

# Incidence of nephrocalcinosis in Norwegian farmed salmon

**A survey conducted in autumn 2019 and spring 2020 shows that nephrocalcinosis is common in farmed salmon in Norway. The condition causes impaired kidney function and is well known as a welfare challenge in Norwegian salmon industry.**

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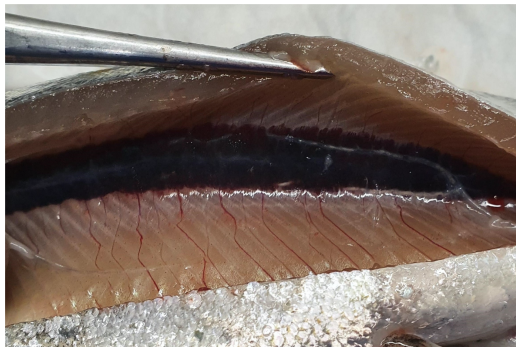
Nephrocalcinosis has been a known production-related disease in farmed salmon for more than 20 years. The condition is described as the accumulation of calcium and magnesium deposits in the kidneys. The extent to which nephrocalcinosis causes increased mortality is not known. However, the condition presents welfare challenges for salmon, and it is likely that impaired kidney function weakens individuals and make them more vulnerable to disease and stress. The lack of systematic registration of nephrocalcinosis makes it challenging to evaluate the extent of the condition. It is also not clear whether nephrocalcinosis is more common in flow-through systems or RAS systems. It is often reported that the severity of nephrocalcinosis is reduced after the transfer of salmon to sea, even though there is a lack of scientific evidence for this. The etiology of nephrocalcinosis in hatcheries is poorly understood. However it is believed that sub-optimal environmental conditions may cause the disease, and several possible reasons have been suggested such as exposure to high CO<sub>2</sub> concentrations.

## Material and methods

A total of 420 fresh salmon from 13 different fish groups from 12 hatcheries in Norway were examined from October 2019 to August 2020. Among the 12 facilities, 4 had RAS technology, while the others were traditional flow-through facilities. All samplings were made as close to sea transfer as possible, so that the degree of smoltification of the fish groups was as high as possible at the time of sampling. A total of 360 fish from the same groups were examined 1 month after transfer to sea.

## Assessment of nephrocalcinosis

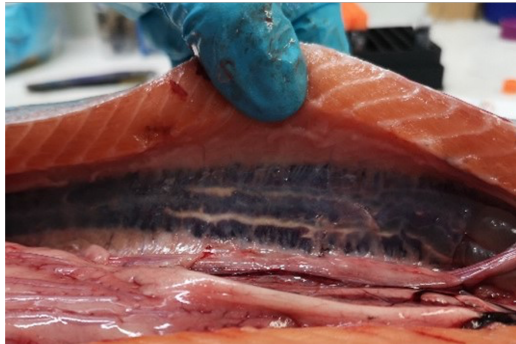
Nephrocalcinosis was assessed visually and a score from 0 to 4 was given, where 0 indicates no visible deposits, while at score 1 (mild) the collecting ducts are visible and opaque (Fig. 1). Score 2 (mild) and 3 (moderate) were given depending on the visible amounts of deposits. Score 4 (severe) was given to individuals with extensive amounts of deposits, as well as loss of normal kidney structure.



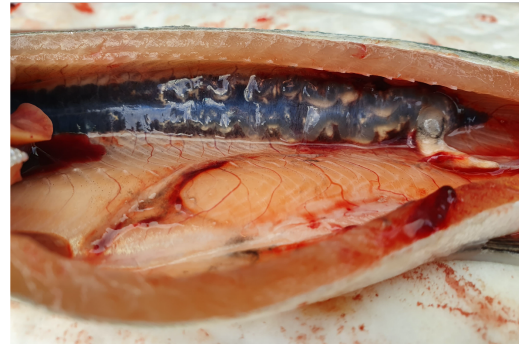
(a) Score 1.



(b) Score 2.



