


# Radiological detection of nephrocalcinosis in farmed Atlantic salmon *Salmo salar* L.

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## Funding information

Fiskeri - og havbruksnæringens forskningsfond; Norges Forskningsråd; Norwegian Seafood Research Fund; Norwegian Research Council

**Keywords:** Atlantic salmon, nephrocalcinosis, radiology

## 1 | INTRODUCTION

Nephrocalcinosis is a common disorder in nurseries in Norway (Klykken, Reed, et al., 2022) and was reported as one of the main welfare challenges in farmed salmon by The Norwegian Fish Health Report of 2019 (Sommerset et al., 2020).

Nephrocalcinosis is described as deposits of minerals within the kidneys (Bruno, 1996), that can disturb kidney function, which in turn can have dramatic consequences on fish performance and survival. The aetiology is most likely related to environmental factors, and suboptimal water quality has been indicated in several studies as the main risk factor (Fivelstad et al., 1999; Fivelstad et al., 2003; Khan et al., 2018; Fivelstad et al., 2015; Good et al., 2010; Lewisch et al., 2013; Chen et al., 2001). Newly conducted research suggested that osmoregulatory stress may be the trigger for nephrocalcinosis (Boissonnot et al., 2022).

Regardless of the severity of the condition, fish rarely present external signs, and it is thus challenging to monitor its prevalence and development. Present diagnostic methods require euthanasia as they consist of visually scoring the accumulation of deposits and the severity of lesions. Macroscopic assessments of necropsied fish are often imprecise, since small deposits are rarely visible to the naked eye, and histopathology is therefore considered as the best existing

diagnostic method (Klykken, Boissonnot, et al., 2022). Research on, and monitoring of, nephrocalcinosis has been greatly hampered by the lack of non-invasive methods of assessing the presence and severity of this condition, as it is not possible to follow the development of the disease in single individuals, and as the number of sampled fish is limited due to ethical reasons.

Radiology has previously been used for assessing vertebral deformities in Atlantic salmon (Drábiková et al., 2021; Holm et al., 2020), based on the classification scheme developed by Witten et al. (2009), and there has been a rapid development of the technology (Ou et al., 2021) including portable systems, which allow efficient in situ diagnosis. Nephrocalcinosis in Atlantic salmon is mainly identified as amorphous carbonate apatite, a calcium-dominated mineral (Klykken, Reed, et al., 2022), and it has previously been demonstrated that this mineral composition is suitable for x-ray detection (Smith & Lehr, 1966). Radiology is non-invasive and can be performed on anaesthetized fish, enabling assessment of nephrocalcinosis without euthanizing the fish. A non-invasive method for assessing nephrocalcinosis would allow for monitoring of the condition over time on an individual level and would be ethically and economically preferable. We have therefore explored radiology as a possible tool to detect and evaluate the severity of nephrocalcinosis, comparing it with histological scoring.

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## 2 | MATERIAL AND METHODS

A total of 80 farmed Atlantic salmon were sampled from two recirculating aquaculture system facilities in Mid-Norway in April 2022. They were randomly sampled among visually healthy individuals (normal swimming behaviour, absence of external injuries/lesions and no sign of emaciation). The fish were not starved before sampling, and they were killed with an overdose of Benzoak VET (200–400 mg/L) followed by a sharp blow to the head according to Norwegian legislation (Akvakulturdriftsforskriften, 2008). Apart from nephrocalcinosis, no health issues were observed. Size measurements of individual fish included body weight in g ( $\pm 1$  g), fork length in cm ( $\pm 0.5$  cm), body thickness in cm ( $\pm 0.1$  cm) and condition factor:  $CF = 100W/L^3$ .

Left anterior lateral view radiographs were taken of smolts (average weight 198 g) using a standard portable x-ray unit (Econet meX+40; calibration: kV: 40, mAs: 5) with a digital plate measuring 25 × 32 cm and subsequently digitized using an VIVX-V2532P and VXvue. Distance between shutter opening and the fish was approximately 80 cm. The staff wore a lead apron and stood 2 m from the digital plate, resulting in negligible radiation exposure. After radiological imaging, the fish were dissected and eviscerated, and a picture was taken of the kidney tissues. Distribution and density of mineral deposits within the kidney was evaluated on radiographs in DICOM format in QXLink Portable Viewer (Vieworks, Republic of KOREA).

