in many pelagic fishes, i.e., tuna species (Hoar & Randall, 1984).

Energy consumption for the ventilatory mechanism was elaborated in several studies and consensus decision is that 5-10% of all energy produced, is used for gill ventilation (Holeton, 1980; Hughes, 1973; Jones & Schwarzfeld, 1974). Similar values of energy are also used for the cardiac pump which is moving blood from the heart, thru gills and to the organs (Hoar & Randall, 1984).

### 2.2.2. Gill specific mechanics – gill morphometry

Morphometry is a term used to describe dimensions of structures in living organisms. Structural entities in live organisms are not easy to measure because it is very time-consuming. Before starting any measurements, it is vital to choose most representative measurements for the particular analysis. It is always good to consult physics scientists and then select which measures should be done.

Many measurements are available for the gills, but gill mechanics uses two main ones in describing gill morphometry. Those are the resistance of the water flow and blood through the gill system, and the second is defining the resistance made by cells and tissues that are on the way of gas exchange (Hoar & Randall, 1984).

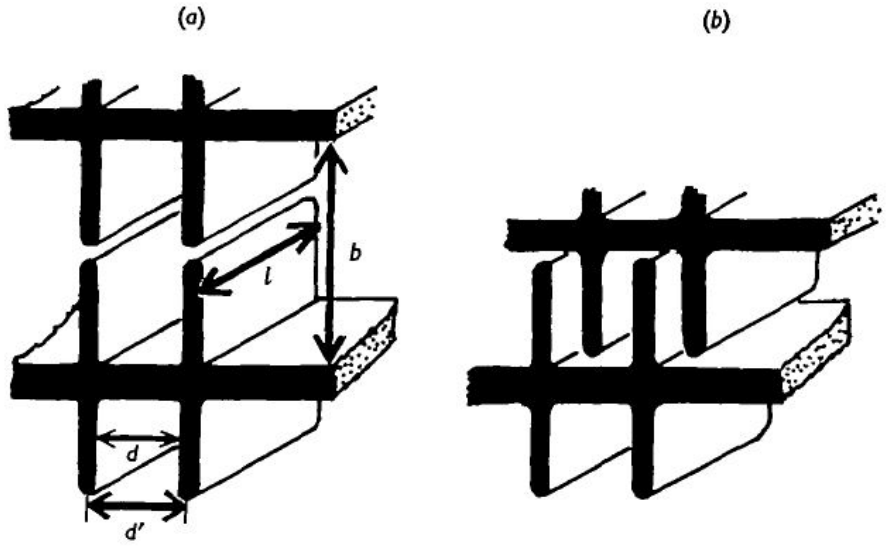



*Figure 2-6 Diagram of the lamellae attached to two filaments of the same gill arch that are used for showing dimensions for the modified Poiseuille equation. In(a) lamellae are shown apart which is presuming in most fishes, and (b) minimum possible size as a result of the close interdigitation of the lamellae (Hughes, 1966)*

Flow-thru of the water and blood in the gill system takes place in relatively narrow spaces, which affects the flow characteristics in connection between pressure difference and flow (Figure 2-6). The flow should not be too slow or too fast to obtain the adequate gas exchange in the gills. The flow-thru the gills is laminar with very low (<10) Reynolds number (Hoar & Randall, 1984). Water flow is presented and calculated by Poiseuille equation. Poiseuille equation is for laminar

flow in the cylindrical tube, but Hughes (1966) adapted the equation for using it in a rectangular cross-section,

$$q = \frac{P_1 - P_2}{\eta} \, x \frac{5d^3 b}{24l}, \qquad (2.1)$$

where $q$ is a flow thru rectangular tube of length $l$, the width $d$, and height $b$. $P_1 - P_2$ is the difference between pressures, and $\eta$ is the water viscosity.

Hughes also gave an expectation of another model of gills which was not found, but scientifically possible, with lamella that is very close to each other (Figure 2-6 b).

Scanning the trout gills with an electron microscope (Figure 2-7), it was discovered that there are many microvilli and micro ridges on the surface of a lamella. That discovered structure can create turbulent water movements in the area of the tissue (Figure 2-7 B). There is no answer, are those structures involved in hydrodynamics, but it is more likely that the spaces between are

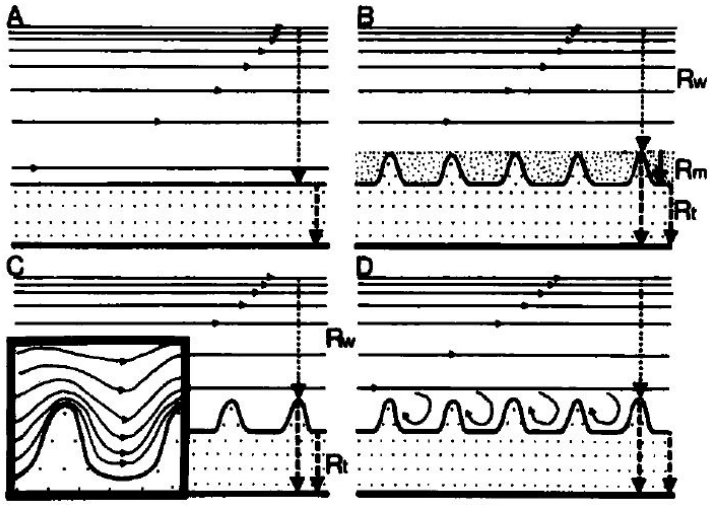



*Figure 2-7 Schematic diagram to indicate possible relationships between water flow and the surface of a lamella. A – Laminar flow across a flat lamellar surface is showing that the two primary resistances to gas transfer are one from the water and the other from the lamellar tissue. B – laminar flow across microvilli filled with mucus. In this example, three resistances are present from water, tissue, and mucus. C – slow laminar flow over microstructures with low Reynolds number and no turbulent flow. D – laminar flow with microturbulent between microstructures on the surface of the lamella (Hughes, 1979)*

filled with the mucus which is providing a smooth water flow (Hughes & Wright, 1970; Lewis & Potter, 1976).

### 2.2.3. The resistance of different components for gas transfer from water to blood

The gasses must pass several components and barriers (Figure 2-8). Gasses from the water are moving thru the multiple layers of tissue, entering the blood, and into the red blood cell. The red blood cell is the location where the gas transfer takes place. As mentioned before, the red blood cells in lamella are the place of exchanging oxygen for carbon dioxide.

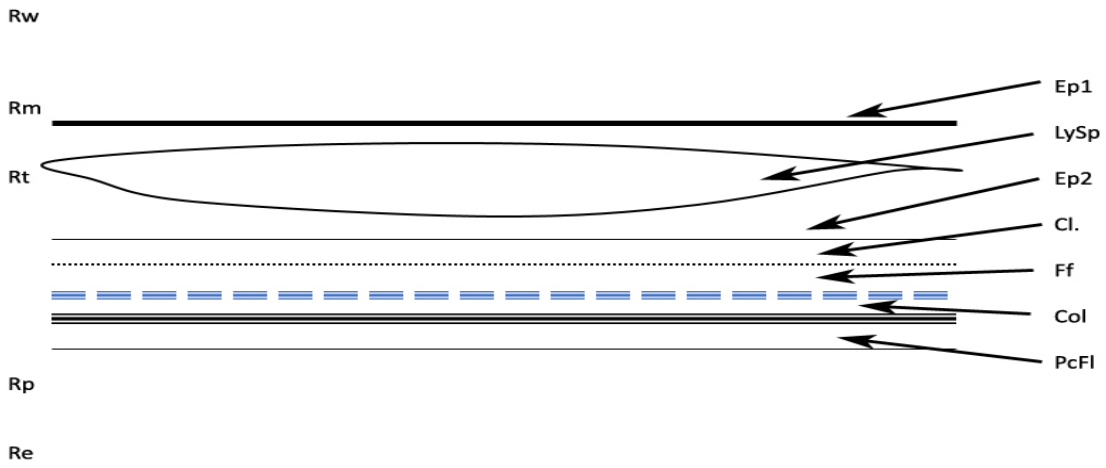



*Figure 2-8 Diagram is showing different components of the resistance to gas transfer from water to blood in lamella of the fish. Rw – water, Rm – mucus layer, Rt – tissue layer, Rp- plasma and within the Re – erythrocyte. Rt – tissue resistance has seven layers. Ep1 and Ep2 for epithelial layers, which can be separated by LySp – lymphoid spaces. The basement membrane is composed of Cl – clear layer, middle Ff – fine fibrous layer and Col – inner collagen layer. The outer two Cl and Ff are forming basal lamina. The innermost layer of tissue is formed by a flange of the PcFl – flange of the pillar cell (Hughes, 1972).*

Diffusing rate can be evaluated knowing which components are included. Diffusion capacity is the capability of gas exchange organ to transmit gasses, and it is the gas volume ratio transferred thru the barrier in the unit of time to the mean partial pressure difference of that gas in two sides of the boundary. This diffusion capacity is directly proportional to the surface of the area and inversely proportional to the thickness. That gives the formula (eq. 2.2) for oxygen diffusing capacity in the gills:

$$D_g = \dot{V}_{O_2} \Big/ \Delta P_{O_2}, \qquad (2.2)$$

where $D_g$ is diffusing capacity, $\dot{V}_{O_2}$- is the rate of the oxygen taken with the gills, $\Delta P_{O_2}$- the difference in partial pressures outside and inside of the gill tissue.

The transfer of oxygen from the gills to the haemoglobin molecule involves several stages of diffusion (eq. 2.3),

$$1/D_g = 1/D_w + 1/D_m + 1/D_t + 1/D_p + 1/D_{e,} \qquad (2.3)$$

where the subscripts are for gill diffusing capacity($g$), water($w$), a mucus layer($m$), tissue($t$), plasma($p$) and erythrocyte($e$), respectively (Hughes, 1984).

## 2.3. Gas exchange in the lamella

### 2.3.1. Introduction

Gas exchange of $O_2$ and $CO_2$ in blood is a complex mechanism that consists of diffusion, mixing and chemical reactions. Some processes are coinciding, some are in parallel, but none of the operations can be isolated on its own because a slight change in only one reaction can change the whole result of the exchange process (Perry & Tufts, 1998).

### 2.3.2. Oxygen transport from water to the tissues

Oxygen solubility in the water is only 1/30 compared to the air, and the rate of diffusion compared to the air is 1/10000 (Perry & Tufts, 1998). The oxygen consumption of fish is doubling

for every 10° of temperature increase in water (Brett & Glass, 1973). The exercise of the fish at the optimal temperature can increase the oxygen consumption by more than ten times. Uptake of the oxygen must be very efficient because of facts mentioned, and it goes thru several steps (Perry & Tufts, 1998):

- Breathing movements
- Oxygen diffusion from water to the organs respiratory capillaries
- Binding with haemoglobin
- The oxygen transport from the gills to the target tissues – consumption sites
- Disconnecting from haemoglobin
- Distribution of the oxygen to the cells, mainly to the mitochondria (cell organ) where the cell respiration happens

The gas exchange from water to red blood cell starts in the mouth, where the oxygenated water enters the system by the breathing movements described in detail by Hughes (1960a) and (1960b). Then, the oxygenated water goes thru the gills and diffuses down its partial pressure gradient from water to the capillaries. Water goes over the gills in blood countercurrent exchange system, which makes this system very efficient. When the oxygen enters the blood, it goes to the red blood cell (RBC), where he binds with haemoglobin (2.4) and makes a structure called oxyhemoglobin (Figure 2-9). The amount of connected oxygen molecules depends on the number of red blood cells, the haemoglobin concentration within the red blood cell, and the oxygen binding properties of the haemoglobin molecule itself. Haemoglobin is a quaternary protein, which means that the protein has four spots for oxygen binding and its transfer to the target cells. Oxygen partial pressure in tissue capillaries is lower than the pressure inside the transporting vessels and oxygen disconnects from haemoglobin. When released from haemoglobin it goes to the mitochondria which are the target cell organs (Perry & Tufts 1998).

$$Hb + O_2 \rightarrow HbO_2\ (Oxyhaemoglobin) \qquad (2.4)$$

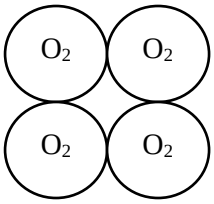



*Figure 2-9 $HbO_2$ - Oxyhaemoglobin*

### 2.3.3. Carbon dioxide transport from tissues to the water

In the tissue capillaries, the metabolically produced carbon dioxide ($CO_2$) is entering the series of chemical reactions. Carbon dioxide is hydrated to carbonic acid, eq.(2.5), and then to hydrogen ($H^+$) and bicarbonate ion ($HCO_3^-$) eq.(2.6).

$$CO_2 + H_2O \xleftrightarrow{Carbonic\ anhydrase} H^+ + HCO_3^- \qquad (2.5)$$

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \qquad (2.6)$$

At a physiological blood pH, which is between 7.4 and 8.1, the chemical reaction will be in favour to produce $H^+$ and $HCO_3^-$ ions (Figure 2-11). The initial step in the transport of $CO_2$ from the tissues to the water is diffusion to the plasma of interstitial fluid and the red blood cell (Henry et al., 1997; Perry & Tufts, 1998).

In trout and another teleosts presumably, the reaction from $CO_2$ to $HCO_3^-$ and $H^+$ catalysed by carbonic anhydrase only happens in the red blood cell. Some authors discovered that there is also an activity in the blood plasma of skeletal muscle in rainbow trout, *Oncorhynchus mykiss* (Henry et al., 1997). They also realised that production of $H^+$ ions in the interstitium is creating $NH_4^+$ ions and facilitating ammonia ($NH_3$) diffusion from the tissue by sustaining the $pNH_3$ gradient (Figure 2-10).

Enzyme carbonic anhydrase (CA) catalyses the carbon dioxide dissociation. Uncatalyzed dissociation reaction is fast itself with the speed of $3.5x10^{-2}$ $s^{-1}$ (Edsall, 1968). In dehydration, the rate of the reaction is quite slow, approximately 20 $s^{-1}$ (Edsall, 1968). With carbonic anhydrase in the reaction, the reaction happens almost instantly.

$$H^+ + HCO_3^- \leftrightarrow H_2CO_3 \xleftrightarrow{Carbonic\ anhydrase} CO_2 + H_2O \qquad (2.7)$$

The $HCO_3^-$ ions produced in $CO_2$ reactions are removed from the red blood cell by electroneutral $Cl^-/HCO_3^-$ exchange. When the blood arrives at the gill, it contains carbon dioxide in the form of $HCO_3^-$ in plasma, and $H^+$ ion connected to the haemoglobin. By chloride shift, $HCO_3^-$ is changed for $Cl^-$ again, and inside the red blood cell $HCO_3^-$ dehydrates to the $CO_2$. Carbon dioxide is excreted into the water from the gills by diffusion and in a ratio that supports its production (Perry & Tufts, 1998).

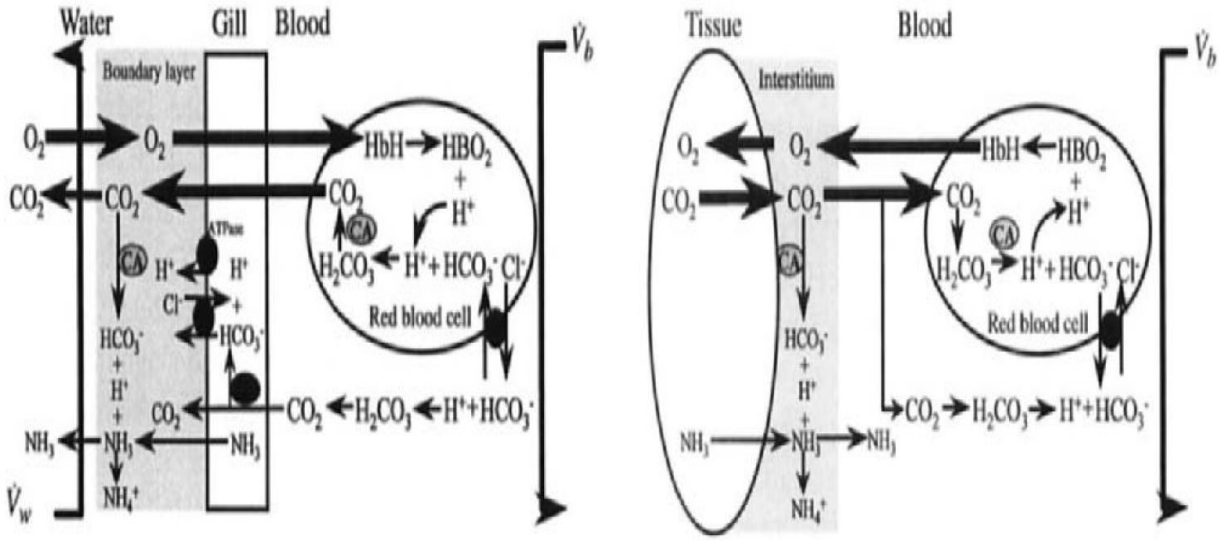



*Figure 2-10 A schematic model depicting the movements of carbon dioxide, oxygen, and ammonia between the blood, water, and tissue in teleost fish. CA for carbonic anhydrase; Vw - water flow; Vb – blood flow; From (Perry & Tufts, 1998)*

## 2.4. Physiological aspects of free $CO_2$ in water. Low pH and use of $CO_2$ as an anaesthetic. Carbon dioxide as a stunning medium

### 2.4.1. Too low pH, acidic water, and connection to carbonates

Most of the water that is coming from well or spring sources is influenced by dissolved carbonates and carbon dioxide and has pH between 5 and 8. Groundwater can be low in buffering capacity because of the contact with silicate minerals, low pH, and a significant amount of $CO_2$ (up to 100mg/l). On the other side, the water that comes from carbonate substrates, passing thru limestone (sediment rock composed mainly from calcium-carbonate($CaCO_3$) as mineral calcite), has an excellent buffering capacity and pH closer to 8 but can cause the problems with calcium precipitation in the equipment. Acid sulfate soils are deficient in pH, less than 4. Acid rains are also the source of low pH, which can influence the overall pH of the water resource (Boyd, 1990). A secondary low pH source in water is the metabolic activity of fishes and other aquatic organisms. In a closed system, this action is happening slowly, but it can turn the system to pH=5.5, which is lethal if not maintained on time (Noga, 2011).