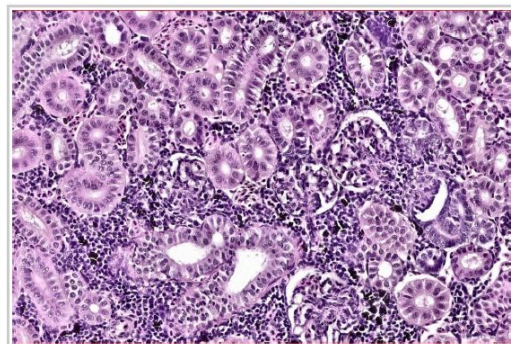
(c) Score 3.



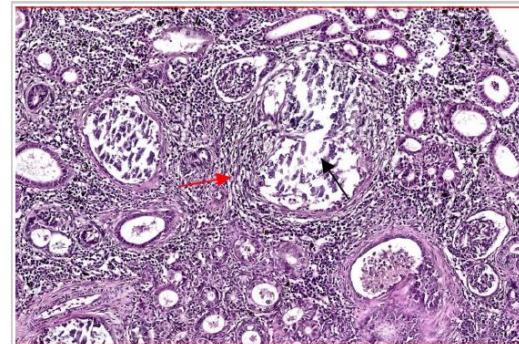
(d) Score 4.

**Figur 1:** Visual assessment of different degrees of nephrocalcinosis.

To confirm visual findings, mid-kidney samples from all individuals were analyzed by Pharmaq Analytiq AS according to standard histological methods (Fig. 2). The diagnosis of nephrocalcinosis was made based on findings of deposits in the tubules and collecting ducts (category 1). Furthermore, damage to the epithelium (category 2), changes in the glomeruli (category 3) and changes in hematopoietic tissue (category 4) were also considered. Each individual was given an overall score, based on the type of injury found in the kidneys, where injuries of categories 3 and 4 were weighted higher than injuries of categories 2 and 1. The overall score was given as: mild (score 1-10), moderate (score 11-20) and severe (score > 20).



(a) Normal kidney.



(b) Kidney with severe degree of nephrocalcinosis indicated by large amounts of precipitation (black arrow) and inflammation (red arrow).

