CO_2 fluctuations in the rearing water cause acid-base perturbations and may increase the likelihood of kidney stone formation in Atlantic salmon and Rainbow trout

NephroReduce

FHF Project: Risk Factors in Norwegian Hatcheries Related to the Development of Nephrocalcinosis in Atlantic Salmon and Rainbow Trout – Reduction and Preventive Measures.

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Introduction

Maintaining normal pH levels in body fluids (acid-base balance) is essential for all animals and represents one of the most fundamental and regulated physiological processes in vertebrates. Fish have evolved a flexible system for regulating pH, utilising manipulation of hydrogen (H^+), ammonium (NH_4^+), hydroxide (OH^-), bicarbonate (HCO_3^-), ammonia (NH_3), phosphate (HPO_4^{3-}) and carbon dioxide (CO_2) levels to adjust to a range of environmental changes. CO_2 and NH_3 are produced by the fish as the end products of metabolizing proteins, fats, and carbohydrates. The HCO_3^-/CO_2 system is the primary buffer system; indeed, it is the ratio of the HCO_3^- concentration to the dissolved CO_2 concentration in the plasma that sets the blood pH; the higher the ratio, the higher the pH, and vice versa. Although the gills are the primary organ for pH regulation, the kidney plays a very important and often overlooked role in acid-base regulation. In this article, we will explain the kidney's function and role in acid-base regulation and how it complements that of the gills in dealing with CO_2 fluctuations in the fish's environment, and indirectly with oxygen (O_2) fluctuations. In this context, when blood is acidified (pH is reduced) during exposure to high CO_2 and/or O_2 (termed respiratory acidosis: "respiratory disturbance"), adjustments in HCO_3^- concentration by the fish can play an important role in bringing the pH back to normal. However, variations in one or both of these environmental parameters can have adverse physiological effects. Finally, we aim to put this in a commercially relevant context by pointing out various risk factors in intensive hatchery production and how such risk factors may be linked to the increasing incidents of kidney stone formation (from now on referred to as nephrocalcinosis) from the recent fish health reports in Norway (2020-2023). Increased understanding of the kidney's role in acid-base regulation in salmonid aquaculture will enable operators at the facilities to make valuable production adjustments to better align with the physiological requirement of the fish and reduce nephrocalcinosis in the hatchery phase.

Several hypotheses have previously been proposed as risk factors for development of nephrocalcinosis. These include high constant CO_2 (hypercapnia) and/or in combination with super-oxygenation (hyperoxia >110% O_2). This combined with the use of additional salinity added to the water, as well as

low magnesium or vitamin E and high selenium or vitamin A concentrations in the diet are all thought to exacerbate nephrocalcinosis. The causative links underlying the pathogenesis and etiology of nephrocalcinosis in teleosts are speculative at best, but acid-base perturbations are assumed to be a central pathophysiological cause. In our recently published review article on acid-base regulation in the fish kidney, an alternative hypothesis for the trigger mechanisms behind nephrocalcinosis was put forth. Based on current knowledge about the kidney's function and role in acid-base regulation we believe that rapid changes from high to normal CO_2 and/or from high to normal O_2 provides the first trigger for fish to develop nephrocalcinosis. Such rapid changes are common during transfer of fish between tanks and systems within land-based facilities. In many cases, nephrocalcinosis is observed histologically in fish as small as 5-7 grams. Nephrocalcinosis occurs in both flow-through systems (FTS) and recirculation aquaculture systems (RAS) using freshwater (FW) and brackish water (BW), and often becomes more severe in larger fish (80-100 grams). Facilities with particularly large changes from high to low CO_2 over time can result in very severe cases of nephrocalcinosis. Such changes, in combination with elevated salinity via the environment (from water or feed) and the use of calcium-based buffers (particular in RAS), can consequently have adverse cumulative effects on the fish following such rapid drops in CO_2.

In this article, we will first discuss physiological functions of the gills and kidneys in relation to acid-base regulation under normal conditions and during a respiratory acidosis (associated with exposure to high CO_2 and/or O_2). Then, we will highlight the kidney's role during rapid changes from high to low CO_2 and outline why this creates adverse physiological changes locally in the kidneys, providing favorable conditions for different types