Low pH can affect some other factors as ammonia and aluminium and make them toxic in specific pH level (Lekang, 2013; Timmons, 2010). Low pH is also changing the composition of carbonates in the water which leads to more $CO_2$ in water according to Bjerrum plot (Figure 2-11). Accumulated $CO_2$ in the water can produce fish disease, hypercarbia (later described).

Water saturated only with $CO_2$ has the lowest pH of 5.6. If the pH value less than 5.6, some other components are contributing (Noga, 2011).

Acute low pH leads to dyspnoea - breathing problems, tremors – fast, involuntary muscle moves, and hyperactivity, and eventually the death of fishes. The critical stress response is present when the pH lowers down off the fish tolerable boundaries. In chronical exposure to high pH, mucus production increases and chronic stress response as a first mechanism of defence (Noga, 2011). Both of those two situations can be seen when $CO_2$ is used as a stunning media in fish maintenance (Gräns et al., 2016).

### 2.4.2. Hypercarbia

Hypercarbia is a fish disease caused by the high amount of dissolved $CO_2$ in water. Carbon dioxide solubility is very high in water, and it can somewhat exceed the atmospheric concentrations. Causes of hypercarbia are overcrowding in combination with high oxygen usage, and poorly buffered groundwater (high in $CO_2$, up to 100 mg/l, and low in pH) (Noga, 2011). Increase in $CO_2$ in water is inhibiting the diffusion rate of $CO_2$ from the blood, because the concentration between the two is changed. If the concentration of $CO_2$ is higher in the water, it will enter the cells and disturb the blood acid-base balance. High $CO_2$ concentration lowers the pH of the blood. The result is decreased affinity of the haemoglobin for oxygen – Bohr effect, and reduced oxygen-carrying capacity – Root effect (Noga, 2011; Perry & Tufts, 1998). Both effects are reducing the amount of oxygen that diffuses to the tissues. Typically for salmonids exposed to the low pH in the water caused by high $CO_2$ is *Nephrocalcinosis* and systemic granuloma - a multifocal deposition of white mineral in the epaxial muscles (muscles associated with ribs and vertebrae), kidneys, and stomach (Noga, 2011).

In salmonids, the concentration of approximately 20mg/l is affecting the fish health (Fivelstad, 2013; Wedemeyer, 1996). Some warm water species, i.e., Channel catfish - *Ictalurus punctatus*, can temporarily handle the concentration of 50mg/l and more if dissolved oxygen is near to the saturation level 10-14ppm (Tucker, 2013). Some fish species, i.e., Rainbow trout, have the mechanisms to avoid the high level (60-120mg/l) of $CO_2$. Clingerman et al. (2007) used this behaviour to transfer fishes from one tank to another. They stated that 99% of fishes voluntarily swim from the tank with high $CO_2$ concentration to tank with low $CO_2$ concentration(<15mg/l) and with oxygen saturation between 110 and 130% (Clingerman et al., 2007).

### 2.4.3. Anaesthesia and stunning with $CO_2$

In fish management, transport and handling easily stress fish, which can cause a significant stress response or even death. Anaesthetics in aquaculture can be used for fish transport to prevent

injuries and to reduce organism metabolic rate, for fish handling during harvesting, sampling or spawning procedures (Shawn D. Coyle, 2004). A wide range of anaesthetics are available, but the economic safety and regulatory considerations are discussed and especially the regulatory considerations which are pointing to some inappropriate use in fish for food.

A suitable alternative for synthetic anaesthetics firstly described by Fish (1943) is carbon dioxide gas. The gas was used as an anaesthetic because it is affordable and safe for use if used in the adequately ventilated room. It is good also as a stunning media because $CO_2$ is not leaving toxic residues in the fish tissues (Bernier & Randall, 1998). Negative sides of $CO_2$ usage as a stunning medium are that loss of consciousness is not immediate, and fish makes aversive movements and the stress response (Benson, 2004; Gräns et al., 2016). In salmonids, deep anaesthesia cannot be reached, and the stress indicators levels in the blood, cortisol, and adrenaline achieve high concentrations (Bernier & Randall, 1998).

Stunning of fish with $CO_2$ was a method of choice because it is affordable and no implications for the meat quality after slaughtering process, but with negative stress response reaction. One of the strategies of European Commission is to implicate animal welfare and especially the animal welfare of animals-fish kept in aquaculture systems (Commission, 2012). A study by Gräns et al. (2016) is questioning abandoning of $CO_2$ stunning because of implication to the fish health and welfar. According to the study, the stunning with electricity, as a stunning leading method in slathering lines for fish in EEA (European Economic Area), is making the higher response of cortisol level than $CO_2$. Cortisol is the primary hormone used for presenting stress reaction. The explanation for this cortisol level they found in similar mammal reaction to electrical stunning, where electricity triggers cortisol releasing from deposits in chromaffin cells.

The experiment conducted on Arctic char showed that $CO_2$ has harmful properties. Fishes tried to escape, and after 10 min of exposure the recovery was impossible, and all fish died. When the electricity is used for stunning, some fishes can only be tetanus-like immobile but fully conscious, meaning that they feel everything and cannot react. The reverse to full equilibrium reached after 7 minutes with electrical stunning. The experiment suggests that the use only of electricity and abandoning of $CO_2$ as a stunning medium should be investigated a bit more to find

a better and more secure way to obtain a good solution for fish health and welfare (Gräns et al., 2016).

In the study Kugino et al. (2016) gave a proposition that can lead to the use of $CO_2$ anaesthesia again, controlling it with oxygen nano-bubbles which are more dilute in water and could stay longer before they are “equilibrated” with the air. Experiment was hold on fish Chicken grunt - *Parapristipoma trilineatum*. The fish handled anaesthesia for 22 hours with nano-bubbles oxygen supply. The experiment fish fully recovered after 2-3 hours when they stopped the anaesthesia, and 24 hours later no abnormalities found. For study preparation, they used $CO_2$ gass with 100% saturation oxygen supply without nano-bubbles in one group of fishes. In that case, all of the fishes died in 25 minutes. The data about sudden is missing because the water was always supplied with 100% oxygen saturaion. Fishes and other vertebrates that are living in the water are adapted to near the 100% saturation. If the heart rhythm and gas exchange is more lowered down by anaesthesia, the normal oxygenation is not enough. The supply of the water with nano-bubbles oxygen is creating high pressure oxygen bubbles for efficient diffusion across the fish gills during carbon dioxide induced anaesthesia (Kugino et al., 2016).

## 2.5. Carbon dioxide in water

### 2.5.1. Carbon dioxide cycle

Carbon dioxide is circulating in nature. Amount of $CO_2$ in the atmosphere is different, and it depends on biological and human activity. The average amount of $CO_2$ in the whole air volume is 0,035%. In the water, $CO_2$ is about 200 times more soluble than oxygen, and that depends mainly on water temperature and pressure. Carbon dioxide dissolves better in colder water, and with the pressure increase, the solubility of carbon dioxide increases too. Carbon dioxide is essential for living, and it is one of the most important components which is determining the water composition. In the reaction with water, one part of $CO_2$ is forming carbonic acid – $H_2CO_3$. Carbonic acid

dissolves calcium-rich stone, forming calcium bicarbonate $CaCO_3$, which is soluble in the water and it dissociates to calcium and carbonate ions:

$$CaCO_3 = Ca^+ + CO_3^{2-} \qquad (2.8)$$

This trait is often used to maintain and raise the pH of the water with the addition of $CaCO_3$.

Carbon dioxide origins in the water can be from inorganic and organic sources. Production of inorganic $CO_2$ is often very complicated, and it happens in geochemical and magmatic processes in the earth ground. Tiny amounts of $CO_2$ in the water are from the atmosphere. Organic origins are from different biochemical processes which are taking place in the water.

In nature several processes happen at the same time and affect lowering down of $CO_2$ concentration in the water:

- Diffusion from over-saturated water to the atmosphere
- Using $CO_2$ to convert carbonates to bicarbonates
- Photosynthesis

Photosynthesis and respiration are the main factors which are influencing the amount of $CO_2$ in the surface waters. Water pH is not affected until there is a balance between carbonate and bicarbonate ions. Adding $H^+$ ions neutralise $OH^-$ ions formed by dissolution of bicarbonates and carbonates and creates molecules of water. These molecules will form with carbonates $OH^-$ ions in excess until the reserves of carbonates are not spent. That is the reason that the pH is not changing until the carbonates and bicarbonates are present.

Amount of $CO_2$ in water variates from several milligrams to several grams in a litre. The smallest amounts are in the sea water, and the biggest is in ground waters. In rivers and lakes, the level of $CO_2$ is between 20 and 30mg/l.

In recirculating aquaculture systems, carbon dioxide is added by fish breathing and microbial activity. Several parameters need to be checked out regularly in closed systems like pH level, alkalinity or $CO_2$ level (Stumm & Morgan, 2012; Timmons, 2010; Wetzel, 2001).

### 2.5.2. Carbon dioxide chemistry in water – Carbon dioxide equilibrium

Carbon dioxide is a part of a carbonate system which includes several components and chemical reactions (Stumm & Morgan, 2012):

$$CO_2(gas) \Leftrightarrow CO_2(aq) + H_2O \Leftrightarrow H_2CO_3 \quad (2.9)$$

$$H_2CO_3 \Leftrightarrow H^+ + HCO_3^- \quad (2.10)$$

$$HCO_3^- \Leftrightarrow H^+ + CO_3^{2-} \quad (2.11)$$

Carbon dioxide dissolves in water, and forms a chemical reaction with water and builds carbonic acid-$H_2CO_3$ eq. (2.9). Carbonic acid dissolves then in two ions, $H^+$ ion and bicarbonic ion-$HCO_3^-$, eq.(2.10). Carbonic ion dissolves again in a carbonate ion - $CO_3^{2-}$, and another $H^+$ ion, eq. (2.11). Every one of these reactions is guided by pH. Bjerrum plot (Figure 2-11) explains these relations in different pH conditions:

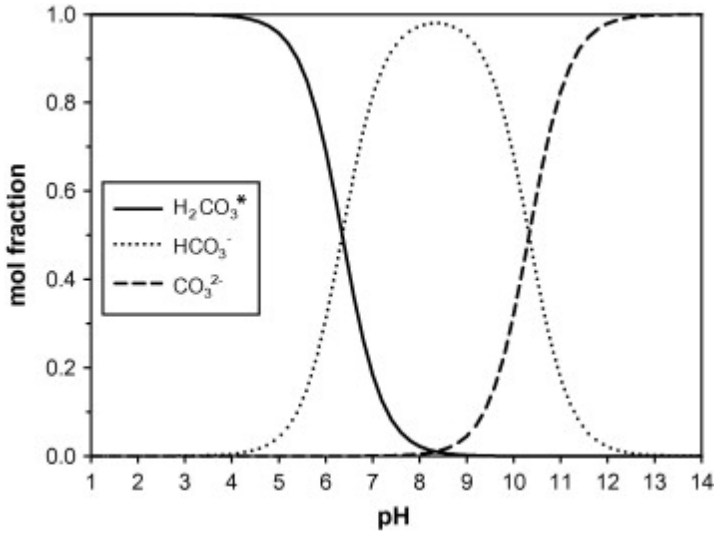



*Figure 2-11 Bjerrum plot of equilibrium distribution of $H_2CO_3$, $HCO_3^-$ and $CO_3^{2-}$*

As a dissolved gas, carbon dioxide exists in aqueous and its hydrated form carbonic acid. The ratio of these two components $CO_2(aq)/H_2CO_3$ is 848 to 1, at 25°C (Soli & Byrne, 2002), meaning that

the most are like carbon dioxide aqueous. Carbon dioxide in aqueous form is very difficult to distinguish with carbonic acid in a solution, so it is better to present it as a sum of carbon dioxide gas in water-$CO_2$(aq) and carbonic acid by the single equation - $H_2CO_3$* (Colt et al., 2012).

### 2.5.3. Alkalinity

Alkalinity is an important parameter to look for because it can affect the water capability to handle the change in pH. The alkalinity is defined as a water capacity to neutralise acids. The usual way of presenting alkalinity is in mg/l of $CaCO_3$ (Clesceri et al., 1998). Alkalinity in water comes from mineral bicarbonates – $HCO_3^-$, carbonates – $CO_3^{2-}$, and hydroxides – $OH^-$ (Stumm & Morgan, 2012):

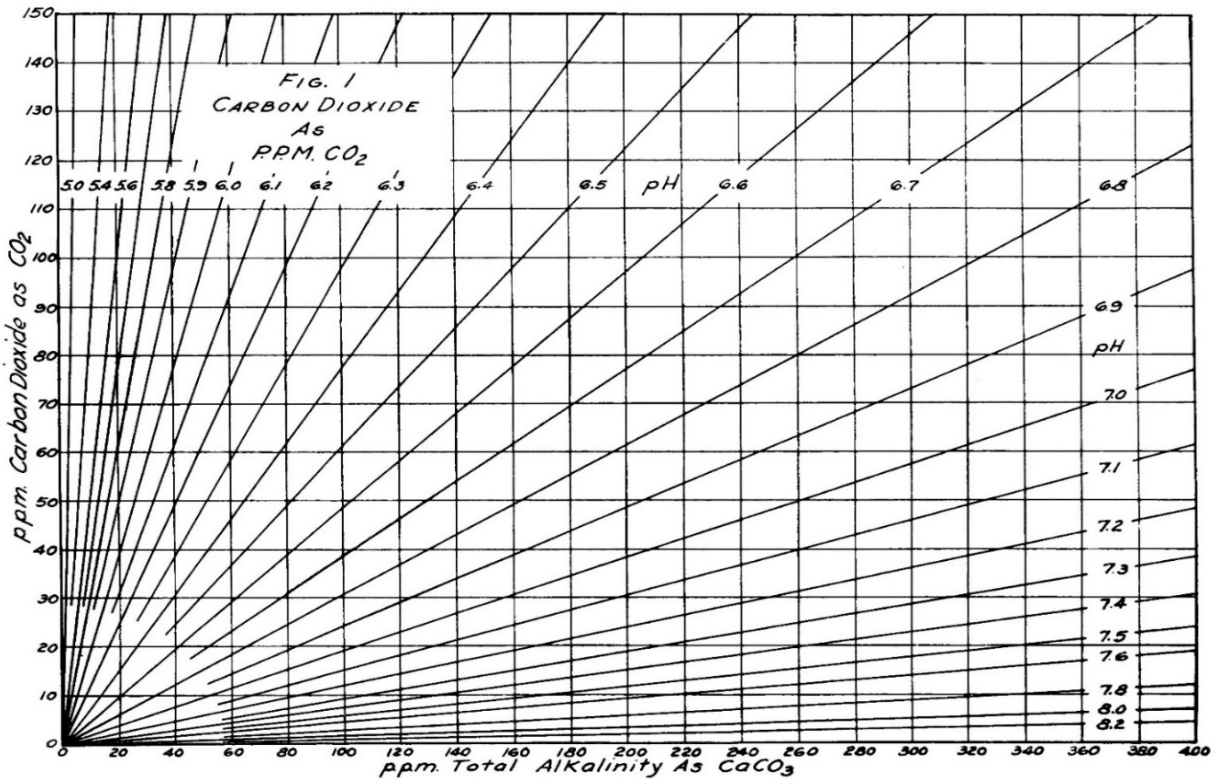
$$[Alk] = [HCO_3^-] + 2[CO_3^{2-}] + [OH^-] - [H^+], \qquad (2.12)$$

where brackets [ ] are presenting molar concentrations.

Sometimes water alkalinity can be misunderstood with hardness because the hardness is also expressed as mg/l of $CaCO_3$. According to, hardness presents the amount of Ca and Mg concentration which do not depend on water alkalinity. In areas of the world where the water hardness builts from limestone, alkalinity, and hardness both have high levels. Waters with high alkalinity have a pH that is above 7 and a high concentration of total dissolved solids (Stickney & Thomson, 2000). Norwegian fresh waters are shallow in alkalinity (Summerfelt et al., 2015).