**Figur 2:** Histological assessment of fish without and with nephrocalcinosis.

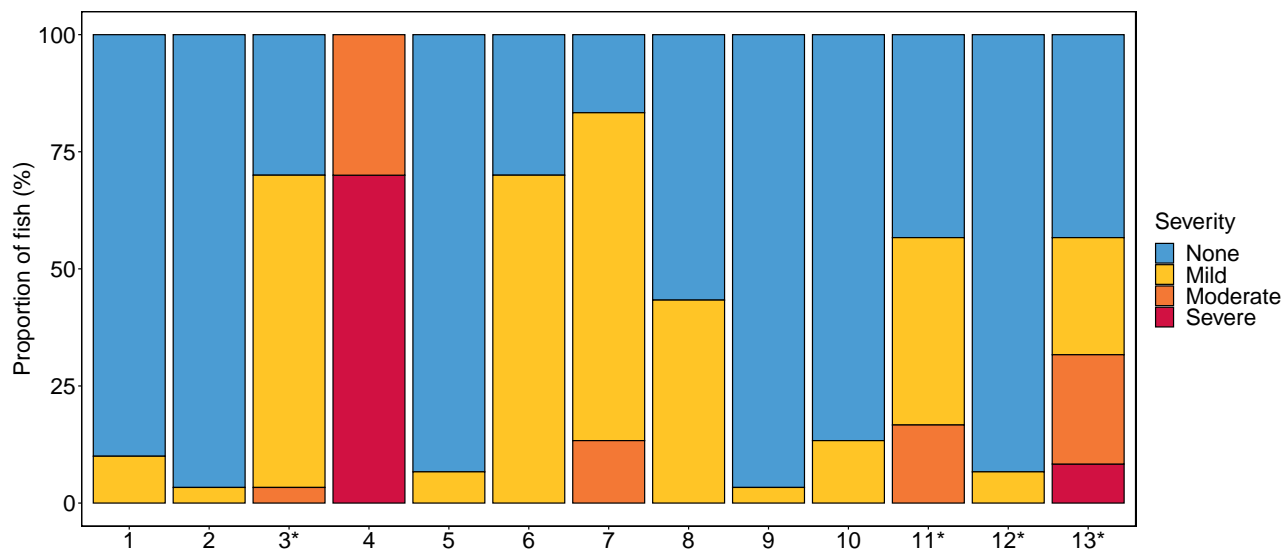
## Water quality

On each visit to hatcheries, water samples were taken in triplicates in the same basins where the fish were sampled from. CO<sub>2</sub> and O<sub>2</sub> concentration and as well as pH were measured *in situ* with suitable tools (Oxyguard CO<sub>2</sub>/Hache multimeter). The following parameters were measured at Aqua Kompetanse's lab: with spectrophotometry (PHotoFlex): concentration of various gases, alkalinity, conductivity/salinity, concentrations of salts and metals.

## Results and discussion

### Nephrocalcinosis is common in Norwegian hatcheries

Nephrocalcinosis was detected in all facilities examined, but there were large variations in the severity between them (Fig. 3). In half of the facilities, more than 50 % of the sampled fish were diagnosed with nephrocalcinosis. The different facilities had varying distribution of severity, however most of the fish had mild changes in the kidney tissues. On average, the results indicated that approximately 40 % of farmed salmon were diagnosed with nephrocalcinosis towards the end of the hatchery phase.



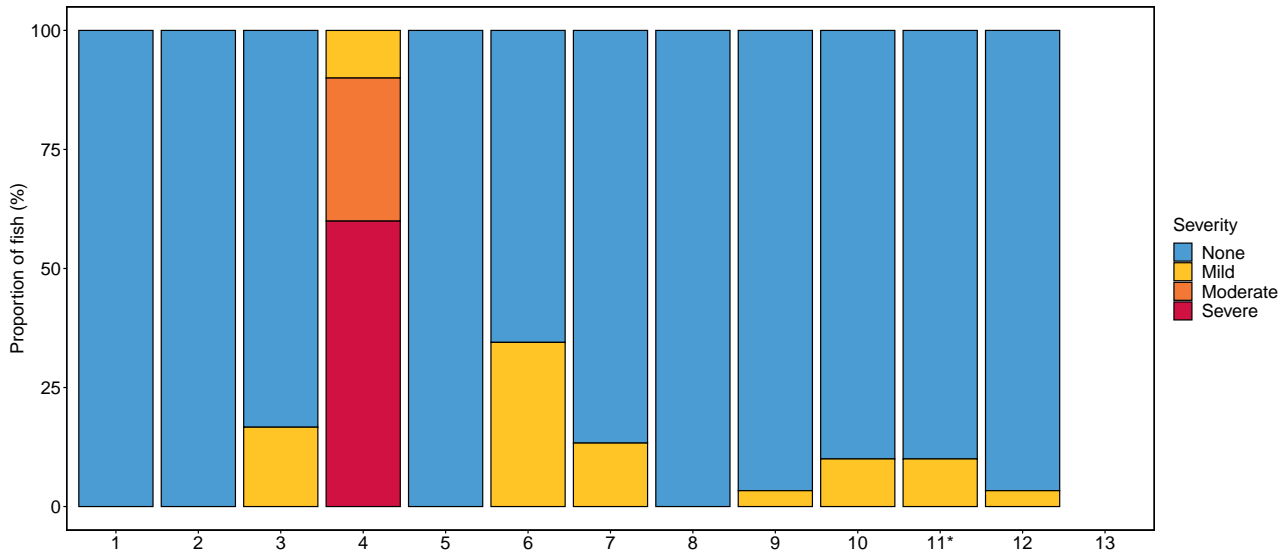
**Figure 3:** Proportion of fish (%) without and with nephrocalcinosis distributed by severity in different hatcheries. The systems with RAS technology are marked with a star, the others are flow-through systems.