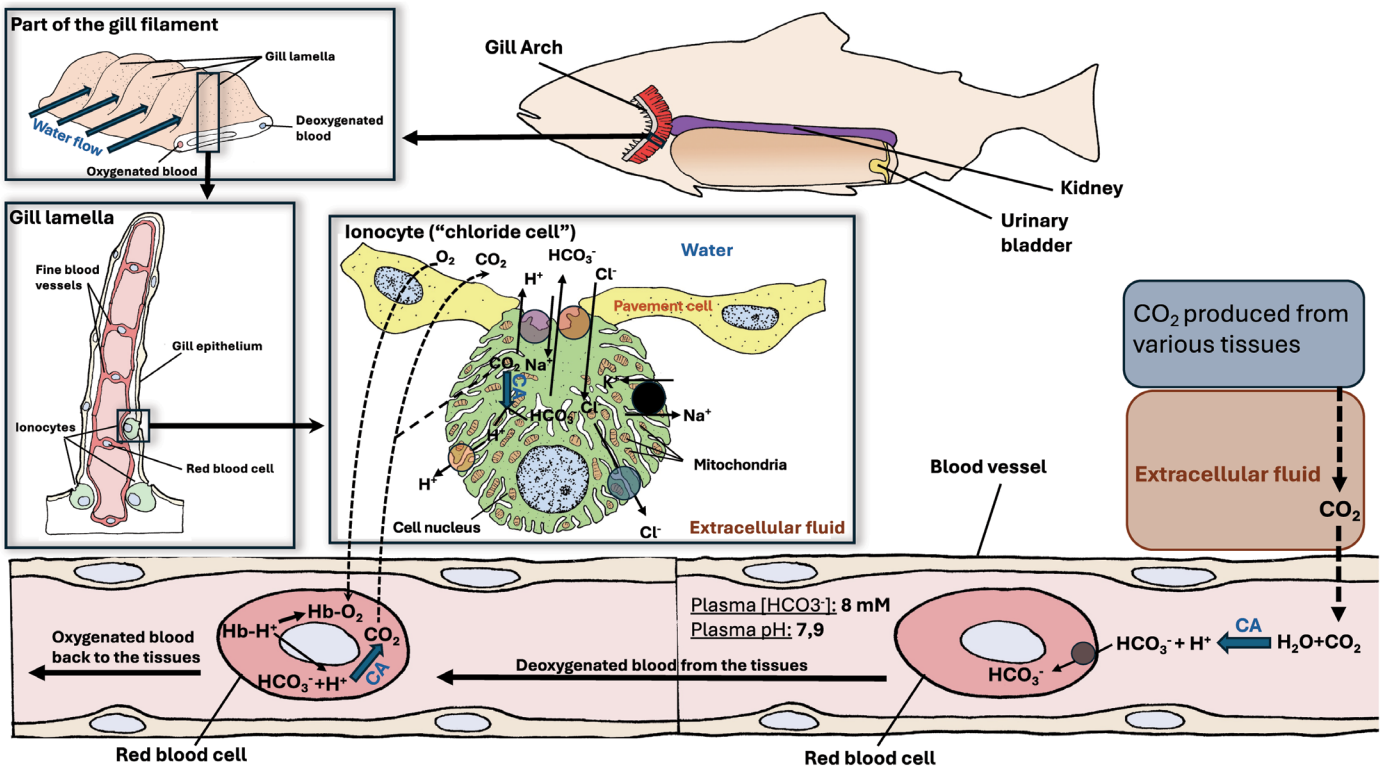

Figure 1: The gills play an important role in removing CO_2 and transporting acidic and basic equivalents.
CO_2 produced via metabolic processes is loaded into the blood as it passes through the metabolic tissues. Once CO_2 enters the blood, the enzyme Carbonic Anhydrase (CA) in the red blood cells rapidly catalyses the conversion of CO_2 to HCO_3^-. Under normal conditions, typical pH values of blood plasma are around 7.9 while HCO_3^- concentrations is approximately 8 mM. When the blood reaches the gill epithelium CA also facilitates the conversion of HCO_3^- back to CO_2, permitting CO_2 to rapidly diffuse through the cell membranes of the pavement cells and into the water, while O_2 diffuses from the water into the blood. As O_2 diffuses into the red blood cells, H^+ ions are released and O_2 binds to the haemoglobin (Hb), which further promotes HCO_3^- dehydration within the red blood cells and CO_2 excretion. The countercurrent flow of water and blood in the gills ensures an effective exchange of CO_2 and O_2 over the gills. The chloride cells normally take up sodium (Na^+) from the external water in exchange for acidic equivalents, and $Cl^-$from the water in exchange for basic equivalents at about the same rate so the pH of the blood is not affected and normal concentrations of Na^+ and Cl^- in the blood plasma are maintained. Under acid-base disturbances the exchange rates of basic and acidic equivalents can be changed to restore normal pH. The HCO_3^-/exchanger (red circle), the Na^+/H^+ exchanger (purple circle), H^+ pump (orange circle), Na^+/K^+ ATPase (NKA; black circle) and Cl^- channel (blue circle) are shown in the ionocyte. CA is shown in blue colour.

of kidney stones to precipitate resulting in nephrocalcinosis. Finally, we will discuss risk factors as well as immediate measures and long-term actions that may prevent and reduce the occurrence of nephrocalcinosis.

Acid-base balance in fish

Acid-base balance generally represents the excretion of acidic equivalents (typically H^+ or NH_4^+) and exchange of base equivalents (OH- or HCO_3^-). Respiratory gases such as CO_2 and NH_3 definitely affect pH in body fluids (CO_2 acidifies while NH_3 alkalinizes). In classical acid-base terms these are not considered acid or base equivalents, but rather "respiratory factors" that affect acid-base balance. Under normal conditions, the typical pH value of body fluids (such as blood plasma) in salmonids is around 7.9 and normal HCO_3^- concentration is about 8 mM. At this pH, the dominating acid-base equivalents in fish body fluids are NH_4^+ and HCO_3^- (generally >95%).

CO_2 is continuously produced via various metabolic processes in all tissues of fish, and must be transported by the blood to the gills, where it can be efficiently removed. This transport is facilitated by a specific enzyme in red blood cells called carbonic anhydrase (CA), which rapidly catalyzes the conversion of CO_2 to HCO_3^- at the tissues (known as CO_2 hydration). This occurs when CO_2 is loaded into the blood as it passes through the metabolic tissues (see **Figure 1**). Conversely, CA also facilitates the conversion of HCO_3^- back to CO_2 (known as HCO_3^- dehydration process) at the gill epithelium **(Figure 1)**. Both CO_2 hydration and HCO_3^- dehydration are crucial to maintaining normal function and can be summarised by this simplified chemical equation: $CO_2 + H_2O \leftrightarrow H^+ + HCO_3^-$. In the absence of CA, the reaction will occur, but very slowly in relation to the transit time of blood through the circulatory system.