Titration with standardized acid measures total alkalinity to the methyl orange endpoint of pH=4.3, or by using phenolphthalein in a kit for aquaculture, which measures to the endpoint of pH=8.3. Kits with phenolphthalein are measuring total $OH^-$ (hydroxide) and $CO_3^{2-}$ (carbonate). If in measure calculation with phenolphthalein the value in mg/l of $CO_2$ is close to zero, that means that all alkalinity comes from bicarbonates and the pH will be around 8.3. Total alkalinity, eq. (2.12), is measuring all the bicarbonates, carbonates, and hydroxides (Clesceri et al.). When

alkalinity, water temperature, and pH are measured - known, those can be used to calculate the concentration of dissolved $CO_2$ using Nomograph developed from De Martini formulas (Figure 2-12).




$$(CO_2) = 9.70 \times 10^{10}(H^+) \times \frac{\left[\frac{Alk}{50{,}000} + (H^+) - \frac{10^{-14}}{(H^+)}\right]}{1 + \frac{11.22 \times 10^{-11}}{(H^+)}}$$

*Figure 2-12 Nomograph presenting relationship between pH, alkalinity of CaCO3 in ppm and Carbon dioxide as CO2 in ppm. Relationship are related to De Martini equation where (Alk) is in ppm as calcium carbonate(CO2) in ppm as carbon dioxide, and ($H^+$), hydrogen ion concentration, in moles per litre (Moore, 1939)*

Fishes do not have the needs for bicarbonates directly, but it can indirectly influence the fish health because it is not supporting enough alkalinity for buffering the fluctuations in water pH made by carbon dioxide in water (Stickney & Thomson, 2000).

### 2.5.4. Carbon dioxide removal – chemical and physical

Carbon dioxide solubility is very high in water, but pure water $CO_2$ concentrations are deficient, as low as 0.54mg/l at 20°C (Timmons, 2010), because the level in the atmosphere is low, only 0,035% of total air volume. According to Henry's law, the amount of dissolved gas in the water is equal to the pressure of his gas phase. Production of $CO_2$ in one aquaculture system depends on the fish number, size and metabolism, and bacterial activity. Both are producing significant amounts of carbon dioxide by respiration or dissolving of the organic compounds. Fishes exposed to high carbon dioxide concentrations have reduced gas exchange over the gills, causing the increment of $CO_2$ in the fish blood, leading to blood acidification, , chronic problems with calcinosis, anaesthesia, and mortality (Hoar & Randall, 1984; Noga, 2011; Roberts, 2012; Timmons, 2010)

When Carbon dioxide enters the water, it starts to react with the water and forms carbonic acid, and after that, it goes to the series of chemical reactions which are pH dependent. According to pH dependency, that can be used to determine which components of carbon dioxide are present (Figure 2-13). Carbon dioxide ammount in water is in function of pH and the total dissolved inorganic carbon, $C_tCO_3$, eq. (2.13) which is the sum of all carbon components (Deffeyes, 1965; Stumm & Morgan, 2012; Timmons, 2010):

$$[C_tCO_3] = [CO_2] + [HCO_3^-] + [CO_3^{2-}], \qquad (2.13)$$

where the species with brackets [ ] are presenting molar concentrations.

The water is mostly classified based on its alkalinity than on $C_tCO_3$. The accumulation of carbon dioxide will increase the total carbonate, create more carbonic acid, and decrease the concentration of carbonate, which will cause the pH drop (Stickney & Thomson, 2000; Stumm & Morgan, 2012).

### 2.5.5. Chemical approach to carbon dioxide removal

Using the relationship with the pH, the chemistry of the water can be manipulated in a way to increase the pH and chemically remove the $CO_2$. The change in pH is not eliminating carbon dioxide, it only changes its form from $CO_2$ to $HCO_3^-$ or $CO_3^{2-}$, depending on water pH and Bjerrum plot (Figure 2-11). Adding $OH^-$ ions into the water, the total carbon remains on the same level, but the pH is increased. Lie and calcium carbonate are common in alkalinity, pH and carbon dioxide regulation. If the lie is added (NaOH), alkalinity is added to the water in the form of $OH^-$ ions and translates the $CO_2$ to less toxic form for the fish by increasing pH (Figure 2-13). Sodium hydroxide is highly soluble in the water, it gives a fast reaction, but in misuse can be overdosed. Overdosing with $CaCO_3$ is reduced because it starts to precipitate on certain pH level (Timmons & Losordo, 1994). When using calcium carbonate (2.14) as a carbon dioxide regulator, $CaCO_3$ transforms carbonic acid in a less toxic form:

$$CaCO_3 + H_2CO_3 = Ca^{2+} + HCO_3^- \qquad (2.14)$$

The problem with the $CaCO_3$ is that adding it to the water, total carbonate is also going up (Figure 2-13), which in recirculation can lead to a problem with $CO_2$, because of the carbon chemistry in different pH levels (Stumm & Morgan, 2012). Several chemicals can be used beside mentioned $CaCO_3$ and NaOH as pH control and the addition of alkalinity (Table 1).

*Table 1 Chemicals used as alkalinity supplement and their properties - adapted from (Timmons & Losordo, 1994)*

| **Chemical formula** | **Common name** | **Solubility** | **Rate of solubilization** |
|---|---|---|---|
| NaOH | Sodium hydroxide Caustic soda | high | rapid |
| $Na_2CO_3$ | Sodium carbonate Soda ash | high | rapid |
| $NaHCO_3$ | Sodium bicarbonate Baking soda | high | rapid |

| | | | |
|---|---|---|---|
| $CaCO_3$ | Calcium Carbonate Lie | moderate | moderate |
| CaO | Slaked lime | high | moderate |
| $Ca(OH)_2$ | Calcium hydroxide Hydrated lime | high | moderate |
| $CaMg(CO_3)_2$ | Dolomite | moderate | slow |
| $Mg\ CO_3$ | Magnesium carbonate Magnesite | moderate | slow |
| $Mg(OH)_2$ | Magnesium hydroxide Brucite | moderate | slow |

### 2.5.6. A physical approach to carbon dioxide removal

Carbon dioxide is not a problem in waters with flow thru system and stocking densities that are less than 30 – 60 $kg/m^3$ (Timmons, 2010). Increased capacity is increasing the amount of $CO_2$ that is produced by the unit of volume, and it needs to be removed from the system. The physical way of removing is by using gas striping – degassing. In an intensive production system, the concentration of $CO_2$ is 20 to 100 times the ambient concentration. With its significant level in the water, it is easy to degas it, but it needs substantial amounts of air in contact with water, because of the high solubility of $CO_2$ gas in the water. The $CO_2$ stripper is positioned in the place where the total amount of $CO_2$ is the highest, and that is after the biofilter. Packed column and gravity flow thru the column are the easiest way to strip off the $CO_2$. Most used today is a forced-ventilated cascade aeration with a volumetric ratio between 5:1 and 10:1 volume air to the volume of water. Stripping using the stripping column is the separate process of the oxygenation process, but in that process, some amounts of carbon dioxide are also removed. Stripping carbon dioxide in chemistry affects the total amount of carbonate, $C_tCO_3$ which is decreasing, and the pH is going up to a higher value (Deffeyes, 1965; Stickney & Thomson, 2000; Summerfelt & Sharrer, 2004; Timmons, 2010).

### 2.5.7. Open or closed $CO_2$ system

The carbonate system in aquaculture production system can be observed as an open or closed system. What it depends on, is the possibility to exchange the aqueous carbon dioxide and equilibrate it with the atmospheric gas. Recirculation systems with high fish densities, above 40kg/$m^3$, are the closed systems because of several facts affecting the gas exchange. Production of $CO_2$ in one fish tank is quite high. The gas itself is very soluble in the water, and the degassing power of the system is relatively low across the water surface in the culture tanks (Aqion(1a), 2018; Aqion(1b), 2018; Timmons, 2010).

### 2.5.8. How to measure the amount of $CO_2$ in the water

There are many methods present to measure the amount of dissolved carbon dioxide in the water solutions. Titration, using wetted $CO_2$ probe analyser, some standard nomographic methods and calculation by direct measurements of temperature, alkalinity, and pH. Titration is the most precise way to measure it, with a standard base to phenolphthalein endpoint of pH=8.3. This method is suitable for freshwater systems where the number of dissolved solids, silicates and other minerals dissolved in water cannot interfere with the result. (Pfeiffer et al., 2011; Timmons, 2010).

### 2.5.9. $CO_2$ influence on alkalinity, pH, and total carbonate carbon

The correlation between carbon dioxide and different chemical properties of the water can be explained using vector diagram which Kenneth S. Deffeyes described and published in 1965. In this diagram (Figure 2-13) adding different chemicals to the water can be calculated and it is defined as a vector property. From the graph, it can be seen how most common solutions for $CO_2$ removal or regulation of the alkalinity are affecting.

- Adding acid or base to the water will have vertical linear activity affecting only the pH and not the total carbonate carbon.
- Adding $CaCO_3$, $Na_2CO_3$ and $NaHCO_3$ will affect adding alkalinity, total carbonate carbon, and pH.
- Adding only $CO_2$ to the water will affect total carbonate carbon and pH, but not the alkalinity.

Adding $CO_2$ to the water is explained by the fact that carbon dioxide is entering the series of equations for carbonate system. The net reaction produces the same number of equivalents of positively charged ions ($H^+$) and negative ones in the form of bicarbonate ($HCO_3^-$) and carbonate ($CO_3^{2-}$). However, the ratio of carbonate to bicarbonate ions depends on the pH of the solution and adding $CO_2$ to the water is lowering the pH. Horizontal linear vector presents adding $CO_2$ into the water (Stumm & Morgan, 2012).

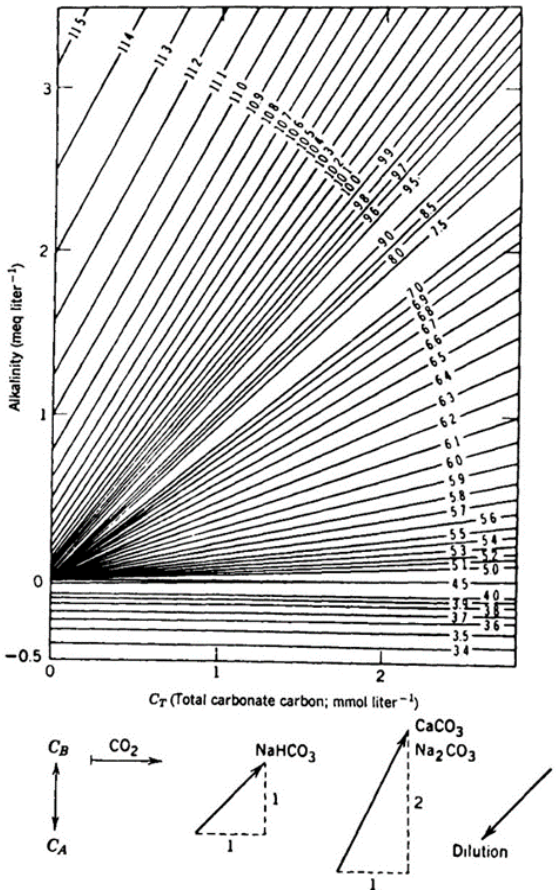
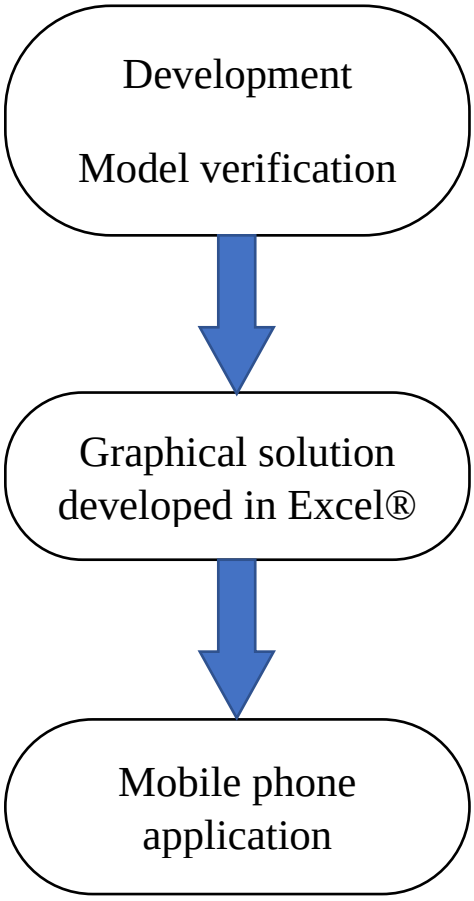



*Figure 2-13 Deffeyes diagram of pH contours in alkalinity versus Ct. Defining point of the solution composition, moves as a vector in the chart as a result of the addition or removal of $CO_2$, $NaHCO_3$, $CaCO_3$, $Na_2CO_3$ or $C_B$ – bases and $C_A$ - acid (Deffeyes, 1965; Stumm & Morgan, 2012)*

# 3. MODEL DEVELOPING

This chapter will represent the stages in a model developing for pH, alkalinity and carbon dioxide prediction in Recirculation Aquaculture System. The carbon dioxide is considered as the primary limiting factor for inland fish production (Gaumet, 2018), and the model is based on $CO_2$ prediction production from different input properties. Model inputs are water parameters, overall farm capacity, fish weight, amount of proteins, carbohydrates, and lipids in food to predict how much oxygen is used and in connection how much carbon dioxide is produced. The biological filter uses some alkalinity from the water and produces carbon dioxide which is also included in the model. Next stage is carbon dioxide stripping. In the stripping unit, the value for the unit efficiency is set up. The final step is adding different chemicals after the carbon dioxide stripper to correct the pH before water enters to the next circle. The model developing passed thru several stages:



Model developing is supported by knowledge from different mathematical models developed by Sanni, Forsberg, Bergheim, and Timmons (Sanni & Forsberg, 1996; Sanni, 1993; Timmons, 2010) and advice from personal communication (Gaumet, 2018) in Kruger-Kaldnes. Deffeyes diagram (Deffeyes, 1965) is used to obtain the amount of the specific chemical to rich the certain pH. The model is verified with values obtained from two farms designed and constructed by Kruger-Kaldnes as a turnkey delivery solution and values from NMBU fish laboratory.

Next step was arranging formulas in Excel, and designing the interface to look user-friendly and intuitive to enter the measured values.

The final stage in model developing is an application for mobile phone. The application is easy to use, and all numbers are presented logically for the user. The user can decide which parameters to change to obtain good production results without affecting the fish health and the current farm operations only by using of the prediction tool.

## 3.1. Model formulas – mathematical background

The model has several input values and after that the calculations. Assumptions for this model are:

1. The fish excretes $CO_2$ according to the respiration coefficient
2. Respired $CO_2$ is not affecting the total alkalinity, but it is changing the entire carbonate carbon defined by Deffeyes diagram (Figure 2-13)
3. The retention time in whole recirculation system is concise, and the whole system is then described as a closed system without $CO_2$ exchange with the atmosphere, except the $CO_2$ stripper where the entire process is partly controlled
4. Fish metabolism ads alkalinity to the water and contributes to overall calculations

5. Loss of alkalinity in biofilter - MBBR[1] is calculated in the table to know how much is lost and how much to add

### 3.1.1. Input values – values defined by the user

Input values are measured directly from the water sample, or they can be assumed to test which water quality fits the best the constructed production unit or project unit proposal.

Input values for the model are:

1) Water physical properties:
    - pH inlet
    - Alkalinity
    - Temperature
    - Salinity
    - Oxygen inlet
2) Technical parameters of the farm:
    - Water flow
    - Total tank(s) or farm capacity
3) Fish parameters:
    - Average single fish weight
    - Total biomass
    - Fish density – calculated from total biomass and tank(s) volume
    - The total number of fishes – derived from total biomass and fish weight
4) Fish food parameters:
    - Amount of proteins, carbohydrates, and lipids
    - Food Conversion rate
    - Amount of the food for one day – calculated by a program
    - SGR – specific growth rate from the table

[1] MBBR - Moving bed biofilm reactor – biofilter with microorganisms growing on a specially designed plastic carriers, form a biofilm, and they are kept suspended in a reactor. Product was developed by professor Hallvard Ødegaard and commercialised by Kruger - Kaldnes

*Table 2 User-defined values and their units*

| ***User-defined values*** | ***Value units*** |
|---|---|
| ***pH inlet*** | - |
| ***Total alkalinity inlet*** | *mg * l $^{-1}$ $CaCO_3$* |
| ***Temperature*** | *°C* |
| ***Salinity*** | *ppt* |
| ***Water flow*** | *$m^3$ * min $^{-1}$* |
| ***Tank volume*** | *$m^3$* |
| ***Fish weight*** | *gr* |
| ***Biomass*** | *kg fish $^{-1}$* |
| ***Fish density*** | *kg/$m^3$* |
| ***Total number*** | - |
| ***p (protein) – protein amount in food*** | *0 ≤ f ≤ 1* |
| ***f (fat) – lipid amount in food*** | *0 ≤ f ≤ 1* |
| ***c (carbonates) – carbohydrates in food*** | *0 ≤ f ≤ 1* |
| ***FCR*** | *Food conversion rate* |
| ***Food for one day*** | *kg* |
| ***SGR from Biomar table*** | *According to fish size in (g) and temperature(°C)* |

Food for one day is indirectly given. It is calculated according to the expected or measured SGR and FCR (3.1),

$$Food\ for\ one\ day = \left(\frac{SGR * biomass}{100}\right) * FCR, \qquad (3.1)$$

where $SGR$ is a specific growth rate for given temperature and fish weight and then red from the specific table, $biomass$ is a total fish weight in kilos (kg), and $FCR$ is a food conversion rate.