### Nephrocalcinosis is as common in flow-through as RAS systems

The question of whether different forms of production technologies increase the risk of nephrocalcinosis is under debate among the salmon farming industry. Based on the findings in this survey, it does not appear that nephrocalcinosis is related to production forms (Fig. 3). Nephrocalcinosis was detected in 48 % salmon smolts in RAS facilities, with mainly mild forms. In flow-through facilities, a lower proportion of fish with nephrocalcinosis was found (33 %), however in more severe degrees.

## Nephrocalcinosis appears to be reversible after transfer to sea

The results of the study confirm feedback from salmon farmers that nephrocalcinosis mainly is an issue in the hatcheries and not the sea farms, except for severe degrees of the condition. The proportion of fish with nephrocalcinosis mainly decreased after transfer to sea (23 % vs. 36 % in hatchery, Fig. 4). Among the 12 fish groups that were diagnosed with nephrocalcinosis in the hatchery phase, 4 were not diagnosed with nephrocalcinosis after transfer to sea. There was also a decrease in fish with mild and moderate degrees of nephrocalcinosis (10 % vs. 30 % in the hatchery phase). On the other hand, the proportion of fish affected by severe forms was unchanged after transfer to sea. These results suggest that small changes in the kidneys are likely to be reversible while severe kidney damages are not believed to heal.

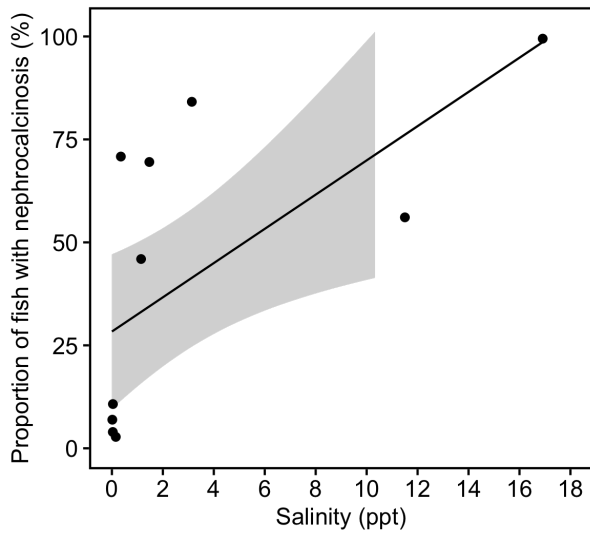


**Figure 4:** Proportion of fish (%) without and with nephrocalcinosis distributed by severity in different sea farms. Note that fish from hatchery 13 was not followed after transfer to sea.

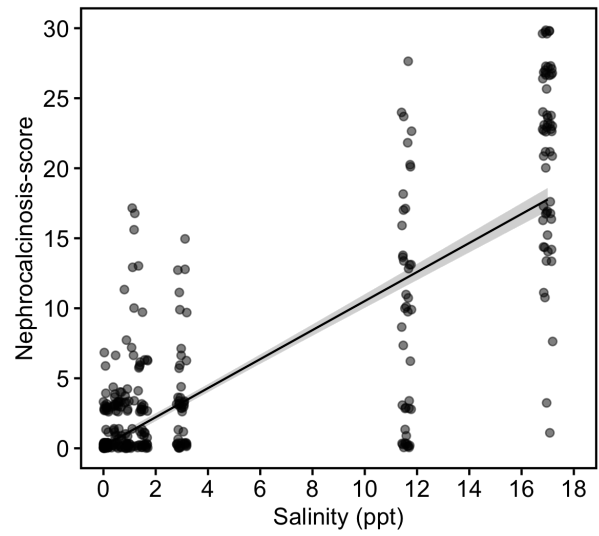
## Nephrocalcinosis seems to be related to the use of seawater in hatcheries