Gills

The general understanding of how fish excrete CO_2 over the gills is well defined. The process begins with CO_2 rapidly diffusing from the blood across the gill epithelium into the water, with O_2 diffusion from the water into the blood. Effective CO_2 excretion is dependent upon HCO_3^- dehydration within the red blood cells that is facilitated by CA (see description above) **(Figure 1)**. At the same time, as O_2 diffuses into the red blood cells, H^+ ions are released from the haemoglobin (Hb), which further promotes CO_2 excretion **(Figure 1)**. When blood cells move through the fine blood vessels in the gills, CA enables the conversion of HCO_3^- to CO_2. As CO_2 easily diffuses through cell membranes, it readily moves across the simple pavement cells that constitute

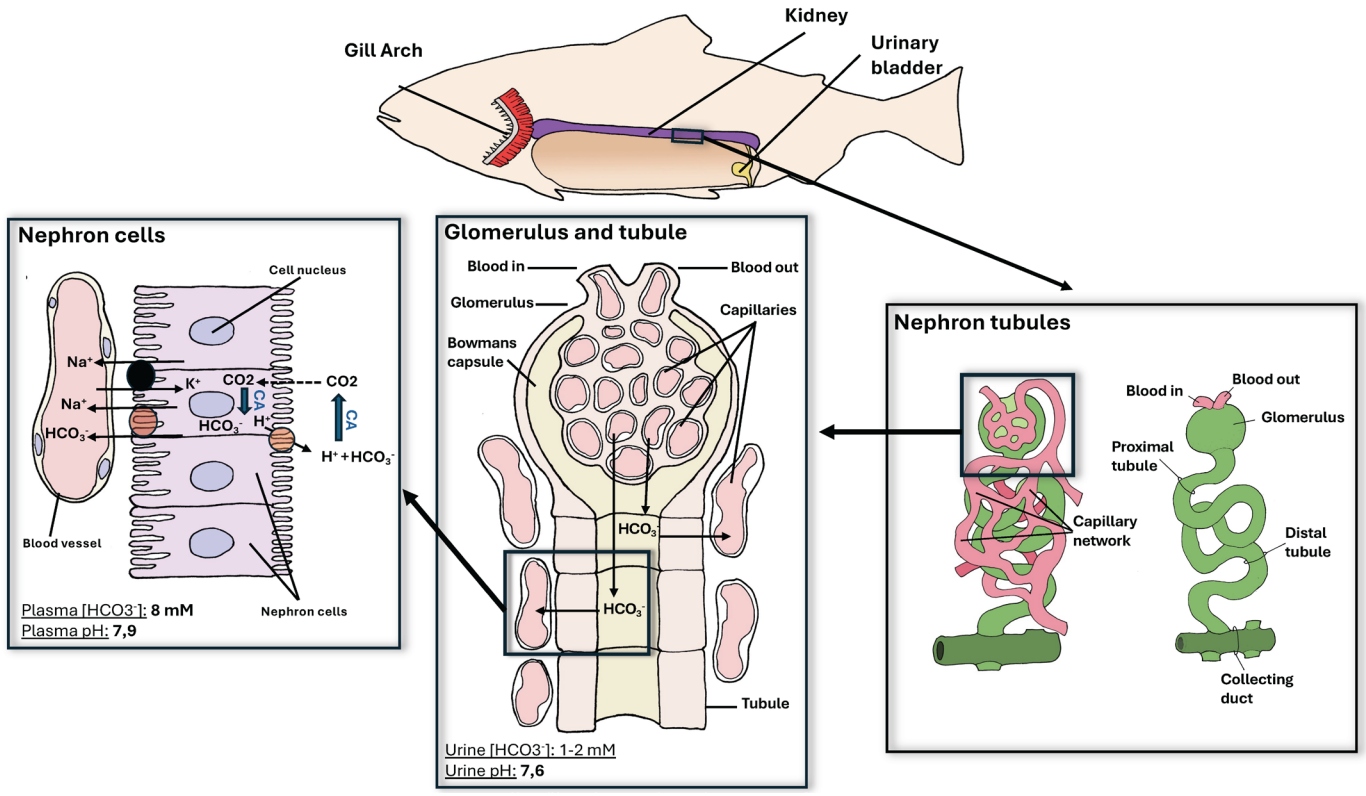

Figure 2: The nephrons in the kidneys play an important in filtering the blood, producing pre-urine and reabsorbing HCO_3^-.
When the pre-urine further moves through the proximal and distal tubules the kidneys effectively take up almost all HCO_3^- in the pre-urine and return it to the blood, resulting in HCO_3^- concentration of 1-2 mM and pH around 7.3 in the urine. The amount of the two major urinary buffers, phosphate and NH_4^+, also increases to minimise pH reduction in urine. The CA enzyme also facilitates dehydration of HCO_3^- to CO_2 so it can diffuse into the cells of the nephron. Once inside the nephron cells CA also facilities the hydration back to HCO_3^-, which can then be returned to the blood, thereby contributing to maintain the plasma HCO_3^- levels at 8 mM and stable blood pH of around 7.9. The kidney will also increase the reabsorption rate of HCO_3^- if blood pH becomes too low.

most (about 90%) of the gill epithelium. In this way, fish can remove CO_2 built up via metabolic processes.