The inlet oxygen value is not affecting directly to the results of prediction $CO_2$, but it is used to see is the water flow suitable for production and how much oxygen must be added to meet the required outlet value. Inlet values of oxygen are in $Sat[\%]$, and to obtain a table value for how much oxygen is available in certain conditions (3.2).

$$O_2 = [table\ value\ t, sal] * \frac{O_2 Sat[\%]}{100} \qquad (3.2)$$

Amount of oxygen depends on water temperature, salinity, and oxygen pressure. All of these values are in the table (Table 3) developed by John Colt (Colt, 2012), and Excel function is used to take the amount of oxygen for the given values automatically:

*Table 3 Standard Air Saturation Concentration of Oxygen as a Function of Temperature and Salinity in mg/L, 0,40 g/kg (Seawater, 1 atm moist air) (Colt, 2012)*

| | **Salinity (g/kg, g/L, ppt)** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Temp(°C)** | **0** | **5** | **10** | **15** | **20** | **25** | **30** | **35** | **40** |
| **0** | 14,621 | 14,120 | 13,635 | 13,167 | 12,714 | 12,276 | 11,854 | 11,445 | 11,050 |
| **1** | 14,216 | 13,733 | 13,266 | 12,815 | 12,378 | 11,956 | 11,548 | 11,153 | 10,772 |
| **2** | 13,829 | 13,364 | 12,914 | 12,478 | 12,057 | 11,649 | 11,255 | 10,875 | 10,506 |
| **3** | 13,460 | 13,011 | 12,577 | 12,156 | 11,750 | 11,356 | 10,976 | 10,608 | 10,252 |
| **4** | 13,107 | 12,674 | 12,255 | 11,849 | 11,456 | 11,076 | 10,708 | 10,352 | 10,008 |
| **5** | 12,770 | 12,352 | 11,946 | 11,554 | 11,174 | 10,807 | 10,451 | 10,107 | 9,774 |
| **6** | 12,447 | 12,043 | 11,652 | 11,272 | 10,905 | 10,550 | 10,205 | 9,872 | 9,550 |
| **7** | 12,138 | 11,748 | 11,369 | 11,002 | 10,647 | 10,303 | 9,970 | 9,647 | 9,335 |
| **8** | 11,843 | 11,465 | 11,098 | 10,743 | 10,399 | 10,066 | 9,743 | 9,431 | 9,128 |
| **9** | 11,559 | 11,194 | 10,839 | 10,495 | 10,162 | 9,839 | 9,526 | 9,223 | 8,930 |
| **10** | 11,288 | 10,933 | 10,590 | 10,257 | 9,934 | 9,621 | 9,318 | 9,024 | 8,739 |
| **11** | 11,027 | 10,684 | 10,351 | 10,028 | 9,715 | 9,411 | 9,117 | 8,832 | 8,556 |
| **12** | 10,777 | 10,444 | 10,121 | 9,808 | 9,505 | 9,210 | 8,925 | 8,648 | 8,379 |
| **13** | 10,536 | 10,214 | 9,901 | 9,597 | 9,302 | 9,016 | 8,739 | 8,470 | 8,209 |
| **14** | 10,306 | 9,993 | 9,689 | 9,394 | 9,108 | 8,830 | 8,561 | 8,299 | 8,046 |
| **15** | 10,084 | 9,780 | 9,485 | 9,198 | 8,920 | 8,651 | 8,389 | 8,135 | 7,888 |
| **16** | 9,870 | 9,575 | 9,289 | 9,010 | 8,740 | 8,478 | 8,223 | 7,976 | 7,736 |
| **17** | 9,665 | 9,378 | 9,099 | 8,829 | 8,566 | 8,311 | 8,064 | 7,823 | 7,590 |
| **18** | 9,467 | 9,188 | 8,917 | 8,654 | 8,399 | 8,151 | 7,910 | 7,676 | 7,448 |
| **19** | 9,276 | 9,005 | 8,742 | 8,486 | 8,237 | 7,996 | 7,761 | 7,533 | 7,312 |
| **20** | 9,092 | 8,828 | 8,572 | 8,323 | 8,081 | 7,846 | 7,617 | 7,395 | 7,180 |
| **21** | 8,914 | 8,658 | 8,408 | 8,166 | 7,930 | 7,701 | 7,479 | 7,262 | 7,052 |
| **22** | 8,743 | 8,493 | 8,250 | 8,014 | 7,785 | 7,561 | 7,344 | 7,134 | 6,929 |
| **23** | 8,578 | 8,334 | 8,098 | 7,868 | 7,644 | 7,426 | 7,215 | 7,009 | 6,809 |
| **24** | 8,418 | 8,181 | 7,950 | 7,726 | 7,507 | 7,295 | 7,089 | 6,888 | 6,693 |
| **25** | 8,263 | 8,032 | 7,807 | 7,588 | 7,375 | 7,168 | 6,967 | 6,771 | 6,581 |

Values from the table are then multiplied by the percentage of saturation $Sat[\%]$ to obtain how much oxygen is available in the tank from the inlet. Outlet value is calculated with eq. (3.3),

$$[O_2]_{out} = [O_2]_{in} - (sOD * sQ^{-1}) \qquad (3.3)$$

where $[O_2]_{in}$ is a value from the table, $sOD$ is specific oxygen demand calculated and, $sQ^{-1}$ water flow for the production unit. To calculate oxygen outlet value in $Sat[\%]$ use the eq. (3.4.)

$$Oxygen\ outlet\ in\ Sat[\%] = \left[\frac{[O_2]_{out}}{table\ value\ t,sal}\right] * 100 \qquad (3.4)$$

### 3.1.2. Metabolic processes parameters – prediction of $CO_2$ levels in the outlet of the fish tank

Processes that are affecting water quality from fish metabolism in the production units are oxygen consumption and carbon dioxide production, nitrogen from food which accumulates during the time in the tanks in the form of total ammonia nitrogen - TAN. Sanni, Forsberg, and Bergheim (Sanni, 1993) developed a method for predicting these amounts in a flow-thru system. This assumption is used in this model for the prediction outlet of the fish tank. In the secondary production units, biological filter and carbon dioxide stripper, alkalinity, total carbonate carbon, and pH are calculated using Deffeyes diagram (Deffeyes, 1965). Deffeyes diagram calculator is developed in Excel for this purpose by NMBU associate professor Kristian Hovde Liland. In the model developing the focus is on carbon dioxide production. Total ammonia nitrogen prediction is used to describe how safe is the system and, to see how much biological filter contributes to the production of carbon dioxide. Bacteria that transfers ammonia to less toxic nitrates are producing some amount of $CO_2$ (Summerfelt & Sharrer, 2004).

Indirect calorimetry method by Brett and Groves (Hoar et al., 1979) calculates oxygen utilisation and carbon dioxide production. Forsberg (1993) developed model according to that work eq.(3.5) and eq.(3.6),

$$sOD = 6{,}94 * 10^{-4} * F * (33{,}5f + 16{,}3p + 13{,}4ch) * (39{,}5f + 20{,}7p + 17{,}4ch), \qquad (3.5)$$

$$sCO_{2R} = 8{,}59 * 10^{-3} * T * W^{-0{,}4} * (23{,}7f + 14{,}7p + 13{,}4ch) * (39{,}5f + 20{,}7p + 17{,}4ch), \quad (3.6)$$

where $sOD$ is specific oxygen demand and $sCO_{2R}$ specific carbon dioxide production affected by feeding rate $F$ , and amount of specific parts of the food, lipids (f), proteins (p), and carbohydrates (ch). Feeding rate is dependent of fish size and water temperature (Austreng et al., 1987), and this particular approach is recalculated eq. (3.7) to fit the Biomar feeding table (Table 4).

$$F = 9T * W^{-0{,}4}, \quad (3.7)$$

where $T$ is water temperature and $W$ is fish weight.

*Table 4 Biomar SGR table*

| **SGR** BIOMAR | | | | | | |
|---|---|---|---|---|---|---|
| **Weight (g)** | | | | | | |
| **Temp (°C)** | **0** | **1** | **5** | **15** | **30** | **60** |
| **6** | 2,6 | 1,9 | 1,8 | 1,5 | 1,1 | 1 |
| **7** | 3,2 | 2,3 | 2,1 | 1,8 | 1,4 | 1 |
| **8** | 3,5 | 2,6 | 2,4 | 2,1 | 1,6 | 1,2 |
| **9** | 3,9 | 3 | 2,7 | 2,3 | 1,8 | 1,3 |
| **10** | 4,2 | 3,3 | 3 | 2,6 | 2 | 1,5 |
| **11** | 4,6 | 3,6 | 3,3 | 2,9 | 2,2 | 1,6 |
| **12** | 5 | 3,9 | 3,6 | 3,1 | 2,4 | 1,8 |
| **13** | 5,4 | 4,3 | 3,9 | 3,4 | 2,6 | 1,9 |
| **14** | 5,9 | 4,6 | 4,2 | 3,7 | 2,8 | 2,1 |
| **15** | 6,2 | 5 | 4,5 | 4 | 3 | 2,3 |
| **16** | 6,4 | 5,2 | 4,8 | 4,3 | 3,2 | 2,5 |
| **17** | 6,6 | 5,5 | 5 | 4,5 | 3,4 | 2,6 |

Nitrogen from the food the food proportionally increases total ammonia nitrogen in the water eq. (3.8):

$$sTANp = 0{,}3pT * W^{-0{,}4}, \quad (3.8)$$

where $sTANp$ is ammonia excretion rate of unionized and ionized ammonia and $p$ is protein amount, $T$ -water temperature and $W$- fish weight in grams.

Fish contribution to total carbonate out of respired $CO_2$ is calculated with a model derived from carbon dioxide production (eq. 3.9).

$$Cr = \frac{1}{44} * sCO_{2R} * sQ^{-1}, \quad (3.9)$$

with $sCO_{2R}$ – specific carbon dioxide production and $sQ^{-1}$ is a specific water flow for the whole production unit.

Specific water flow is calculated from $Q$ - total waterflow for the whole farm in l/min and the total biomass (eq. 3.10).

$$sQ = Q * \left(\frac{1}{total\ biomass}\right) \quad (3.10)$$

The user defines water inlet pH, but the outlet is calculated according to how much carbon dioxide is added to the water (eq. 3.11). From the Deffeyes diagram (Figure 2-13), adding $CO_2$ is lowering the pH,

$$pH_{out} = -\log\left(K_1 * [CO_2]_{out} * [HCO_3^-]_{out}{}^{-1}\right), \quad (3.11)$$

where $K_1$ is a first dissociation constant for carbonic acid, $[CO_2]_{out}$ – amount of carbon dioxide in moles and $[HCO_3^-]_{out}$ bicarbonates in moles out of the production component (Sanni & Forsberg, 1996; Stumm & Morgan, 2012).

Total alkalinity inlet is defined by the user in mg/l of $CaCO_3$ and divided by 50 to obtain the amount in meq/l used for Deffeyes calculations (eq. 3.12).

$$AC_{in} = \frac{Alkalinity\ in \frac{mg}{l}\ CaCO_3}{50} \quad (3.12)$$

Alkalinity in the outlet is the same as the alkalinity in the inlet (3.13) which is true if the alkalinity is not spent in the system. The amount of the carbon dioxide is not affecting the total alkalinity defined by the Deffeyes diagram (Deffeyes, 1965; Stumm & Morgan, 2012).

$$AC_{out} = AC_{in} \tag{3.13}$$

Total carbonate carbon (eq. 3.14 and 3.15) is calculated for inlet and outlet values (Stumm & Morgan, 2012):

$$C_{t\,in} = \frac{Ac_{in}}{\alpha_{1i} + 2\alpha_{2i}}, \tag{3.14}$$

where $Ac_{in}$ is alkalinity inlet in meq/l and $\alpha_{1i}$ and $\alpha_{2i}$ are constants.

Total carbonate carbon in the outlet is a sum of fish contribution to the total carbonate out of respired carbon dioxide - $C_r$ and $C_{t\,in}$:

$$C_{t\,out} = C_{t\,in} + C_r \tag{3.15}$$

To calculate the pH that is in the outlet of the fish tank, there are three more components to calculate (eq. 3.16,3.17 and 3.18)

$$[H_2CO_3^*] = C_{Tout} - \left(\frac{Ac_{out} + [HCO_3^-]_{out}}{2}\right), \tag{3.16}$$

$$[HCO_3^-]_{out} = \frac{-b \pm \sqrt{-4ac + b^2}}{2a}, \tag{3.17}$$

$$[CO_3^{2-}]_{out} = \frac{(Ac_{out} - [HCO_3^-]_{out})}{2}, \qquad (3.18)$$

where $[CO_3^{2-}]_{out}$ is carbonate in the outlet, $[HCO_3^-]_{out}$ is bicarbonate in the outlet and $[H_2CO_3^*]$ is a sum of carbonic acid and carbon dioxide dissolved in water(Stumm & Morgan, 2012). Factors $a, b\ and\ c$ will be described later.

Total ammonia nitrogen is a value depending on fish metabolism and the amount of Nitrogen added by the food (eq. 3.19). The value obtained this way is an indicator when ammonia is becoming a problematic value for the system. For the fish itself only the unionised ammonia is toxic (Alabaster & Lloyd, 2013). The ratio of unionised ammonia depends on temperature and pH. The concentration of total ammonia depends on ammonia excreted from the fish (Forsberg, 1993):

$$[TAN]_{out} = [TAN]_{in} + (1000 * sTANp * sQ^{-1}), \qquad (3.19)$$

where $[TAN]_{in}$ is expected to be zero on the beginning of the recirculation, $sTANp$ is calculated and waterflow $sQ^{-1}$, given by the production unit.

Finally, the carbon dioxide is calculated (3.20) as a function of alkalinity - $Ac_{out}$, pH - $pHout$, carbonic system dissociation constants - $K_1, K_2$ and water ionisation constant - $Kw$ (Timmons, 2010):

$$CO_{2\,out} = \left( \left( Ac_{out} + 10^{-pHout} - \frac{Kw}{10^{-pHout}} \right) * \left( \frac{1}{\left(\frac{K_1}{10^{-pHout}}\right) + \left(\frac{2K_1 * K_2}{(10^{-pHout})^2}\right)} \right) \right) * 44000 \qquad (3.20)$$

### 3.1.3. Factors and constants used for calculations

In calculations, different factors and constants are used. Factors $a, b \; and \; c$ are derived from quadratic equation (Sanni & Forsberg, 1996):

$$a = 4K_2 - K_1 \tag{3.21}$$

$$b = 2K_1 \tag{3.22}$$

$$c = K_1 Ac(Ac - 2C_{T\,out}) \tag{3.23}$$

, where $K_1$ (eq. 3.27 and 3.28) and $K_2$ (eq. 3.29 and 3.30) are dissociation constants for carbonic acid, $Ac$ is carbonate alkalinity and $C_{T\,out}$ – is the total sum of the total carbonate in the inlet water and carbon dioxide contribution from the fish.

Constants $\alpha_{1i}$ (3.24) and $\alpha_{2i}$ (3.25) from Stumm&Morgan (2012):

$$\alpha_{1i} = (\frac{[H_{in}^+]}{K_1} + 1 + \frac{K_2}{[H_{in}^+]})^{-1}, \tag{3.24}$$

$$\alpha_{2i} = (\frac{[H_{in}^+]^2}{K_1 K_2} + \frac{[H_{in}^+]}{K_2} + 1)^{-1}, \tag{3.25}$$

where expression $[H_{in}^+]$ defines molar concentration of $H^+$ ions:

$$[H_{in}^+] = 10^{-pH_{in}}. \tag{3.26}$$

Dissociation constants can be read from the table, but instead of using the table the process is automated in Excel. Dissociation constants are different for fresh water and for the saltwater with salinity that is above zero (Harned, 1943; Harned, 1941; Timmons, 2010). Using "IF" function in the table according to the salinity value, the program then chooses the right value:

- Freshwater constants:

$$K_1 = 10^{-((0{,}519899/T)+6{,}43897+(-0{,}00394989*T))} \qquad (3.27)$$

$$K_2 = 10^{-((0{,}681109/T)+10{,}46162+(-0{,}00583285*T))} \qquad (3.28)$$

- Saltwater constants:

$$K_1 = 10^{-(3405/(273+T)+0{,}03279*(273+T)-14{,}71-0{,}1918*sal^{0{,}33})}, \qquad (3.29)$$

$$K_2 = 10^{-(2902/(273+T)+0{,}02379*(273+T)-6{,}471-0{,}4693*sal^{0{,}33})}, \qquad (3.30)$$

and water ionization (eq. (3.31) constant which is the same in fresh water and saltwater conditions.

$$K_w = 10^{-((4470{,}99/(T+273{,}15))-6{,}0875+(0{,}01706*(T+273{,}15)))}, \qquad (3.31)$$

where $T$ is the temperature in °C, and $sal$ is salinity ppt.