Whole kidney tissues were sampled from all individuals for histological analysis of nephrocalcinosis. The kidney tissues were fixed in 4% phosphate-buffered formaldehyde solution, embedded in paraffin wax and routinely processed (Suvarna et al., 2018). The kidney tissues were sectioned in the anterior–posterior direction in the para-sagittal plane so that the whole length of the kidney was evaluated for each fish. Due to space restrictions, segments of approximately 2.5 cm were made and oriented in a consecutive fashion from anterior to posterior on the resulting slide. All sections were stained by haematoxylin and eosin. The histopathological diagnosis of nephrocalcinosis was defined as the presence of amorphous, basophilic deposits in tubules, collecting ducts and excretory ducts. Histopathological nephrocalcinosis scoring was performed according to Klykken, Reed, et al. (2022).

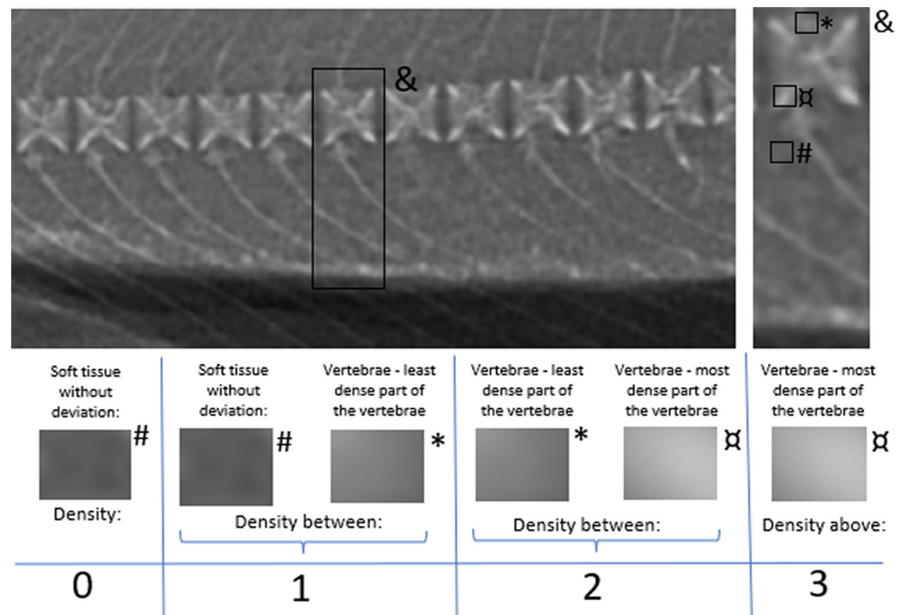
Initially, histopathological nephrocalcinosis scoring was undertaken for all 80 fish. From this, a subsample of 17 fish was chosen to encompass all degrees of histological manifestation of nephrocalcinosis, and the radiographs of the corresponding fish were investigated. Based on those, we proposed five different categories for radiological scoring of nephrocalcinosis (herby referred to as radiological score) as given in Table 1 and Figure 1, with the sum of scores from each category making up the radiological score.

To validate the preliminary scoring system based on radiology, 63 fish were diagnosed on radiographic images only and then checked by comparing the results with the histological assessment. The validity of the preliminary model was assessed as the degree to which

TABLE 1 Categories for radiographic assessment of nephrocalcinosis

Parameter	Score	Assessment
Density of deposits	0	Not detected (Figure 1-0)
	1	Densities comparable to the spongiosa or less (Figure 1-1)
	2	Densities between densities comparable to spongiosa and the endplates of a random vertebrae (Figure 1-2)
	3	Densities comparable to the endplates of a random vertebrae (Figure 1-3)
Spatial distribution of mineral deposits	0	Not detected
	1	Only present in the collecting ducts
	2	Present in half of the space between the vertebrae column and the swim bladder (Figure 2-B2)
	3	Present in 75% of space between the vertebrae column and the swim bladder (Figure 2-C2)
Presence of deposits in the total length of the kidney	0	Not detected
	1	<25% of the total length
	2	25%–50% of the total length (Figure 2-B3)
	3	50%–75% of the total length (Figure 2-C3)
Presence in urinary bladder	0	Not detected
	1	Detected (Figure 2-B3)
	0	No deviation detected
	1	Deviation detected (see supplementary)