In addition to eliminating CO_2, the gills also play an important role in transporting acidic and basic equivalents via cells called ionocytes or "chloride cells" which make up less than 10% of the gill epithelium **(Figure 1)**. These cells are packed with mitochondria and consume a lot of ATP to fuel ion transport processes. These chloride cells normally take up sodium (Na^+) from the external water in exchange for acidic equivalents, and Cl^- from the water in exchange for basic equivalents at about the same rate, so the pH of the blood is not affected and normal concentrations of Na^+ and Cl^- in the blood plasma are maintained **(see Figure 1)**. However, when the blood pH is too low (for example during respiratory acidosis), the fish needs to excrete more acidic equivalents and retain more basic equivalents, thereby increasing the concentration of HCO_3^- in the blood plasma, so as to re-establish the correct ratio of HCO_3^- concentration to the dissolved CO_2 concentration in the plasma, and thereby restore blood pH to 7.9. At this time, the fish slows the rate of Cl^- uptake so less HCO_3^- is excreted. It also slightly accelerates the rate of Na^+ uptake so that more H^+ is excreted. As a result, the concentration of HCO_3^- in the blood plasma rises greatly, the concentration of Cl^- falls greatly, and the concentration of Na^+ rises slightly. Later we will explain why these processes are so important under high CO_2 and/or high O_2, where fish must compensate for a respiratory acidosis from the environment. Now, let's see how the kidneys complement the gills to maintain acid-base balance.

Kidneys

Generally, the kidneys continuously filter blood through the many thousands of active nephron tubules (the functional units that regulate acid-, base-, salt-, and water- balance) in the main part of the kidneys **(see Figure 2)** in this first step of urine production. The levels of HCO_3^-, dissolved CO_2, and pH will be identical in plasma and pre-urine. Thus, under normal conditions, the nephrons filter everything present in the plasma except blood cells and proteins. When the pre-urine further moves through the various sections of the nephrons (called proximal and distal tubules), the kidneys play a very important role in maintaining stable pH levels in the blood. They do so by reabsorbing (uptake) of HCO_3^- from the pre-urine and returning it to the blood **(see Figure 2)**. Also, in the kidney the CA facilitates conversion of HCO_3^- to CO_2 so it can diffuse into the cells of the nephron. Once inside the cell CA facilitates the conversion back to HCO_3^- which then can be returned to the blood via specialised transporters **(Figure 2)**. To illustrate this point with an example, under normal filtration rates in rainbow trout, roughly 48 µmol/kg/h HCO_3^- (pre-urine) is filtered, which will effectively be reabsorbed so that almost no HCO_3^- is present in the final urine. This may seem minor, but if the kidneys stopped reabsorbing HCO_3^-, the entire pool of basic equivalents (buffer capacity) of the blood would be lost within just eight hours! Therefore, the kidneys play a crucial but often underestimated role in maintaining plasma HCO_3^- levels at 8 mM, thereby ensuring a stable blood pH of around 7.9 and urine pH of around 7.6 **(Figure 2)**. When blood pH is too low (for example during a respiratory acidosis) the kidneys increase the reabsorption rate of HCO_3^- through these same mechanisms.

Physiological responses to high CO_2 and O_2 levels in land-based aquaculture systems

CO_2 is almost 30 times more soluble in water compared to O_2, which consequently affects gas exchange over the gills in fish. As fish grow larger, density increases in the tanks. In turn, O_2 consumption will be higher and CO_2