## 3.2. Biological reactor calculations – $CO_2$ and alkalinity levels prediction out of the biological filter

Ammonia is removed in the biological filter using the autotrophic bacteria which are converting ammonia to nitrite and then to non-toxic nitrates. Fishes are producing ammonia thru the metabolism of proteins and excreting it thru the gills. If the products of metabolism stay in the RAS system, this will be very poisonous, and fish will die. A biological filter is using alkalinity from the water and producing some amount of carbon dioxide (Summerfelt & Sharrer, 2004; Timmons, 2010). These contributions are used in prediction calculations out of the MBBR - biological filter.

Primary carbon source for nitrifying bacteria as the obligate autotrophs and aerobes is carbon dioxide (Hagopian & Riley, 1998). However, the free $H^+$ ions produced during nitrification process react with bicarbonates ($HCO_3^-$) from alkalinity in the water and creates more $CO_2$ than is used for nitrification. Stochiometric reaction shows the correlation of ammonia, oxygen, and bicarbonates and gives the cell mass, nitrates, water, and carbon dioxide:

$$NH_4^+ + 1{,}86\ O_2 + 1{,}98\ HCO_3^- \rightarrow 0{,}0206\ C_5H_7O_2N + 0{,}0980 NO_3^- + 1{,}041\ H_2O + 1{,}88\ H_2CO_3{}^* \quad (3.32)$$

Carbon dioxide is in the form of carbonic acid ($H_2CO_3^*$) because there is 848 times more carbon dioxide than carbonic acid in the chemical equilibrium. In $H_2CO_3^*$ the most of it is in the form of $CO_2$. When the stoichiometric reaction is explained in numbers, nitrification consumes 4.6mg/l of oxygen for every 1mg/l of TAN consumed and produces 5,85mg/l of carbon dioxide (Summerfelt & Sharrer, 2004).

Fishes in the fish tank are producing alkalinity. Ammonia that is produced from metabolism acts as a weak base and contributes to the overall alkalinity and reduces the amount spent in the biofilter. Biofilter spends approximately 8 mg of alkalinity per every mg of TAN reduced, but with the alkalinity from the fish tank, the value is 3,57mg per every mg of TAN. (Summerfelt & Sharrer, 2004; Timmons, 2010).

Producers of Atlantic salmon smolts should aim the alkalinity value of approximately 70mg/l because of good pH stability and because biofilter uses alkalinity during nitrification process (Summerfelt et al., 2015).

In the calculations the fish tank outlet values are used: alkalinity in mg/l of $CaCO_3$, pH, total carbonate carbon and carbonate carbon dissociation constants $K_1$, $K_2$ and $K_W$. Dissociation constants values will be the same thru whole system because the temperature and salinity will not change thru the system components.

Alkalinity in the outlet - $ALK_{outMBBR}$ is reduced by the alkalinity spent in the biofilter and alkalinity produced in the fish tank:

$$ALK_{outMBBR} = AC_{out} - \left( TANout \left(\frac{mg}{L}\right) * 3{,}57 \right), \qquad (3.33)$$

where $AC_{out}$ is alkalinity out of the fish tank and $TANout$ – total ammonia nitrogen out of the fish tank.

From personal communication in Kruger-Kaldnes (Gaumet, 2018) , it was pointed out that 5% of total carbon dioxide is taken out in the biofilter of the total produced carbon dioxide. This reaction is happening because of the significant area of the biofilter and the aeration process in the tanks which are physically taking the $CO_2$ out of the tank. This is calculated, and the total carbon dioxide produced in the outlet of biofilter - $CO_{2\ out\ of\ MBBR}$, is:

$$CO_{2\ out\ of\ MBBR} = (5{,}85 * TAN_{out}) + CO_{2\ out\ of\ fish\ tank}, \qquad (3.34)$$

where $TANout$ – total ammonia nitrogen out of the fish tank and $CO_{2\ out\ of\ fish\ tank}$ – total amount of carbon dioxide produced in the fish tank according to the pH in the inlet water.

To calculate the loss of 5%, this contribution is defined as a total carbonate carbon out of total $CO_2$ in the biofilter $CO_{2\ out\ of\ MBBR}$. Contribution is equal to 5% out of all $CO_2$ produced:

$$C_{R\ out\ of\ MBBR} = \left(CO_{2\ out\ of\ MBBR} *\ 0{,}05\right) * \left(\frac{1}{44}\right), \qquad (3.35)$$

where 44 is a molar mass of carbon dioxide in one mmol of the solution.

Contribution to $C_{R\ out\ of\ MBBR}$ is subtracted from total carbonate carbon to obtain the value for the new total carbonate carbon out of the biofilter. New $C_{t\ out\ MBBR}$ and $ALK_{outMBBR}$ are used with Deffeyes diagram to obtain the new pH value out of the biofilter.

The new values for total carbonate carbon and carbon dioxide are calculated in two steps in Excel.

Carbon dioxide value in new pH, alkalinity and total carbonate carbon calculates using formula (3.20) but with the outlet values from biofilter.

## 3.3. Prediction of $CO_2$ and alkalinity out of the $CO_2$ stripper

In the prediction for carbon dioxide out of the stripping unit, the same principle is used as for biofilter outlet. Inlet values are output values from the biofilter and then corrected for numbers obtained by a stripper. When the carbon dioxide is stripped out, alkalinity is not changing (Figure 2-13), but the total carbonate carbon is decreasing (Stumm & Morgan, 2012). Using this relationship, the sheet is calculating the new value of the carbon dioxide in the outlet and the new pH. Carbon dioxide is calculated with the same formula (3.20), and the new pH is derived from new carbonate carbon value and alkalinity using Deffeyes diagram formula relationship. The carbon dioxide stripper efficiency must be set up, and the total amount is subtracted from carbon dioxide that is entering the stripping unit, and it is called a contribution to the overall carbonate carbon out of total $CO_2$ in the stripping unit - $C_{R\ out\ of\ striper}$:

$$C_{R\ out\ of\ striper} = \left(CO_{2\ out\ of\ stripper} *\ SE\right) * \left(\frac{1}{44}\right), \qquad (3.36)$$

where $SE$ is a percentage of carbon dioxide stripper efficiency, and $CO_{2\ out\ of\ stripper}$ is calculated with eq. (3.20), with values for pH and Alkalinity from the stripper.

## 3.4. Mass balance for TAN in the recirculation system

To predict how much total ammonia nitrogen is in the recirculation system the mass balance equation is used. The equation is telling when the system becomes stable, meaning when the values are in steady state.

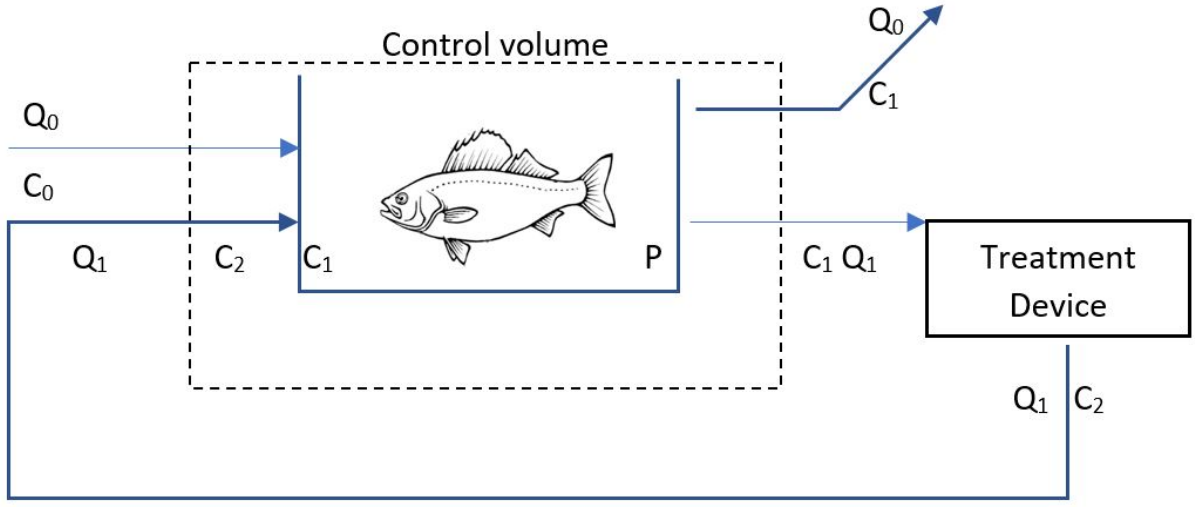



*Figure 3-1 General mass balance of a fish tank with the treatment process out of the fish tank (Timmons, 2010)*

The mass balance formula in steady-state conditions is:

$$Q_1C_2 + Q_0C_0 + P = Q_0C_1 + C_1Q_1, \qquad (3.37)$$

where $Q$ is a water flow, $P$ – product added by fish metabolism, $C$ – produced $CO_2$ or TAN

Mass balance equation is programmed in the Excel. The Figure 3-1 shows the part of the table with calculated values using inlets for the first case farm (later described). The RAS has 95% re-use degree and 80% efficiency of the biological filter. In the given example system stabilises on the value of 1.08mg/l of TAN, after four loops.

| RAS LOOPS Prediction | | | | | |
|---|---|---|---|---|---|
| | Outlet of fish tank | | | | |
| N° loops | pH | Alkalinity | CO2 | TAN | |
| 1st | 6,58 | 25 | 15,6 | 0,88 | mg/l |
| 2nd | 6,62 | 25 | 14,7 | 1,04 | mg/l |
| 3rd | 6,63 | 26 | 14,4 | 1,07 | mg/l |
| 4th | 6,63 | 26 | 14,3 | 1,08 | mg/l |
| 5th | 6,64 | 26 | 14,3 | 1,08 | mg/l |
| 6th | 6,64 | 26 | 14,3 | 1,08 | mg/l |
| 7th | 6,64 | 26 | 14,3 | 1,08 | mg/l |
| 8th | 6,65 | 27 | 14,4 | 1,08 | mg/l |
| 9th | 6,65 | 27 | 14,4 | 1,08 | mg/l |
| 10th | 6,64 | 27 | 14,5 | 1,08 | mg/l |

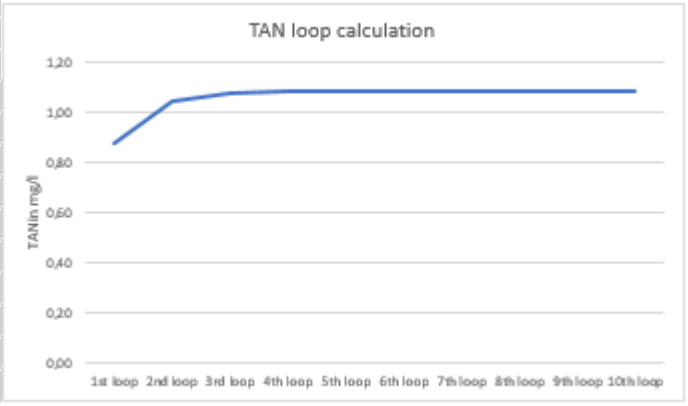



*Figure 3-1 Graphical view of the table part with values for TAN with inlet values from the first case farm*

## 3.5. Correction of pH before water enters the new cycle

Before water enters the new cycle, it is corrected for alkalinity or pH to achieve the best performance of the recirculation system. For correcting pH several components can be used (Table 1), but for the testing purposes, the most used will be tested (Table 5), Lye (NaOH) and calcium carbonate ($CaCO_3$). The calculation is done using Deffeyes diagram formula and the correlations with both chemicals:

- NaOH - one meq of added alkalinity is not adding carbonate carbon,
- $CaCO_3$ - for one meq of alkalinity adds two moles of carbonate carbon

The calculation is done according to the fixed pH that is defined by the user, and then the toll calculates the new total carbonate and alkalinity. When the correction is turned on it is positioned between the outlet of the fish tank and inlet to the biofilter. Inlet values for the biofilter are corrected for the chemical that is added.

*Table 5 Alkalinity supplements and their characteristics (Timmons & Losordo, 1994)*

| Chemical | Alkalinity added (meq/l) |
|---|---|
| **Lye – NaOH** | 40 |
| **Calcium carbonate – $CaCO_3$** | 83 |

## 3.6. Mixing of recirculated water with makeup water

In this step, the water from the recirculation is mixed with the water that is coming from water inlet-makeup water. The new water will have new pH, alkalinity, and level of $CO_2$ according to the recirculation percentage. If the prediction tool is only used as a tool for the flow-thru system, the RAS percentages for re-use, carbon dioxide and biofilter efficiency are set-up to zero.

The calculation is:

- For pH calculation, the amount of $H^+$ ions need to be calculated in one litre of water because of $pH = -\log(H^+)$ mol/l
- In the mixing of two different pH values, the new amount of $H^+$ ions are calculated in one litre of water mix which is then a new pH value of the new make-up water.

For example, a recirculation system has a 100l capacity and 95% of recirculation. The inlet water has the pH=8, and the water after carbon dioxide stripper has pH=7.5.

a) What is the new pH of the new makeup water?

1. The amount of makeup water is 5 l with pH=8
   $pH = -\log (H^+)$ mol/l
   $pH = -\log (10^{-8})$ mol/l
   $H^+ = 5*10^{-8}$ mol
2. The amount of new makeup water is 95l with pH=7.5
   $pH = -\log (10^{-7.5})$
   $H^+ = 95*10^{-7.5}$ mol
3. New pH
   $pH= -\log ((5*10^{-8} \text{ mol} + 95*10^{-7.5} \text{ mol})/100l)$
   pH= 7.51

a) What is the new alkalinity of the water mix:

With the same example, inlet water has the alkalinity of 100mg/l $CaCO_3$ and the water after carbon dioxide stripper has 80mg/l of $CaCO_3$ alkalinity. New makeup water alkalinity is:

0,95*80mg/l + 0,05*100mg/l = 81mg/L

The alkalinity of new makeup water is 81mg/l of $CaCO_3$.

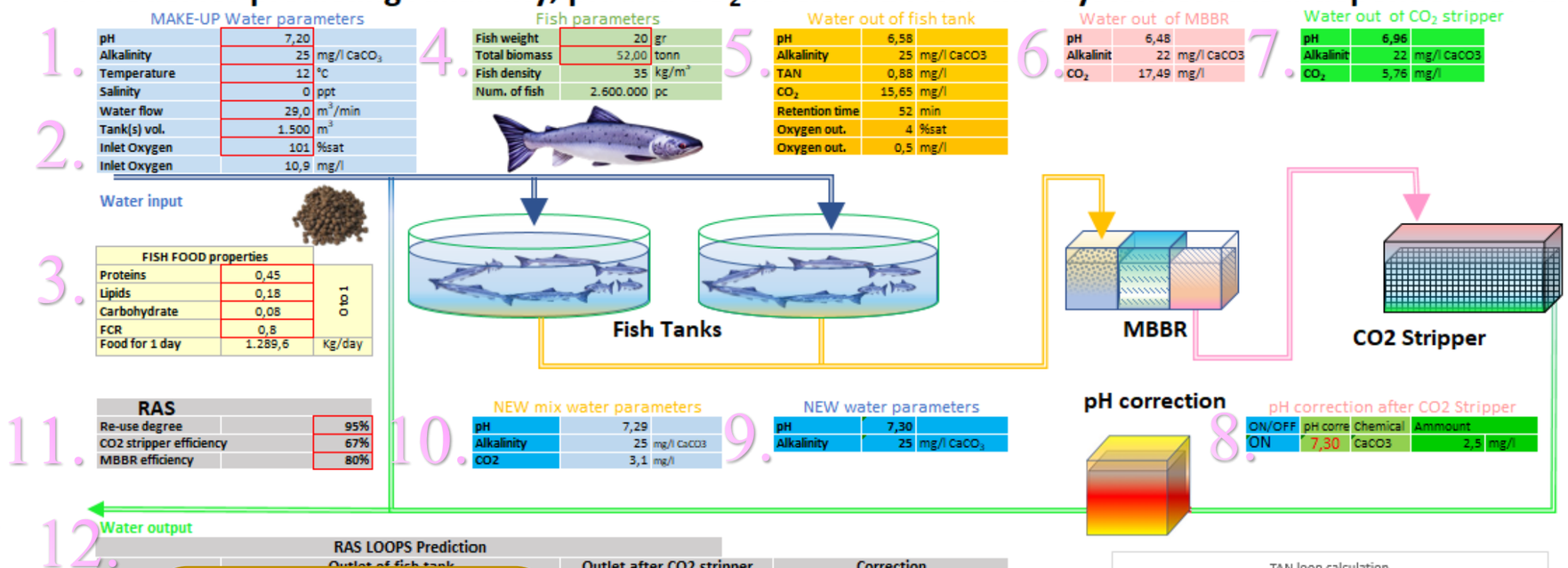
The equations are programmed in Excel to obtain an automatic value for every loop in the model.