**FIGURE 1** Density assessment of mineral deposits compared with density of soft tissue (#) and vertebral structures: spongiosa (\*) and endplates (⌘).



scores based on radiology were correlated with the histology score, based on the assumption that histology scoring was sufficiently objective. Correlation was checked with Pearson correlation test.

### 3 | RESULTS AND DISCUSSION

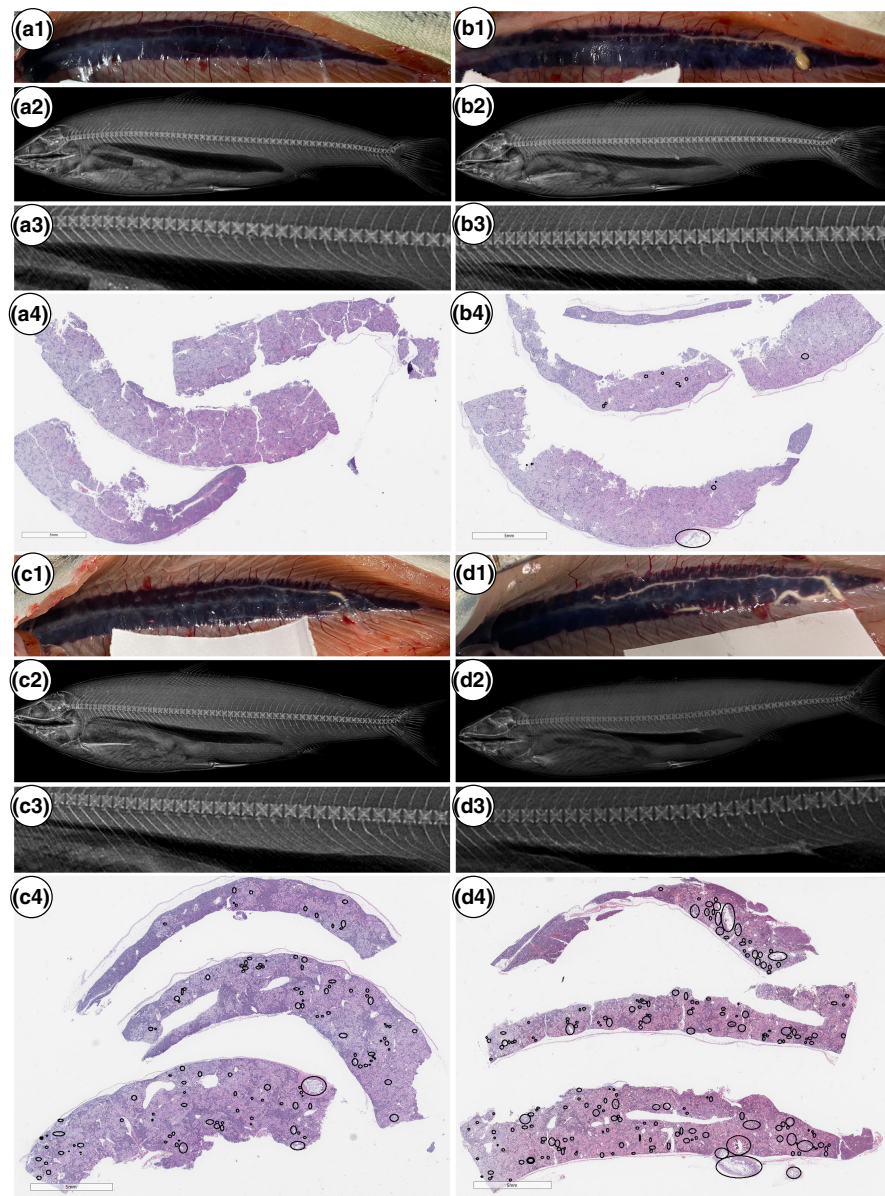
The distribution and density of mineral deposits were clearly observed on the x-ray images (Figure 2), which offers promising prospects for the development of a non-invasive assessment of nephrocalcinosis in farmed salmon.