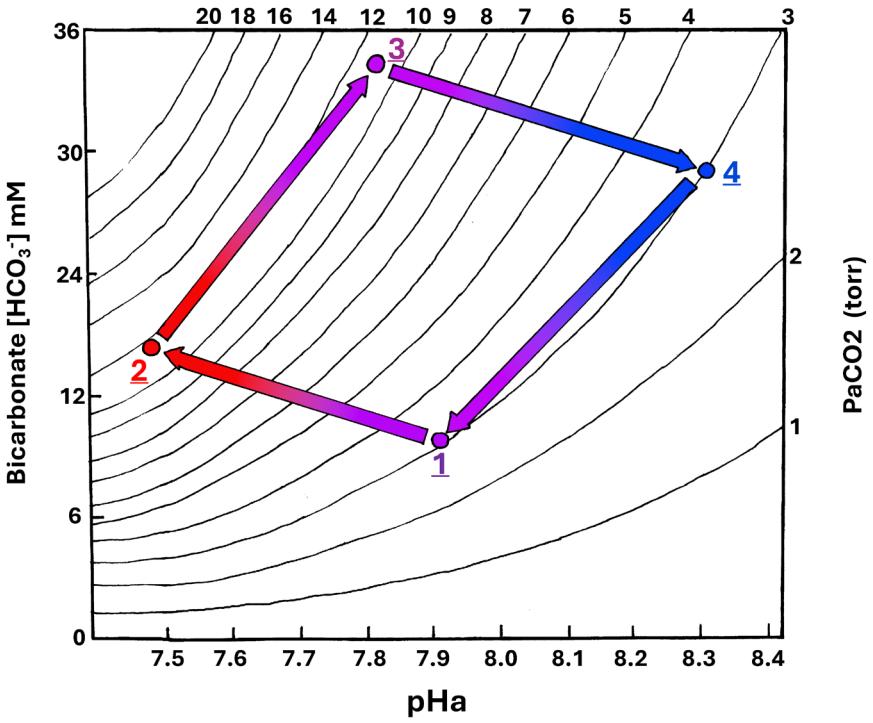

Figure 3: Davenport diagram visualising changes in HCO_3^-, pH, and partial pressure of CO_2 ($PaCO_2$ mmHg) in the blood.
Cultured fish may experience elevated CO_2 concentrations, for example during respiratory and metabolic acidosis or in the rearing water. If the $PaCO_2$ is 10-12 mmHg (approx. 32-40 mg/l) of the rearing water, blood pH will rapidly decrease, reaching 7.53 (stage 2; red colour). The fish require approximately three days to compensate by increasing the concentration of plasma HCO_3^- with blood HCO_3^- levels reaching as high as 30-35 mM (Stage 3; purple colour). If CO_2 levels suddenly drop, for example when fish are transferred to new tanks, the pH will quickly increase while the blood plasma (HCO_3^-) will remain high and the pH in blood will rapidly increase to alkaline 8.3-8.4 (Stage 4; blue colour), thus creating optimal conditions for nucleation and precipitation of kidney stones. Approximately 2-3 days later fish will return back to normal pH and HCO_3^- levels (stage 1; purple colour).

production will increase and accumulate in the water. CO_2 is challenging to remove from the water because of its high solubility and it dissociates to H^+ and HCO_3^-. At typical pH levels (usually above 6), most of the dissolved CO_2 converts to HCO_3^- in the tanks. The CO_2 produced by the fish (via metabolic processes; see first section) will slowly be converted to HCO_3^- (slow because there is no CA in the water), which the system (degassing) must first convert back to CO_2 before removal. This dehydration process is slow (much slower than the dehydration occurring in the gills, again due to the lack of CA; see section above), meaning that the removal of CO_2 will always lag behind the CO_2 production from metabolic processes under intensive farming conditions. Hence, aquaculture systems will not be efficient enough to remove CO_2, regardless of whether you use RAS, FTS or a hybrid flow-through system (HFS).

Several facilities experience CO_2 levels between 18-25 mg/l during the final period before fish are transferred to new tanks but even higher levels (25-35 mg/l) are reported during the actual pumping, crowding and transfer of fish. If the high CO_2 levels occur in combination with elevated O_2 levels in the tanks this can have additional negative effects on the fish. It is not uncommon for farmers to increase O_2 levels in the tanks before, during and after a transfer process. During periods of elevated O_2 levels (e.g., 110-120%, often called hyperoxia), the respiration rate in the gills will decrease and the fine blood vessels tend to constrict, which in turn reduces the gills' capacity to remove CO_2 (less water moves across the gills, less gill surface area for CO_2 diffusion). As a result, the fish tend to retain their metabolically produced CO_2, exacerbating the rise of blood CO_2 coming from the high CO_2 in the external water. The result is an even stronger respiratory acidosis when elevated O_2 (hyperoxia) is combined with high water CO_2 (hypercapnia). Fish can compensate by increasing the concentration of HCO_3^- in the blood plasma. This process slowly restores the correct ratio of HCO_3^- concentration to the dissolved CO_2 concentration in the plasma so as to bring the blood pH back to about 7.9. The fish achieves this increase in HCO_3^- concentration mainly by slowing the rate of Cl^- uptake and accelerating the rate of Na^+ uptake via the chloride cells in the gills (**see Gills section above**). This is referred to as the classic Cl^-/ HCO_3^- shift and has been well-described in the literature. At this time, the nephrons in the kidneys must increase the rate of HCO_3^- reabsorption from the pre-urine, or else all this extra plasma HCO_3^- would