## 3.7. The graphical solution

To input and test different values it is more suitable to use the user-friendly graphical interface. This interface is constructed in the Excel, and every input step is described in a short instruction manual flow chart (Figure 3-2) to explain how to trace and write the values. Numbers in the flowchart are following the numbers for input values. In the pH correction tab, the unit can be turned on or off, and the chemical used can be changed to NaOH or $CaCO_3$. Because the model itself is calculating the values thru ten different loops, in every new loop user must decide how much chemical to add and which chemical to use.

The programed Excel sheet calculates all other measurements for every loop. The sheet is going thru 10 different loops. In every other loop programmed table is taking the values for New mix water parameters from the previous loop as the Make-up water. Make-up water has new alkalinity and pH, and all other input values stay the same: tank(s) capacity, temperature, fish size, food parameters, salinity.

# A model for predicting Alkalinity, pH and $CO_2$ levels in a Flow-thru system or RAS independent of salinity




1. **MAKE-UP WATER PARAMETERS** measured or given:
   - pH,
   - Alkalinity in mg/l $CaCO_3$
   - Temperature in °C
   - Salinity in ppt

2. Inlet technical parameters of the farm or production unit
   - Water flow in $m^3$/min
   - Total tank capacity in $m^3$
   - Oxygen inlet in %sat

3. **FISH FOOD PARAMETERS** – food ingredients:
   - Proteins
   - Lipids
   - Carbohydrates
   - FCR
   - Food for one day in kg/day is calculated

4. **FISH PARAMETERS**:
   - Average single fish weight
   - Total biomass
   - Fish density and total number are calculated

5. Program calculates from input data the new values for **WATER OUT OF FISH TANK**
   - pH
   - Alkalinity
   - TAN
   - Total $CO_2$ in mg/l
   - Retention time in minute
   - Oxygen level in mg/l and %sat

6. Program calculates **WATER OUT OF** MBBR®:
   - pH
   - Alkalinity
   - Total $CO_2$ in mg/l

7. Program calculates outlet values **WATER OUT OF $CO_2$ STRIPPER:**
   - pH
   - Alkalinity
   - Total $CO_2$ in mg/l

8. **pH CORRECTION** after the $CO_2$ stripper with NaOH, or $CaCO_3$, and recalculation of the new alkalinity and pH. This step is optional, and it can be turned on or off in loop red fields

9. - **NEW WATER PARAMETERS** according to the pH correction
   - If there is not correction the values are same as $CO_2$ out stripper values

10. - **NEW MIX WATER PARAMETERS** are mixed with the make up water, and this is the new water for the next loop

11. **RAS –** in this field parameters for RAS are written. If all values are set to zero, then it is a flow thru system.

12. In **RAS LOOPS PREDICTION** in the red boxes, the pH correction is set ON or OFF, which chemical is used and value of pH change

*Figure 3-2 Model Excel table presentation and steps for calculations*

## 3.8. Application for mobile phone

The first beta mobile phone application (Figure 3-3) is developed in the online tool OpenAsAPP (OpenAsApp, 2018) for translating the Excel sheet into the mobile phone adapted interface. The application is still in its beta phase (software is still in development and contains known and unknown bugs) because the online tool itself has its limitations. One of the main limitations is the use of iterative formulas, repeating formulas in Excel. The program is fully functional for prediction without correction of pH. The pH correction works only for NaOH as a chemical medium. The phone application is giving the same numbers as the Excel sheet, and the testing and presenting of the results is the same as with the Excel graphical solution.

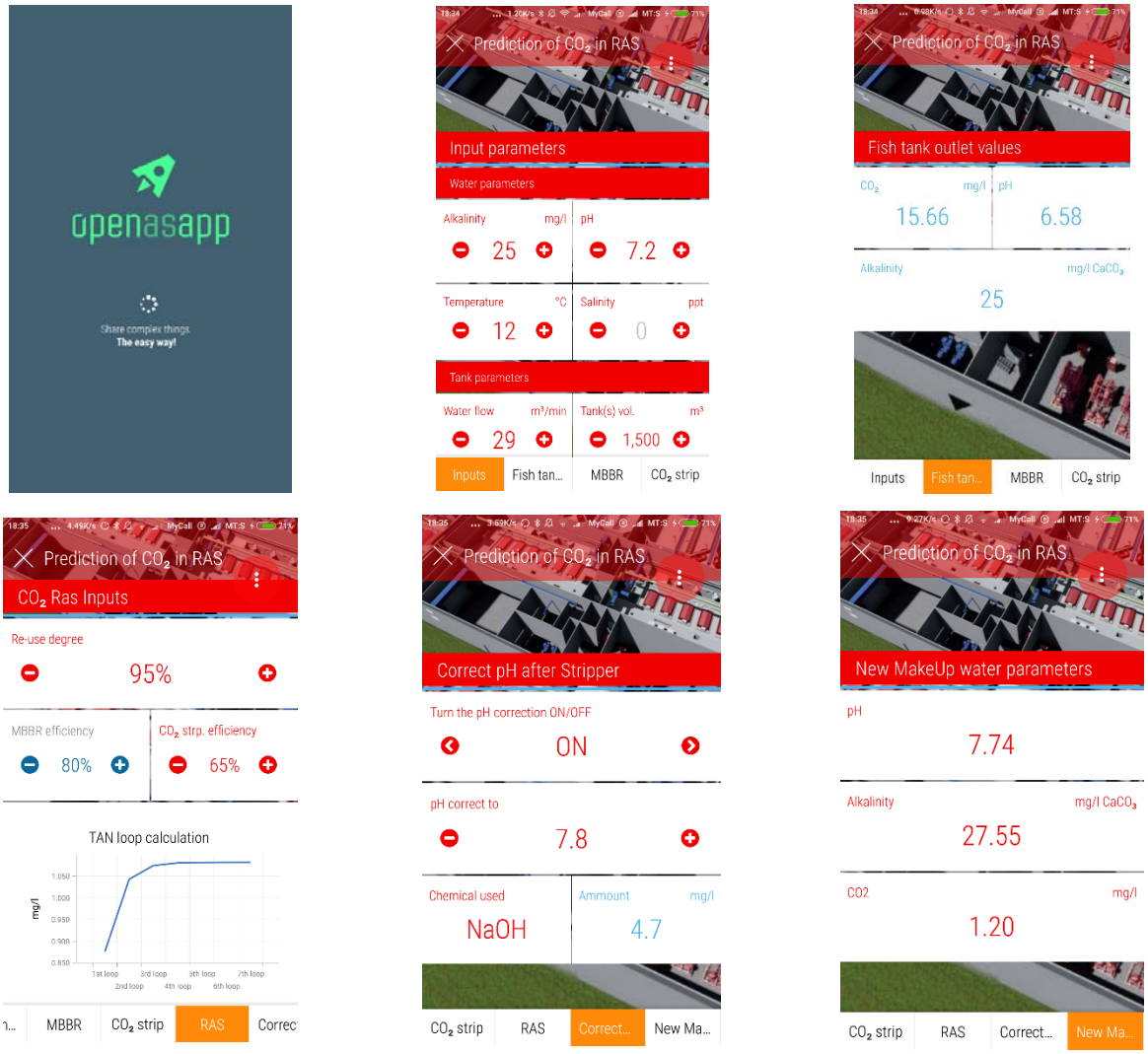



*Figure 3-3 Screenshots of an Android application, view-thru several different screens*

# 4. TEORETICAL MODEL VERIFICATION

Within this chapter, the inlet values obtained from the fish farms and the outlet values that program calculates are presented. All farms have installed RAS systems. Results are obtained from the farm with fresh water, brackish water, and from freshwater systems with ground water inlet source with high alkalinity and pH.

## 4.1. Values for the first case farm

### 4.1.1. Inlet values

Inlet values are entered in the Excel sheet in the red colour boxes to avoid a mistake (Table 6). The RAS system is set up to 95% recirculation, 67% carbon dioxide stripper efficiency 80% biological filter efficiency.

*Table 6 Inlet parameters for water quality, fish farm capacity, fish food and fish size for the first case farm with 0 ppt salinity*

| **Make-up Water parameters** | | |
|---|---|---|
| **pH** | 7,2 | |
| **Alkalinity** | 25 | mg/l $CaCO_3$ |
| **Temperature** | 12 | °C |
| **Salinity** | 0 | ppt |
| **Water flow** | 29 | $m^3$/min |
| **Tank(s) vol.** | 1500 | $m^3$ |
| **Inlet Oxygen** | 101 | %sat |
| **Inlet oxygen** | 10,9 | mg/l |

| **FISH parameters** | | |
|---|---|---|
| **Fish weight** | 20 | gr |
| **Total biomass** | 52 | ton |
| **Fish density** | 35 | kg/$m^3$ |
| **Num. of fish** | 2600000 | pc |

| **FISH FOOD properties** | | |
|---|---|---|
| **Proteins** | 0,45 | 0 to 1 |
| **Lipids** | 0,18 | |
| **Carbohydrate** | 0,08 | |
| **FCR** | 0,8 | |
| **Food for one day** | 1290 | Kg/day |

### 4.1.2. Outlet values calculated by the program

Outlet values are calculated automatically, and the program shows directly on the sheet (Table 7) values for every production unit in the first pass thru recirculation system.

*Table 7 Calculated outlet values for the first case farm water out of the fish tank, out of the biological filter (MBBR) and out of the carbon dioxide stripper for the first loop*

| **Water out of the fish tank** | | |
|---|---|---|
| **pH** | 6,58 | |
| **Alkalinity** | 25 | mg/l $CaCO_3$ |
| **TAN** | 0,9 | mg/l |
| **$CO_2$** | 15,6 | mg/l |
| **Retention time** | 52 | min |
| **Oxygen outlet** | 4,2 | %sat |
| **Oxygen outlet** | 0,5 | mg/l |

| **Water out of MBBR** | | |
|---|---|---|
| **pH** | 6,48 | |
| **Alkalinity** | 22 | mg/l $CaCO_3$ |
| **$CO_2$** | 17,5 | mg/l |

| **Water out of $CO_2$ stripper** | | |
|---|---|---|
| **pH** | 6,96 | |
| **Alkalinity** | 22 | mg/l $CaCO_3$ |
| **$CO_2$** | 5,8 | mg/l |

For other nine loops (Table 8), RAS LOOPS PREDICTION table is used for calculation, and the table is showing values for every next loop in the RAS system. In this prediction example pH is not corrected.

*Table 8 RAS loop calculation for the first case farm without pH correction*

| | **RAS LOOPS PREDICTION** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **OUTLET OF FISH TANK** | | | | | **OUTLET OF $CO_2$ STRIPPER** | | | |
| N° loops | pH | Alkalinity | $CO_2$ | TAN | | pH | Alkalinity | $CO_2$ | |
| 1st | 6,58 | 25 | 15,6 | 0,88 | mg/l | 6,96 | 22 | 5,76 | mg/l |
| 2nd | 6,48 | 22 | 17,6 | 1,04 | mg/l | 6,85 | 19 | 6,36 | mg/l |
| 3rd | 6,40 | 19 | 18,2 | 1,07 | mg/l | 6,77 | 16 | 6,54 | mg/l |
| 4th | 6,33 | 17 | 18,4 | 1,08 | mg/l | 6,69 | 13 | 6,60 | mg/l |
| 5th | 6,26 | 14 | 18,6 | 1,08 | mg/l | 6,59 | 11 | 6,63 | mg/l |
| 6th | 6,17 | 12 | 18,7 | 1,08 | mg/l | 6,48 | 8 | 6,64 | mg/l |
| 7th | 6,07 | 9 | 19,0 | 1,08 | mg/l | 6,35 | 6 | 6,64 | mg/l |
| 8th | 5,95 | 7 | 19,3 | 1,08 | mg/l | 6,16 | 4 | 6,53 | mg/l |
| 9th | 5,78 | 5 | 20,0 | 1,08 | mg/l | 5,93 | 2 | 5,35 | mg/l |
| 10th | 5,56 | 3 | 20,8 | 1,08 | mg/l | 14,00 | 0 | 0,00 | mg/l |

The value for the TAN becomes steady after four loops on the value of 1,08 mg/l.

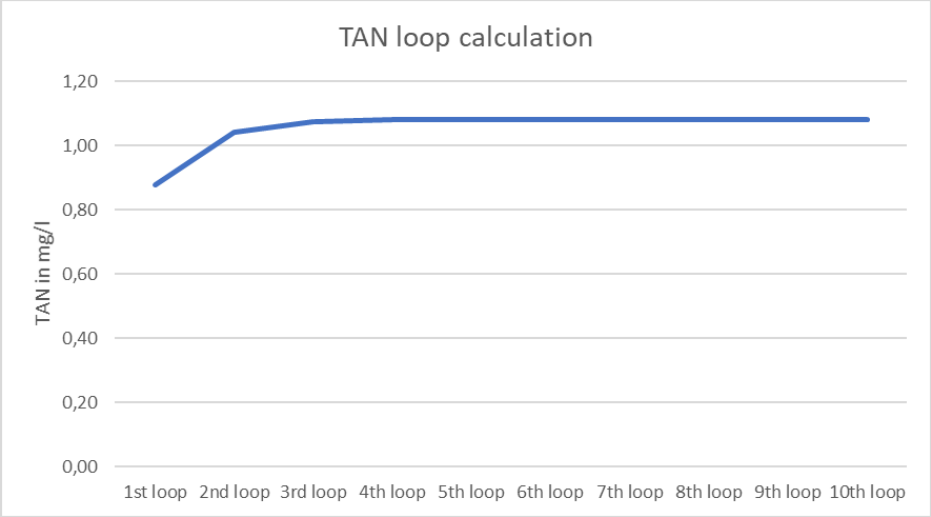



*Chart 1 TAN steady value with biofilter efficiency of 80% in the first case farm*

In the second step, pH is corrected with NaOH as a medium to maintain alkalinity on the level of 25 mg/l of $CaCO_3$ (Table 9). Goal-Seek tool in Excel is searching the optimal value of pH correction to reach a value of 25 mg/l of alkalinity in every loop, changing the pH in the previous loop to affect the next one.

*Table 9 RAS loop calculation with the pH correction using NaOH after the $CO_2$ stripper in the first case farm*

| | **RAS LOOPS PREDICTION** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **OUTLET OF FISH TANK** | | | | | **OUTLET OF $CO_2$ STRIPPER** | | | | **pH CORRECTION** | | | |
| N° loops | pH | Alk | $CO_2$ | TAN | unit | pH | Alk | $CO_2$ | | pH corr. | Chemical | Value | unit |
| 1st | 6,58 | 25 | 15,6 | 0,88 | mg/l | 6,96 | 22 | 5,76 | mg/l | 7,30 | NaOH | 2,50 | mg/l |
| 2nd | 6,60 | 25 | 14,9 | 1,04 | mg/l | 6,98 | 22 | 5,54 | mg/l | 7,33 | NaOH | 2,50 | mg/l |
| 3rd | 6,61 | 25 | 14,7 | 1,07 | mg/l | 6,98 | 22 | 5,47 | mg/l | 7,34 | NaOH | 2,50 | mg/l |
| 4th | 6,61 | 25 | 14,7 | 1,08 | mg/l | 6,98 | 22 | 5,45 | mg/l | 7,34 | NaOH | 2,50 | mg/l |
| 5th | 6,61 | 25 | 14,6 | 1,08 | mg/l | 6,98 | 22 | 5,45 | mg/l | 7,34 | NaOH | 2,50 | mg/l |
| 6th | 6,61 | 25 | 14,6 | 1,08 | mg/l | 6,98 | 22 | 5,45 | mg/l | 7,34 | NaOH | 2,50 | mg/l |
| 7th | 6,61 | 25 | 14,6 | 1,08 | mg/l | 6,98 | 22 | 5,44 | mg/l | 7,34 | NaOH | 2,50 | mg/l |
| 8th | 6,61 | 25 | 14,6 | 1,08 | mg/l | 6,98 | 22 | 5,44 | mg/l | 7,34 | NaOH | 2,50 | mg/l |
| 9th | 6,61 | 25 | 14,6 | 1,08 | mg/l | 6,98 | 22 | 5,44 | mg/l | 7,34 | NaOH | 2,50 | mg/l |
| 10th | 6,61 | 25 | 14,6 | 1,08 | mg/l | 6,98 | 22 | 5,44 | mg/l | | NaOH | | |

The same principle is used for correction of pH with $CaCO_3$ in the RAS loop (Table 10). Targeted value is 25 mg/l of alkalinity in the fish tank outlet. Values that are changed are in the red boxes.