While some hatcheries used only freshwater, others (facilities 3, 4, 6, 7, 11 and 13) added various concentrations of seawater (0.5 - 17 ppt). The proportion of fish affected by nephrocalcinosis in hatcheries that did not add seawater was about 13% while it reached 73% in those that added seawater. The results further indicated a positive correlation between the proportion of fish with nephrocalcinosis and salinity. The severity of nephrocalcinosis also increased with increased salinity (Fig. 5). Similarly the incidence and severity of nephrocalcinosis was positively correlated with seawater typical elements (Cl, Na, SO<sub>4</sub>, Mg, Ca, K). This raises the question whether these salinities typical of brackish water send a mixed up signal to the salmon in regard to what kind of environment it is in (salt or fresh water) and therefore lead to osmoregulatory stress. This may in turn affect ion balance of salmon and therefore may lead to the accumulation of deposits in kidneys.

There is a debate in the literature about whether high CO<sub>2</sub> concentrations in water can cause nephrocalcinosis. The results of this study did not support a correlation between the incidence and severity of nephrocalcinosis and CO<sub>2</sub> concentrations in water (Fig. 6).

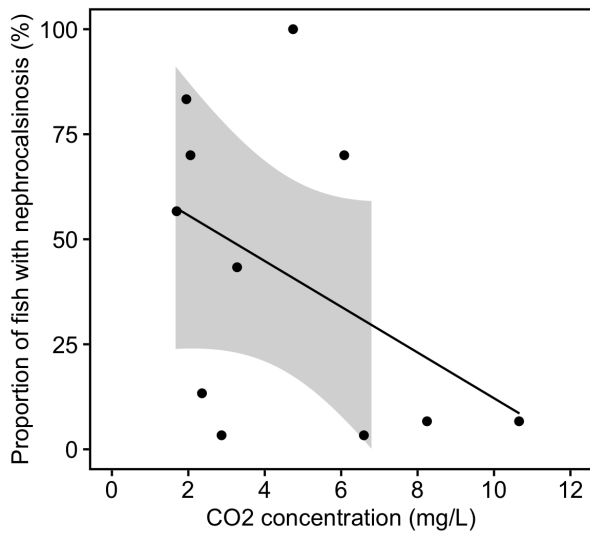


(a) Relation between proportion of fish with nephrocalcinosis (%) and salinity in different hatcheries. Each point represents 1 facility. The line shows linear regression with standard deviation ( $p < 0.05$ ,  $R^2 = 0.42$ ).

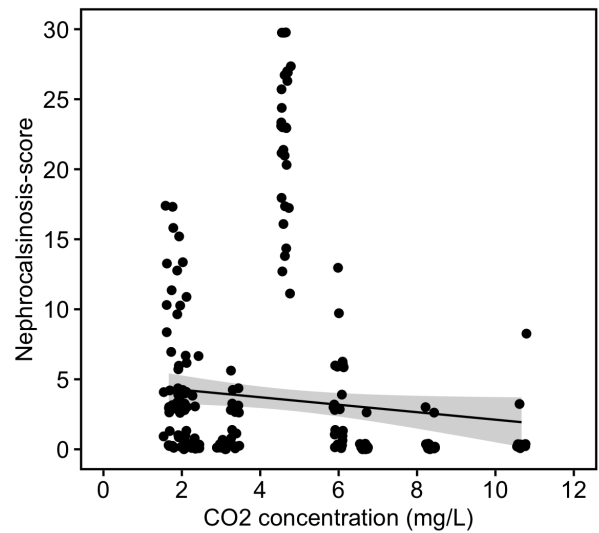


(b) Relation between nephrocalcinosis score and salinity in different hatcheries. Each point represents 1 fish. The line shows linear regression with standard deviation ( $p < 0.0001$ ,  $R^2 = 0.53$ ).

**Figure 5:** Relation between the incidence and severity of nephrocalcinosis and salinity in different hatcheries.



(a) Relation between proportion of fish with nephrocalcinosis (%) and  $CO_2$  concentration in different hatcheries. Each point represents 1 facility. The line shows linear regression with standard deviation ( $p > 0.1$ ,  $R^2 = 0.19$ ).