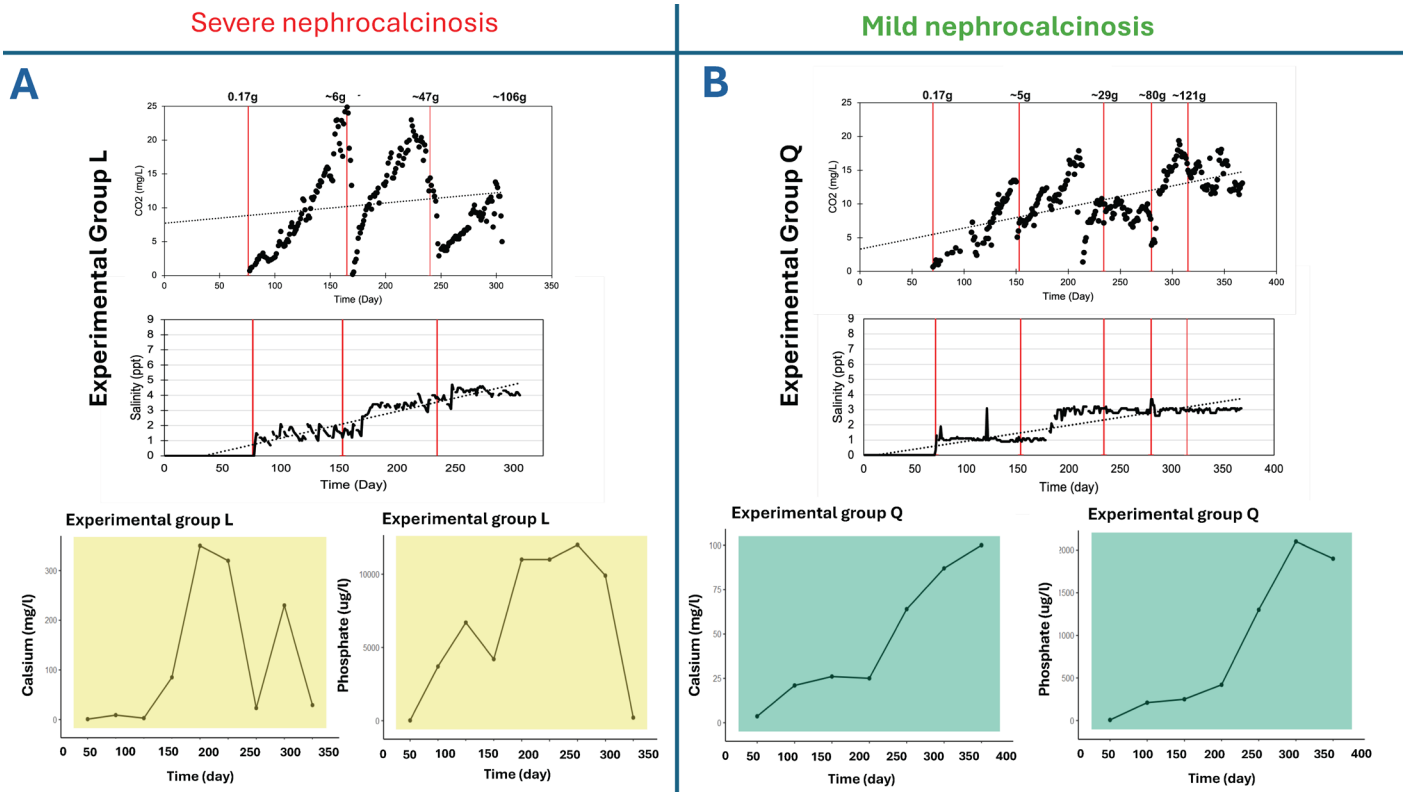

Figure 4: Water quality parameters in commercial RAS facilities where fish display severe (A) and mild (B) degree of nephrocalcinosis. Facilities with severe nephrocalcinosis (A) show larger CO_2 fluctuations than facilities with mild nephrocalcinosis (B). Experimental group L with severe nephrocalcinosis displays a 100-fold increase in water calcium levels compared to only a 20-fold increase in experimental group Q with mild nephrocalcinosis, despite similar salinities between the two facilities. Phosphate is increased by 1,000 times or more in both groups but with experimental group L having around five times higher concentrations compared to Experimental group Q.

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be lost in the excreted urine (**see Kidneys section above**). Studies have shown that when plasma HCO_3^- concentration is experimentally increased (via injection directly into the blood), it results in a 2.5-fold increase in the kidneys' reabsorption rate. Similar increases were observed during classic compensation for a respiratory acidosis. Studies suggest that the total capacity for gills and kidneys to increase the HCO_3^- concentration in the plasma has an upper threshold in salmonids at about 30-35 mM (normal levels about 8 mM).

Under high CO_2, fish will experience a respiratory acidosis followed by a compensation period where the restoration of normal blood pH in a healthy fish will take up to three days depending on the magnitude of the respiratory disturbance (in this case the level of CO_2). Generally, this results in a rapid reduction in plasma chloride (Cl^-), small rise in plasma Na^+, and large increase in plasma HCO_3^- levels, as previously discussed. The dynamics during such acid-base disturbances are often illustrated using a classic Davenport diagram, which allows visualisation of plasma HCO_3^-, pH, and partial pressure of CO_2 (mmHg) in the blood simultaneously **(Figure 3)**. During a respiratory acidosis several factors change which are necessary for kidney stones to precipitate: (**1**) increase in HCO_3^- concentrations in plasma, (**2**) mobilisation of Ca^{2+} and phosphate from the skeleton into the blood and urine and (**3**) increased metabolic production of ammonia (NH_4^+) and associated increase in plasma and urine ammonia levels. Thus, during the respiratory acidosis there is an increase in HCO_3^- (major buffer in plasma to minimise reduction in blood pH), slight decrease in urine pH (from 7.6 to 7.3), an increase in phosphate and NH_4^+ (major urinary buffers to minimise pH reduction in urine), and an increase in Ca^{2+} and Mg^{2+} (mobilisation from bones). Only one last factor (**4**) is missing, namely elevated pH in the plasma and urine. This elevation in pH occurs first when fish are returned to normal water CO_2 **(see Figure 3)** while still all the above factors are present in plasma and urine, creating optimal conditions for kidney stones to form. Limited focus has been directed to such rapid reductions in CO_2 which is surprising given that these changes are very common in aquaculture settings **(see Figure 4)**.

Possible trigger mechanisms associated with nephrocalcinosis

Over a very short period, fish move from a tank with high biomass, density, and CO_2