*Table 10 RAS loop calculation with the pH correction using $CaCO_3$ after the $CO_2$ stripper in the first case farm*

| | **RAS LOOPS PREDICTION** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **OUTLET OF FISH TANK** | | | | | **OUTLET OF $CO_2$ STRIPPER** | | | | **pH CORRECTION** | | |
| N° loops | pH | Alk | $CO_2$ | TAN | unit | pH | Alk | $CO_2$ | | pH corr. | Chemical | Value | unit |
| 1st | 6,58 | 25 | 15,6 | 0,88 | mg/l | 6,96 | 22 | 5,76 | mg/l | 7,12 | $CaCO_3$ | 2,89 | mg/l |
| 2nd | 6,56 | 25 | 16,3 | 1,04 | mg/l | 6,94 | 22 | 5,98 | mg/l | 7,10 | $CaCO_3$ | 2,96 | mg/l |
| 3rd | 6,55 | 25 | 16,5 | 1,07 | mg/l | 6,93 | 21 | 6,03 | mg/l | 7,10 | $CaCO_3$ | 3,09 | mg/l |
| 4th | 6,55 | 25 | 16,5 | 1,08 | mg/l | 6,93 | 21 | 6,03 | mg/l | 7,10 | $CaCO_3$ | 3,10 | mg/l |
| 5th | 6,55 | 25 | 16,5 | 1,08 | mg/l | 6,93 | 21 | 6,03 | mg/l | 7,10 | $CaCO_3$ | 3,10 | mg/l |
| 6th | 6,55 | 25 | 16,5 | 1,08 | mg/l | 6,93 | 21 | 6,03 | mg/l | 7,10 | $CaCO_3$ | 3,10 | mg/l |
| 7th | 6,55 | 25 | 16,5 | 1,08 | mg/l | 6,93 | 21 | 6,03 | mg/l | 7,10 | $CaCO_3$ | 3,10 | mg/l |
| 8th | 6,55 | 25 | 16,5 | 1,08 | mg/l | 6,93 | 21 | 6,03 | mg/l | 7,10 | $CaCO_3$ | 3,10 | mg/l |
| 9th | 6,55 | 25 | 16,5 | 1,08 | mg/l | 6,93 | 21 | 6,03 | mg/l | 7,10 | $CaCO_3$ | 3,10 | mg/l |
| 10th | 6,55 | 25 | 16,5 | 1,08 | mg/l | 6,93 | 21 | 6,03 | mg/l | | $CaCO_3$ | 1,32 | |

## 4.2. Values for the second case farm

### 4.2.1. Inlet values

Inlet values are entered in the Excel sheet in the red colour boxes to avoid a mistake (Table 11). The RAS system is set up to 95% recirculation, 67% carbon dioxide stripper efficiency and 80% biological filter efficiency. It is a brackish water example with 5 ppt salinity setup.

*Table 11 Inlet parameters for water quality, technical capacity, fish food and fish size for the second case farm with 5 ppt salinity*

| **Make-up Water parameters** | | |
|---|---|---|
| **pH** | 7,2 | |
| **Alkalinity** | 70 | mg/l $CaCO_3$ |
| **Temperature** | 7 | °C |
| **Salinity** | 5 | ppt |
| **Water flow** | 77 | $m^3$/min |
| **Tank(s) vol.** | 4.700 | $m^3$ |
| **Inlet Oxygen** | 101 | %sat |
| **Inlet oxygen** | 10,9 | mg/l |

| **FISH parameters** | | |
|---|---|---|
| **Fish weight** | 200 | gr |
| **Total biomass** | 234 | ton |
| **Fish density** | 50 | kg/$m^3$ |
| **Num. of fish** | 1.170.000 | pc |

| **FISH FOOD properties** | | |
|---|---|---|
| **Proteins** | 0,4 | 0 to 1 |
| **Lipids** | 0,2 | |
| **Carbohydrate** | 0,08 | |
| **FCR** | 0,65 | |
| **Food for one day** | 1.521 | Kg/day |

### 4.2.2. Outlet values calculated by the program

Outlet values are calculated automatically, and the program shows directly on the sheet (Table 12) values for every production unit in the first pass thru recirculation system.

*Table 12 Calculated outlet values for the second case farm water out of the fish tank, out of the biological filter and out of the carbon dioxide stripper for the first loop*

| **Water out of the fish tank** | | |
|---|---|---|
| **pH** | 7,02 | |
| **Alkalinity** | 70 | mg/l $CaCO_3$ |
| **TAN** | 0,31 | mg/l |
| **$CO_2$** | 13,3 | mg/l |
| **Retention time** | 61 | min |
| **Oxygen outlet** | 67 | %sat |
| **Oxygen outlet** | 7,9 | mg/l |

| **Water out of MBBR** | | |
|---|---|---|
| **pH** | 6,99 | |
| **Alkalinity** | 69 | mg/l $CaCO_3$ |
| **$CO_2$** | 14,26 | mg/l |

| **Water out of $CO_2$ stripper** | | |
|---|---|---|
| **pH** | 7,47 | |
| **Alkalinity** | 69 | mg/l $CaCO_3$ |
| **$CO_2$** | 4,62 | mg/l |

For other nine loops (Table 13), RAS LOOPS PREDICTION table is used for calculation, and the table is showing values for every next loop in the RAS system. In this prediction example pH is not corrected.

*Table 13 RAS loop calculation for the second case farm without pH correction*

| | **RAS LOOPS PREDICTION** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **OUTLET OF FISH TANK** | | | | | **OUTLET OF $CO_2$ STRIPPER** | | | |
| N° loops | pH | Alkalinity | $CO_2$ | TAN | | pH | Alkalinity | $CO_2$ | |
| 1st | 7,02 | 70 | 13,3 | 0,31 | mg/l | 7,47 | 69 | 4,62 | mg/l |
| 2nd | 7,17 | 69 | 9,2 | 0,36 | mg/l | 7,60 | 68 | 3,39 | mg/l |
| 3rd | 7,23 | 68 | 8,0 | 0,38 | mg/l | 7,64 | 67 | 3,02 | mg/l |
| 4th | 7,24 | 67 | 7,6 | 0,38 | mg/l | 7,65 | 66 | 2,91 | mg/l |
| 5th | 7,24 | 66 | 7,5 | 0,38 | mg/l | 7,65 | 65 | 2,87 | mg/l |
| 6th | 7,24 | 65 | 7,5 | 0,38 | mg/l | 7,65 | 64 | 2,85 | mg/l |
| 7th | 7,24 | 64 | 7,5 | 0,38 | mg/l | 7,65 | 63 | 2,84 | mg/l |
| 8th | 7,23 | 64 | 7,5 | 0,38 | mg/l | 7,64 | 63 | 2,83 | mg/l |
| 9th | 7,23 | 63 | 7,5 | 0,38 | mg/l | 7,64 | 62 | 2,83 | mg/l |
| 10th | 7,22 | 62 | 7,4 | 0,38 | mg/l | 7,63 | 61 | 2,82 | mg/l |

The value for the TAN becomes steady after three loops on the value of 0,38 mg/l.

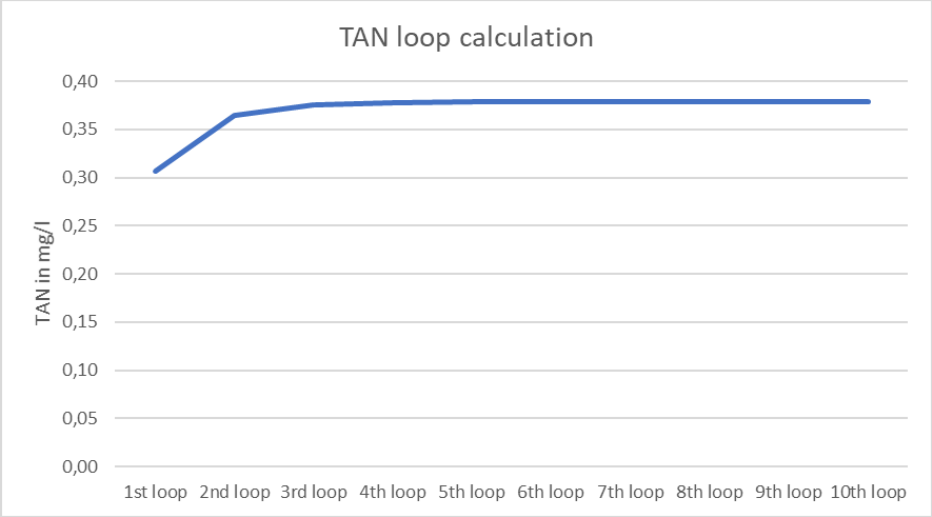



*Chart 2 TAN steady value presented in a chart with biofilter efficiency of 80% in the second case farm*

In the second step, pH is corrected with NaOH as a medium to maintain alkalinity on the level of 70 mg/l of $CaCO_3$ (Table 14). Goal-Seek tool in Excel is searching the optimal value of pH correction to reach a value of 70 mg/l of alkalinity in every loop, changing the pH in the previous loop to affect the next one.

*Table 14 RAS loop calculation with the pH correction using NaOH after the $CO_2$ stripper in the second case farm*

| | **RAS LOOPS PREDICTION** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **OUTLET OF FISH TANK** | | | | | **OUTLET OF $CO_2$ STRIPPER** | | | | **pH CORRECTION** | | | |
| N° loops | pH | Alk | $CO_2$ | TAN | unit | pH | Alk | $CO_2$ | | pH corr. | Chemical | Value | unit |
| 1st | 7,02 | 70 | 13,3 | 0,31 | mg/l | 7,47 | 69 | 4,62 | mg/l | 7,57 | NaOH | 0,88 | mg/l |
| 2nd | 7,22 | 70 | 8,3 | 0,36 | mg/l | 7,64 | 69 | 3,13 | mg/l | 7,78 | NaOH | 0,88 | mg/l |
| 3rd | 7,31 | 70 | 6,9 | 0,38 | mg/l | 7,70 | 69 | 2,70 | mg/l | 7,86 | NaOH | 0,88 | mg/l |
| 4th | 7,34 | 70 | 6,5 | 0,38 | mg/l | 7,72 | 69 | 2,58 | mg/l | 7,88 | NaOH | 0,88 | mg/l |
| 5th | 7,34 | 70 | 6,3 | 0,38 | mg/l | 7,73 | 69 | 2,54 | mg/l | 7,89 | NaOH | 0,88 | mg/l |
| 6th | 7,35 | 70 | 6,3 | 0,38 | mg/l | 7,73 | 69 | 2,53 | mg/l | 7,89 | NaOH | 0,88 | mg/l |
| 7th | 7,35 | 70 | 6,3 | 0,38 | mg/l | 7,73 | 69 | 2,53 | mg/l | 7,89 | NaOH | 0,88 | mg/l |
| 8th | 7,35 | 70 | 6,3 | 0,38 | mg/l | 7,73 | 69 | 2,53 | mg/l | 7,89 | NaOH | 0,88 | mg/l |
| 9th | 7,35 | 70 | 6,3 | 0,38 | mg/l | 7,73 | 69 | 2,53 | mg/l | 7,89 | NaOH | 0,88 | mg/l |
| 10th | 7,35 | 70 | 6,3 | 0,38 | mg/l | 7,73 | 69 | 2,53 | mg/l | | NaOH | | |

The same principle is used to maintain alkalinity on the level of 70 mg/l with $CaCO_3$ as a pH chemical correction (Table 15).

*Table 15 RAS loop calculation with the pH correction using $CaCO_3$ after the $CO_2$ stripper in the second case farm*

| | **RAS LOOPS PREDICTION** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **OUTLET OF FISH TANK** | | | | | **OUTLET OF $CO_2$ STRIPPER** | | | | **pH CORRECTION** | | | |
| N° loops | pH | Alk | $CO_2$ | TAN | unit | pH | Alk | $CO_2$ | | pH corr. | Chemical | Value | unit |
| 1st | 7,02 | 70 | 13,3 | 0,31 | mg/l | 7,47 | 69 | 4,62 | mg/l | 7,50 | $CaCO_3$ | 0,59 | mg/l |
| 2nd | 7,19 | 70 | 9,0 | 0,36 | mg/l | 7,61 | 68 | 3,32 | mg/l | 7,71 | $CaCO_3$ | 1,60 | mg/l |
| 3rd | 7,28 | 70 | 7,3 | 0,38 | mg/l | 7,68 | 69 | 2,83 | mg/l | 7,73 | $CaCO_3$ | 0,69 | mg/l |
| 4th | 7,29 | 70 | 7,2 | 0,38 | mg/l | 7,69 | 69 | 2,78 | mg/l | 7,77 | $CaCO_3$ | 1,19 | mg/l |
| 5th | 7,30 | 70 | 6,9 | 0,38 | mg/l | 7,70 | 69 | 2,71 | mg/l | 7,80 | $CaCO_3$ | 1,42 | mg/l |
| 6th | 7,32 | 70 | 6,8 | 0,38 | mg/l | 7,71 | 69 | 2,67 | mg/l | 7,80 | $CaCO_3$ | 1,29 | mg/l |
| 7th | 7,32 | 70 | 6,8 | 0,38 | mg/l | 7,71 | 69 | 2,67 | mg/l | 7,80 | $CaCO_3$ | 1,28 | mg/l |
| 8th | 7,32 | 70 | 6,8 | 0,38 | mg/l | 7,71 | 69 | 2,67 | mg/l | 7,75 | $CaCO_3$ | 0,58 | mg/l |
| 9th | 7,30 | 70 | 7,1 | 0,38 | mg/l | 7,70 | 69 | 2,75 | mg/l | 7,80 | $CaCO_3$ | 1,50 | mg/l |
| 10th | 7,32 | 70 | 6,8 | 0,38 | mg/l | 7,71 | 69 | 2,67 | mg/l | | $CaCO_3$ | | |

## 4.3. The values from freshwater systems with ground water inlet source with high alkalinity and pH - RAS 1, RAS 2, and RAS 3

Systems are working in different fish size and different densities. In these systems, there is no carbon dioxide stripper and no pH correction for the alkalinity maintenance. Alkalinity is controlled by using the high buffering capacity of the municipal inlet water from 75-100mg/l of $CaCO_3$ and pH=8, and groundwater with alkalinity 175mg/l of $CaCO_3$ and pH=8,3. The mix of the two waters behaves as a pH correction unit (Hansen, 2018). Biological filter efficiency is set up to 80%, and the RAS level will be presented for specific RAS systems in examples.

### 4.3.1. Inlet water values – RAS 1

The inlet water parameters from the fish lab are given after the biological filter. The program is tuned to suit the outlet values in the biofilter with approximate numbers for the water inlet (Table 16). Values after the biofilter are 65 mg/l of alkalinity, 12.8°C, and pH=7.5. The RAS efficiency is calculated 97%. TAN measured is less than 0,05mg/l. Total biomass is calculated from the given food amount. In this example, the total food is 2,5 kg/day which is 2,5% of total biomass which gives 100kg of total biomass. Assumed fish size is 20 grams.

*Table 16 RAS 1 inlet water values, fish size values, fish food properties, and overall capacity*

| **Make-up Water parameters** | | |
|---|---|---|
| **pH** | 8,00 | |
| **Alkalinity** | 100 | mg/l $CaCO_3$ |
| **Temperature** | 12,8 | °C |
| **Salinity** | 0 | ppt |
| **Water flow** | 0,2 | $m^3$/min |
| **Tank(s) vol.** | 10 | $m^3$ |
| **Inlet Oxygen** | 101 | %sat |
| **Inlet oxygen** | 10,9 | mg/l |

| **FISH parameters** | | |
|---|---|---|
| **Fish weight** | 20 | gr |
| **Total biomass** | 0,1 | ton |
| **Fish density** | 10 | kg/$m^3$ |
| **Num. of fish** | 5000 | pc |

| **FISH FOOD properties** | | |
|---|---|---|
| **Proteins** | 0,4 | 0 to 1 |
| **Lipids** | 0,2 | |
| **Carbohydrate** | 0,08 | |
| **FCR** | 0,8 | |
| **Food for one day** | 2,5 | Kg/day |

### 4.3.2. Outlet values calculated by the program – RAS 1

Outlet values are calculated automatically, and the program shows directly in the sheet (Table 17) values for every production unit in the first pass thru recirculation system.

*Table 17 Calculated outlet values for the RAS 1 water out of the fish tank, out of the biological filter (MBBR) and for the first loop in details*

| **Water out of the fish tank** | | |
|---|---|---|
| **pH** | 7,64 | |
| **Alkalinity** | 100 | mg/l $CaCO_3$ |
| **TAN** | 0,22 | mg/l |
| **$CO_2$** | 5,41 | mg/l |
| **Retention time** | 48 | min |
| **Oxygen outlet** | 74 | %sat |
| **Oxygen outlet** | 8,0 | mg/l |

| **Water out of MBBR** | | |
|---|---|---|
| **pH** | 7,61 | |
| **Alkalinity** | 99 | mg/l $CaCO_3$ |
| **$CO_2$** | 5,80 | mg/l |

Outlet values for the other nine loops are calculated automatically by the program (Table 18). There is no carbon dioxide regulation, and the biofilter efficiency is set up to 80%.