(b) Relation between nephrocalcinosis score and  $CO_2$  concentration in different hatcheries. Each point represents 1 fish. The line shows linear regression with standard deviation ( $p > 0.05$ ,  $R^2 = 0.01$ ).

**Figure 6:** Relation between the incidence and severity of nephrocalcinosis and  $CO_2$  concentration in different hatcheries.

## More about the project

This study is part of a larger project led by Aqua Kompetanse AS, whose main goal is to identify risk factors for nephrocalcinosis in farmed salmon in central Norway. It is also an objective to develop measures that can be implemented in the facilities to reduce the incidence of nephrocalcinosis in hatcheries.

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## References

- Attramadal (2020) Vannkvalitet med fokus på sjøvann og fiskehelse. Presentasjon under webinar for nefrokalsinose hos laksesmolt. Digitalt.
- Bruno (1996) Nephrocalcinosis .Aquaculture Information Series.
- Fivelstad et al. (1998) Sublethal effects and safe levels of carbon dioxide in seawater for Atlantic salmon postsmolts (*Salmo salar* L.): Ion regulation and growth. *Aquaculture*, 160(3-4):305–316.
- Fivelstad et al. (1999) Effects of carbon dioxide on Atlantic salmon (*Salmo salar* L.) smolts at constant pH in bicarbonate rich freshwater. *Aquaculture*, 178(1-2):171–187.
- Fivelstad et al. (2003) A major water quality problem in smolt farms: Combined effects of carbon dioxide, reduced pH and aluminium on Atlantic salmon (*Salmo salar* L.) smolts: Physiology and growth. *Aquaculture*, 215(1-4):339–357.
- Fivelstad et al. (2015) Sluttrapport for Postsmolt D: Grenseverdier for karbondioksid for postsmolt. Høgskolen i Bergen. FHF-prosjekt 900895, 34 pp.
- Fivelstad et al. (2018) Growth and nephrocalcinosis for Atlantic salmon (*Salmo salar* L.) post-smolt exposed to elevated carbon dioxide partial pressures. *Aquaculture* 482, 83-89.
- Good et al. (2018) The effects of long-term 20 mg/L carbon dioxide exposure on the health and performance of Atlantic salmon *Salmo salar* post-smolt in water recirculation aquaculture systems. *Aquaculture Engineering* 81, 1-9.
- Hjeltnes et al. (2019) Fiskehelsereporten 2018. Technical report, 2019.
- Hosfeld et al. (2008) Long-term separate and combined effects of environmental hypercapnia and hyperoxia in Atlantic salmon (*Salmo salar* L.) smolts. *Aquaculture* 280, 146-153.
- Khan et al. (2018) The effects of acute and long-term exposure to CO<sub>2</sub> on the respiratory physiology and production performance of Atlantic salmon (*Salmo salar*) in freshwater. *Aquaculture* 491, 20-27.
- Mota et al. (2019) The effects of carbon dioxide on growth performance, welfare, and health of Atlantic salmon post-smolt (*Salmo salar*) in recirculating aquaculture systems. *Aquaculture* 498, 578-586.
- Nylund et al. (2003) Haemorrhagic smolt syndrome (HSS) in Norway: Pathology and associated virus-like particles. *Diseases of Aquatic Organisms*, 54(1):15–27.
- Olsen et al. (2006) Helse situasjonen hos oppdretts fisk 2005. Technical report, 2006.
- Rodger & Richards (1998) Haemorrhagic smolt syndrome: A severe anaemic condition in farmed salmon in Scotland. *Veterinary Record*, 142(20):538–541.
- Woo et al. (2002) Diseases and disorders of finfish in cage culture.
- Xiong et al. (2020) Growth, osmoregulation and energy budget of rainbow and steelhead trout under different salinity acclimation methods and the best transition size of steelhead trout. *Aquac Res.* 2020; 51: 2369– 2378.