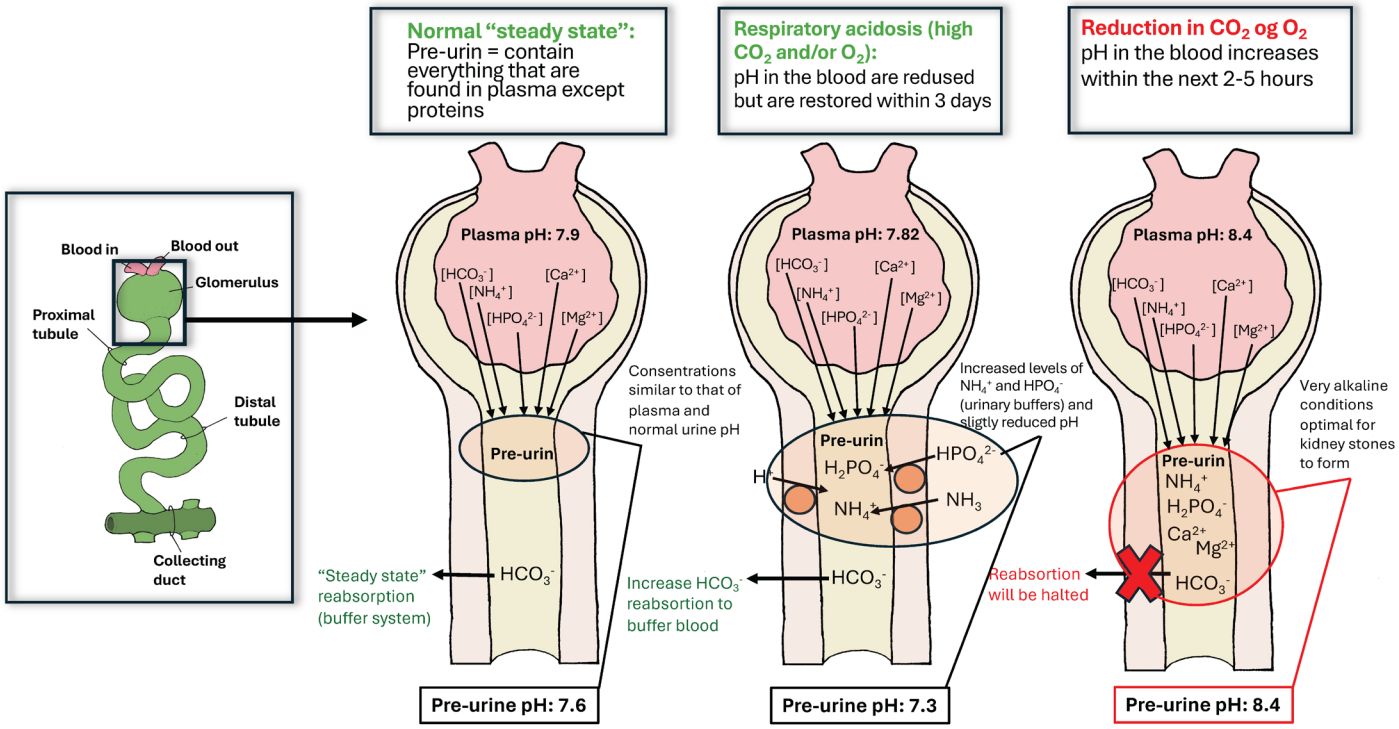

Figure 5: Favourable conditions for precipitation of kidney stones.
Under normal steady state conditions, ion concentrations and pH in plasma and pre-urine are similar. During respiratory acidosis the blood and urine pH decrease slightly, despite the kidney increasing the reabsorption rate of HCO_3^- and the concentration of urinary buffers (NH_4^+, $H_2PO_4^-$), thereby restoring blood pH and preventing pH in the urine from further decreasing. Once the fish experiences a rapid and sufficient drop in CO_2, the pH in blood and pre-urine increases dramatically within a few hours, while the HCO_3^- concentration remains high, thereby creating very favourable conditions for nucleation and precipitation of kidney stones.

levels to a new tank with low biomass, density, and CO_2 levels. Water O_2 levels may also be lower. Through dialogue and extensive collaboration with industry stakeholders in the NephroReduce project, it has become evident that fish usually develop detectable nephrocalcinosis 1-2 weeks after this type of transfer. However, the actual initial nucleation of the kidney stones may have occurred much earlier, immediately following the transfer. The substantial water quality changes (CO_2 in particular) that occur during the transfer to new tanks are often overlooked as a causative factor **(see example in Figure 4)**.

When fish are transferred to new tanks with lower CO_2 and/or O_2 the fish are quickly relieved (within minutes) from the respiratory acidosis (hypercapnia) because CO_2 can now quickly diffuse out across the gills. However, the HCO_3^- level in the blood plasma will stay high for a number of hours as it is only excreted slowly by the gills and kidneys. This means that the ratio of HCO_3^- concentration to CO_2 concentration in the blood plasma is suddenly too high, immediately driving the blood pH up to very alkaline values. Following the initial reduction in water CO_2 we have measured blood pH values around 8.1 five hours after transfer, but we suspect that the blood pH may go as high as 8.4 immediately after transfer **(go back to Figure 3)**. The pH in the pre-urine will be just as high, and the HCO_3^- concentration in the pre-urine will also remain high. Thus, we have a situation in which pre-urine in the nephrons has high pH, high HCO_3^-, high Ca^{2+}, high Mg^{2+}, high $H_2PO_4^-$, and high NH_4^+ – all of which are constituents needed to form kidney stones! The very high pH will promote initial nucleation and precipitation and the whole process of nephrocalcinosis has now started, likely within just a few hours. However, it can take 1-2 weeks before this is observed using macroscore which is commonly used in facilities. **Figure 5** shows how fluctuations in environmental CO_2 affect pH in both plasma and urine. The conclusion is that rapid changes from high to low CO_2 create highly favourable pH conditions in the urine that clearly increase the likelihood of calcium/phosphate-based stones (e.g., carbonate apatite), but also magnesium-based stones (e.g., struvite) to precipitate **(Figure 5C; pH 8.4)** compared to normal and stable CO_2 **(Figure 5A; pH 7.6)** and high CO_2 **(Figure 5B; pH 7.3)**.