*Table 18 Ten RAS loop calculation for the RAS 1*

| | **RAS LOOPS PREDICTION** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **OUTLET OF FISH TANK** | | | | | **OUTLET OF BIOLOGICAL FILTER** | | | |
| N° loops | pH | Alkalinity | $CO_2$ | TAN | | pH | Alkalinity | $CO_2$ | |
| 1st | 7,64 | 100 | 5,4 | 0,22 | mg/l | 7,61 | 99 | 5,80 | mg/l |
| 2nd | 7,42 | 99 | 8,9 | 0,26 | mg/l | 7,41 | 98 | 9,11 | mg/l |
| 3rd | 7,28 | 98 | 12,1 | 0,27 | mg/l | 7,28 | 98 | 12,19 | mg/l |
| 4th | 7,18 | 98 | 15,1 | 0,27 | mg/l | 7,18 | 97 | 15,04 | mg/l |
| 5th | 7,11 | 97 | 17,9 | 0,27 | mg/l | 7,11 | 96 | 17,67 | mg/l |
| 6th | 7,05 | 96 | 20,5 | 0,27 | mg/l | 7,05 | 96 | 20,10 | mg/l |
| 7th | 7,00 | 96 | 22,8 | 0,27 | mg/l | 7,00 | 95 | 22,36 | mg/l |
| 8th | 6,95 | 95 | 25,0 | 0,27 | mg/l | 6,96 | 94 | 24,44 | mg/l |
| 9th | 6,92 | 94 | 27,1 | 0,27 | mg/l | 6,92 | 94 | 26,36 | mg/l |
| 10th | 6,88 | 94 | 28,9 | 0,27 | mg/l | 6,89 | 93 | 28,15 | mg/l |

### 4.3.3. Inlet water values – RAS 2

Inlet values after the biofilter for RAS 2 are 65 mg/l of alkalinity, 13.3°C, and pH=7.4. The program is tuned to suit the outlet values in the biofilter with approximate numbers for the water inlet (Table 19). The RAS 2 efficiency is calculated 98%. TAN measured is less than 0,05mg/l. Total biomass is calculated from the given food amount. In this example, the total food is 2,2 kg/day which is 2,5% of total biomass which gives 80kg of total biomass. Assumed fish size is 20 grams.

*Table 19 RAS 2 inlet water values, fish size values, fish food properties, and overall capacity*

| **Make-up Water parameters** | | |
|---|---|---|
| **pH** | 7,80 | |
| **Alkalinity** | 65 | mg/l $CaCO_3$ |
| **Temperature** | 13,3 | °C |
| **Salinity** | 0 | ppt |
| **Water flow** | 0,4 | $m^3$/min |
| **Tank(s) vol.** | 22 | $m^3$ |
| **Inlet Oxygen** | 101 | %sat |
| **Inlet oxygen** | 10,9 | mg/l |

| **FISH parameters** | | |
|---|---|---|
| **Fish weight** | 20 | gr |
| **Total biomass** | 0,08 | ton |
| **Fish density** | 4 | kg/$m^3$ |
| **Num. of fish** | 4.000 | pc |

| **FISH FOOD properties** | | |
|---|---|---|
| **Proteins** | 0,4 | 0 to 1 |
| **Lipids** | 0,2 | |
| **Carbohydrate** | 0,08 | |
| **FCR** | 0,8 | |
| **Food for one day** | 2,2 | Kg/day |

### 4.3.4. Outlet values calculated by the program – RAS 2

Outlet values are calculated automatically, and the program shows directly in the sheet (Table 20) values for every production unit in the first pass thru recirculation system.

*Table 20 Calculated outlet values for the RAS 2 water out of the fish tank, out of the biological filter (MBBR) and for the first loop in details*

| **Water out of the fish tank** | | |
|---|---|---|
| **pH** | 7,60 | |
| **Alkalinity** | 65 | mg/l $CaCO_3$ |
| **TAN** | 0,10 | mg/l |
| **$CO_2$** | 3,85 | mg/l |
| **Retention time** | 58 | min |
| **Oxygen outlet** | 88 | %sat |
| **Oxygen outlet** | 9,3 | mg/l |

| **Water out of MBBR** | | |
|---|---|---|
| **pH** | 7,58 | |
| **Alkalinity** | 65 | mg/l $CaCO_3$ |
| **$CO_2$** | 3,96 | mg/l |

Outlet values for the other nine loops are calculated automatically by the program (Table 21). There is no carbon dioxide regulation in the RAS 2, and the biofilter efficiency is set up to 80%.

*Table 21 Ten RAS loop calculation for the RAS 2*

| | **RAS LOOPS PREDICTION** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **OUTLET OF FISH TANK** | | | | | **OUTLET OF BIOLOGICAL FILTER** | | | |
| N° loops | pH | Alkalinity | $CO_2$ | TAN | | pH | Alkalinity | $CO_2$ | |
| 1st | 7,60 | 65 | 3,8 | 0,10 | mg/l | 7,58 | 65 | 3,96 | mg/l |
| 2nd | 7,45 | 65 | 5,4 | 0,12 | mg/l | 7,44 | 64 | 5,42 | mg/l |
| 3rd | 7,34 | 64 | 6,8 | 0,12 | mg/l | 7,34 | 64 | 6,77 | mg/l |
| 4th | 7,27 | 64 | 8,1 | 0,13 | mg/l | 7,27 | 64 | 8,03 | mg/l |
| 5th | 7,20 | 64 | 9,3 | 0,13 | mg/l | 7,21 | 63 | 9,19 | mg/l |
| 6th | 7,15 | 63 | 10,5 | 0,13 | mg/l | 7,16 | 63 | 10,26 | mg/l |
| 7th | 7,11 | 63 | 11,5 | 0,13 | mg/l | 7,12 | 63 | 11,24 | mg/l |
| 8th | 7,07 | 63 | 12,5 | 0,13 | mg/l | 7,08 | 62 | 12,16 | mg/l |
| 9th | 7,04 | 62 | 13,4 | 0,13 | mg/l | 7,05 | 62 | 13,00 | mg/l |
| 10th | 7,01 | 62 | 14,2 | 0,13 | mg/l | 7,02 | 62 | 13,78 | mg/l |

### 4.3.1. Inlet water values – RAS 3

Inlet values after the biofilter for RAS 3 are 90 mg/l of alkalinity, the temperature of 12.8°C, and pH=8.1. The program is tuned to suit the outlet values in the biofilter with approximate numbers for the water inlet (Table 22). The RAS 3 efficiency is calculated 87%. TAN measured is less than 0,05mg/l. Total biomass is calculated from the given food amount. In this example, the total food is 0,3 kg/day which is 2,5% of total biomass which gives 12 kg of total biomass. Assumed fish size is 50 grams.

*Table 22 RAS 3 inlet water values, fish size values, fish food properties, and overall capacity*

| **Make-up Water parameters** | | |
|---|---|---|
| **pH** | 8,10 | |
| **Alkalinity** | 90 | mg/l $CaCO_3$ |
| **Temperature** | 12,8 | °C |
| **Salinity** | 0 | ppt |
| **Water flow** | 0,1 | $m^3$/min |
| **Tank(s) vol.** | 6 | $m^3$ |
| **Inlet Oxygen** | 101 | %sat |
| **Inlet oxygen** | 10,9 | mg/l |

| FISH FOOD properties | | |
|---|---|---|
| **Proteins** | 0,38 | 0 to 1 |
| **Lipids** | 0,18 | |
| **Carbohydrate** | 0,08 | |
| **FCR** | 1,2 | |
| **Food for one day** | 0,3 | Kg/day |

| **FISH parameters** | | |
|---|---|---|
| **Fish weight** | 50 | gr |
| **Total biomass** | 0,010 | ton |
| **Fish density** | 2 | kg/$m^3$ |
| **Num. of fish** | 200 | pc |

### 4.3.2. Outlet values calculated by the program – RAS 3

Outlet values are calculated automatically, and the program shows directly in the sheet (Table 23) values for every production unit in the first pass thru recirculation system.

*Table 23 Calculated outlet values for the RAS 3 water out of the fish tank, out of the biological filter (MBBR) and for the first loop in details*

| **Water out of the fish tank** | | |
|---|---|---|
| **pH** | 7,60 | |
| **Alkalinity** | 65 | mg/l $CaCO_3$ |
| **TAN** | 0,10 | mg/l |
| **$CO_2$** | 3,85 | mg/l |
| **Retention time** | 58 | min |
| **Oxygen outlet** | 88 | %sat |
| **Oxygen outlet** | 9,3 | mg/l |

| **Water out of MBBR** | | |
|---|---|---|
| **pH** | 7,58 | |
| **Alkalinity** | 65 | mg/l $CaCO_3$ |
| **$CO_2$** | 3,96 | mg/l |

Outlet values for the other nine loops are calculated automatically by the program (Table 24). There is no carbon dioxide regulation in the RAS 3, and the biofilter efficiency is set up to 80%.

*Table 24 Ten RAS loop calculation for the RAS 3*

| | **RAS LOOPS PREDICTION** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **OUTLET OF FISH TANK** | | | | | **OUTLET OF BIOLOGICAL FILTER** | | | |
| N° loops | pH | Alkalinity | $CO_2$ | TAN | | pH | Alkalinity | $CO_2$ | |
| 1st | 7,99 | 90 | 2,1 | 0,04 | mg/l | 7,99 | 90 | 2,17 | mg/l |
| 2nd | 7,91 | 90 | 2,6 | 0,05 | mg/l | 7,91 | 90 | 2,60 | mg/l |
| 3rd | 7,85 | 90 | 3,0 | 0,05 | mg/l | 7,85 | 90 | 2,98 | mg/l |
| 4th | 7,80 | 90 | 3,3 | 0,05 | mg/l | 7,81 | 90 | 3,29 | mg/l |
| 5th | 7,77 | 90 | 3,6 | 0,05 | mg/l | 7,77 | 89 | 3,55 | mg/l |
| 6th | 7,74 | 90 | 3,8 | 0,05 | mg/l | 7,75 | 89 | 3,78 | mg/l |
| 7th | 7,72 | 89 | 4,0 | 0,05 | mg/l | 7,72 | 89 | 3,96 | mg/l |
| 8th | 7,70 | 89 | 4,2 | 0,05 | mg/l | 7,71 | 89 | 4,12 | mg/l |
| 9th | 7,69 | 89 | 4,3 | 0,05 | mg/l | 7,69 | 89 | 4,25 | mg/l |
| 10th | 7,68 | 89 | 4,4 | 0,05 | mg/l | 7,68 | 89 | 4,35 | mg/l |

## 4.4.Discussion

The first case farm with 20 gr fishes, inlet water of pH 7.2 and alkalinity of 25 mg/l after ten loops the alkalinity level goes down to zero, and the pH in the outlet in the tenth loop is on the very low level of 5.56. Water inlet is shallow in alkalinity and after ten loops all of the alkalinity is spent by the biofilter. The system is then calibrated first with NaOH and then with $CaCO_3$ to maintain the water in the fish tank outlet on alkalinity level of 25mg/l of $CaCO_3$. When NaOH is used to maintain alkalinity level on 25mg/l $CaCO_3$, the level of carbon dioxide in the outlet stays on the acceptable level of 14.6mg/l in the loops. The water outlet pH of the fish tank is 6.61. If $CaCO_3$ used for alkalinity maintenance in the same system, the pH of the tank is around 6.5, and carbon dioxide level is 16.5 mg/l in every loop.

The specific behaving of chemicals can explain the difference in values for pH and carbon dioxide between NaOH and $CaCO_3$ according to the Deffeyes diagram (Figure 2-13).

In the second case farm with brackish water and salinity on 5ppt, fish size is bigger around 200gr, and alkalinity is 70mg/l of $CaCO_3$. In this example, the alkalinity of the inlet water is higher, the buffering capacity of the water is higher, and the whole system remains more stable during the loops in RAS. Of course, if alkalinity is not maintained, the system will collapse because alkalinity is spent in the biofilter. After ten loops the pH stays on the acceptable level of 7.22, and carbon dioxide is on the level of approximately 7 mg/l.

Maintenance of the system alkalinity in the second case farm is necessary, and it is set to 70 mg/l for both used chemicals. Using NaOH as a chemical, the system stays stable on the pH=7.35 water out of the fish tank and the carbon dioxide level around 6.3 mg/l. With the use of $CaCO_3$, the system requires more adjustments with the quantity of chemical in every loop to stay on the same alkalinity level. The outlet of the fish tank is around pH value of 7.3 and carbon dioxide level is slightly higher, around 6.8 compared to NaOH.

In the ground water inlet RAS systems, the size of fish is predicted to suit the values obtained.

In the RAS 1 after ten loops the predicted pH level is 6.88 and alkalinity 94 mg/l. The level of carbon dioxide is 28,4mg/. In the RAS 2 after ten loops the pH level is 7.01, alkalinity 62mg/l of $CaCO_3$, and carbon dioxide level is 14.2 mg/l. In the RAS 3 system after ten loops, the pH values is 7,68 after ten loops, alkalinity is 89 mg/l of $CaCO_3$ and carbon dioxide 4,4 mg/l. In

all three systems, alkalinity is not spent fast because the systems are not stressed with high fish capacities, and the water inlet has high buffering capacity. The level of carbon dioxide in RAS 1 is higher than it is supposed to meet requirements of 15 mg/l after ten loops in the prediction tool. The systems do not have chemical alkalinity and pH maintenance. The inlet water sources are very high in pH and alkalinity, so the system can be maintained only by using more make-up water in one of the circles or reduce the RAS reuse degree. The systems themselves in real life do not have problems with carbon dioxide(Hansen, 2018)), meaning that some of the carbon dioxide is stripped during aeration, but it is not considered for the calculation, because there is no data of how much is lost during the whole recirculation cycle.

# 5. MODEL WEAKNESSES AND STRENGTHS, RECOMMENDATIONS FOR FUTURE DEVELOPMENT

During model testing, the model showed good results in predicting the amount of carbon dioxide, alkalinity, and pH. The concept of the model is based on the analytical chemistry and the models constructed by different authors and their scientific work. Sanni, Forsberg, and Bergheim (Sanni, 1993) made a prediction model of carbon dioxide, alkalinity, and pH for single-pass systems with calculation of carbon dioxide contribution from fish metabolism. Also, M. Timmons (Timmons, 2010) gave a free online source for using several tools in the prediction of carbon dioxide, alkalinity, and pH in recirculation systems with different inlets. Models were tested and according to the authors work gave fair results as prediction tools compared to the values from existing systems. Models do not have the alkalinity correction, pH and carbon dioxide prediction in recirculation cycles. In the model developed, Deffeyes diagram (Deffeyes, 1965) was used for that purpose. Deffeyes diagram predicts also the pH level in the biological filter and in the carbon dioxide stripper with the alkalinity and total carbon as inlet values for every new circle in RAS. When the alkalinity is corrected, the model takes new values and correct alkalinity and pH to correspond to the required values.

The model itself does not include the exact biofilter carbon dioxide production and alkalinity usage. In the model, the calculated values are for maximum efficiency of biofilter and that means that it will use maximum oxygen and produce the maximum amount of carbon dioxide. The biological filter has different effects on different pH values of the system. For example, if the efficiency is set up to 80%, the overall production of carbon dioxide will also be lower according to the efficiency quotient on specific pH, and alkalinity drop will be less in the total recirculation system. This must be elaborated more in future work.

The model can include several different tools as proposed add-ons such as heavy metal precipitation, medicals precipitation, Nitrites and Nitrates precipitation or particle removal.

The weakness is too many tables that are working together and in developing stage demanded to much manual work of entering different formulas. The future upgrades of the model must be developed in a programming language as, i.e. Python, JavaScript or similar. This will give the opportunity for the model developer to include less manual work with the tables by using the logic of the programming language. In that way, mistakes can be avoided. One more advantage with use of the program languages is unlimited space for graphical

interface design. The design of the application itself is also important because the end-user will only write the values that are obtained from the fish farm instruments.

The final stage in model developing will be the model testing. Using the approach of machine learning algorithms in statistical analysis of the data obtained from the testing, the model can give representative results.

# 6. CONCLUSION

The developed model for predicting alkalinity, pH, and $CO_2$ levels in a flow-thru or RAS system independent of salinity is giving an overview what is happening in the various parts of the recirculation system. The model is developed as a tool to simulate and predict values without projecting the whole system. It can be used in fresh, brackish, or saltwater defining the salinity at the beginning of usage. Different food ingredients, mainly proteins, are profoundly affecting the overall results by increasing the respiratory quotient. Using different chemicals for alkalinity and pH maintenance gave a slight difference in the results, and it must be more elaborated with increased amount of data. The size of the fish is important because smaller fish metabolism uses more oxygen per kg compared to bigger fish and produces more carbon dioxide. Biofilter is spending alkalinity, and that is affecting buffering capacity of the water and the amount of carbon dioxide present. Recirculation degree affects the water quality. With every loop water loses alkalinity and buffering capacity. The pH is going down and carbon dioxide will go up because of specific chemistry of carbon dioxide in the water.

If the model develops with recommendations from the previous chapter, it can be a secure and robust tool to use. With add-ins mentioned the model could be transferred to a future overall simulator tool which can be used for optimising the farming system and technical components included.

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