Risk factors and mitigating measures related to CO_2/O_2 during transfer periods

Most studies to date have focused on constant high CO_2 levels (hypercapnia) and/or high O_2 levels (hyperoxia), with variable and ambiguous outcomes. In some studies, nephrocalcinosis was observed, while in others no signs of nephrocalcinosis were detected. It is important to note that high constant CO_2 levels can be problematic for fish, often reducing growth and fish welfare. Furthermore, it can be particularly problematic for fish already suffering from kidney stones, since existing kidney stones locally in the nephrons would negatively impact the transport mechanisms in the nephron **(See Figure 2 and 5)**, potentially leading to an accumulation of the key constituents of kidney stones in the pre-urine described above and ultimately increase urine pH. In the NephroReduce project we have investigated rapid changes in CO_2 and explored immediate measures to minimise such situations. Observations from commercial production indicate that facilities with better CO_2 control before fish are transferred have milder cases of kidney stones **(Figure 4B)** compared with facilities having significant CO_2 fluctuations **(see Figure 4A)** (severity based on histological kidney score). Therefore, the first immediate measure is to reduce biomass, or transfer the fish earlier compared to current practices. Alternatively, CO_2 removal capacity needs to increase in the system and/or there must be better CO_2 removal technology to reduce the high CO_2 peaks during the final weeks before transfer. We are currently discussing with project partners the possibility of increasing CO_2 levels in the tanks to which the fish are being transferred. This way, CO_2 levels can be gradually reduced over several days, minimising rapid reductions in water

CO_2 after transfer and the associated increase in urine pH. We recommend that companies monitor CO_2 and O_2 levels more closely before, during, and after fish transfer within the facility. High O_2 levels before, during, and after transfer can have adverse effects in combination with increased CO_2 levels. Therefore, O_2 levels should remain stable and not exceed 90-95% before, during, and after transfer. We have observed that CO_2/O_2 levels can increase significantly during crowding, pumping and moving, so measurements during the transfer period will be vital to monitor. Finally, the magnitude of the CO_2 drop (5- 35 mg/l) necessary to increase blood and urine pH will be a key focus moving forward, since fish are transferred to tanks with virtually no CO_2 (0-2 mg/l).

Risk factors and mitigating measures related to ion composition in water

Increased salt concentrations via water or feed can contribute to nephrocalcinosis when fish are transferred to new tanks. Water salinity (parts per million; ppt) is identified as a risk factor. Elevated salinity can provide higher access to salts (including calcium, magnesium, and phosphate), which is unfavourable during periods of elevated urine pH. Therefore, operators should be cautious using brackish water (5-12 ppt) or transitional feeds (with higher salt concentrations) in the first week after transfer to new tanks. It is also important to note that use of calcium buffers (relevant for RAS) can increase calcium concentration in the rearing water, exposing fish to elevated calcium levels and thereby increasing the risk of precipitation, as calcium is a major component of kidney stones in salmon. However, salinity (ppt) as a measure alone is insufficient to observe changes in overall ion composition. Preliminary water quality results show some facilities have a 20-fold increase in calcium levels, and in extreme cases over a 100-fold increase, while salinity remains relatively stable in both facilities **(see Figure 4)**. Also, phosphate can increase several hundred times during production **(Figure 4)**. In **Figure 4** experimental group L experiences drastically larger drops in CO_2 in combination with three times higher calcium and five times higher phosphate levels compared to experimental group Q, which experiences smaller drops and lower calcium and phosphate concentrations **(Figure 4)**. We are currently conducting a meta-analysis of water quality and feed composition in several facilities from egg to smolt (100-150 grams), so other risk factors may emerge later during the project. Going forward it would be beneficial for companies to conduct more thorough water analyses in each tank, especially various ions (calcium, phosphate, and magnesium in particular), ammonium (NH_4^+), hardness ($CaCO_3$), pH and alkalinity, providing personnel with a better understanding of the changes in water quality that can occur during different transfer operations in their facility. This is especially important during moving operations as there are large changes in water quality from one tank to the other. This is rarely monitored today, and such water quality changes are likely significantly underreported in today's production.

Future perspective and potential for targeted treatment of nephrocalcinosis

In the long-term, we are working on targeted treatments through environmental or dietary interventions to reduce urine pH in the days following transfer. For example, it has been reported that fish develop fewer kidney stones after transfer to full strength seawater (34-35 ppt). Fish in seawater generally have lower urine pH compared to freshwater

fish, potentially helping to dissolve kidney stones, and thus chances for kidney stone development are lower. In the NephroReduce project we are planning to test this using full strength seawater, giving the fish a sufficient marine signal to reduce pH in the urine. Additionally, targeted dietary interventions can possibly reduce urine pH after transfer by changing the feed composition and/or adding new components that can reduce the pH of the urine in the days after transfer. However, it's likely that kidney stones form only hours after a pH increase and targeted feed treatment would likely need to be administered before transfer or relatively quickly after transfer to dissolve kidney stones at an early stage. The potential effect of such therapies will be closely monitored using non-lethal repetitive x-ray and/or ultrasound imaging on individual fish to see specific treatment effects in mild, moderate and severe nephrocalcinosis groups. The development of imaging technologies such as ultrasound and use of artificial intelligence (AI) in aquaculture could potentially target and sort fish according to severity, for example in connection with vaccination.

This article and ongoing work in the NephroReduce project aim to enhance understanding of the kidneys' role in acid-base and ion regulation and to identify risk factors for nephrocalcinosis. This could significantly enhance fish health and welfare. With the increasing focus on extended land-based production (production of large smolt/post-smolt), measures to address nephrocalcinosis issues are more important than ever, as fish will spend a large proportion of their life in systems that may otherwise be conducive to nephrocalcinosis.

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