The sensitivity of radiology for nephrocalcinosis was high, as 93.2% of the fish ( $n = 55$ ) histologically diagnosed with nephrocalcinosis were also diagnosed with radiology (Table 2). The remaining 6.8% ( $n = 4$ ) that were not detected with radiology were all diagnosed with mild changes with histology. On the opposite, three individuals were diagnosed with nephrocalcinosis with radiology, but not detected with histology. These were assessed with sparse amounts of mineral deposits. The correlation between the total histology score and the radiological assessment (Figure 3) was significant but low (Yadav, 2018). One possible explanation for these inconsistencies could be that mild changes, according to the histological scoring model, usually refer to sparse amounts of mineral deposits in collecting ducts and tubules (Klykken, Reed, et al., 2022). It is, therefore, likely that the density or the proportion of mineral deposits were too small to be detected by radiography. We also observed background artefacts on some of the radiographs that may have affected the radiological assessment of nephrocalcinosis. To increase the specificity of radiology, background artefacts should be minimized. For example, breaching the scale pockets could lead to disturbances with several scales ending on top of each other in an unnatural fashion, causing disturbances to the radiographic image. Background artefacts may also have contributed to the radiological detection of the three fishes, which were considered healthy with histology (Table 2).

Another possible explanation for these inconsistencies is related to the limitations of histopathology itself. The histopathological nephrocalcinosis score is a semi-quantitative scoring model based on changes in the kidney according to the known principles of pathology. Histopathological changes are divided into four subcategories (presence of deposits, epithelial degeneration and/or necrosis, pathological changes in the glomeruli and pathological changes in the interstitial tissue; Klykken, Reed, et al., 2022). The histological section represents a two-dimensional thin slide (approximately  $3 \mu\text{m}$ ) of a relatively large organ, and there might be deposits in a plane that is not included in the section. It is, therefore, possible that we were able to detect deposits with radiology that we failed to observe with histopathology. This should be further investigated as it is not possible to conclude based on our study.

It is also possible that the low correlation could be explained by the fact that radiology can only detect the first category in the histopathologic scoring model, that is, the presence of deposits. Fish with severe changes in the kidney tended to have larger spatial distribution of minerals on radiography. In addition, whether we observed mineral deposits in the urinary bladder was not correlated with the overall histological score, and the radiological categories should be further evaluated.

The use of portable radiography is advantageous as it allows for a fast and cost-effective diagnosis of mineral deposits characteristic for nephrocalcinosis that can be performed on a large number of sedated individuals at each facility. The Norwegian legislation requires, however, that the person conducting radiography (operator) receives a short training and use radiation protection, as radiography consists of x-ray radiation, which can potentially pose a risk for the operator (Zamanian & Hardiman, 2005). The voltage, shutter speed and exposure time settings used in this study resulted in radiation doses of  $0.001 \text{ mSv/h}$  measured at a distance of 20 cm from the digital plate. In this study, the operator was wearing a lead apron, standing 2 m from the digital plate,



**FIGURE 2** Examples of macroscopical presentation (1), radiological imaging (2 (whole fish) and 3 (focus on kidney region)) and histology (4 (section of the whole kidney from anterior to posterior part; regions with changes associated with nephrocalcinosis indicated by black ellipses), from normal fish (a) and fish with different grading of nephrocalcinosis ranging from mild (b), moderate (c) to severe (d) as evaluated from radiological and histological evaluation.

**TABLE 2** Number of fish with nephrocalcinosis detected on radiology and histology

	Radiology positive	Radiology negative	Total
Histology positive	55	4	59
Histology negative	3	1	4
<b>Total</b>	<b>58</b>	<b>5</b>	<b>63</b>

resulting in effective dose of  $0.00002 \mu\text{Sv}$ , which is quite negligible. For comparison, will a single domestic flight in the US give an effective dose of  $3\text{--}6 \mu\text{Sv}$  (United Nations Scientific Committee on the Effects of Atomic Energy, 2010).

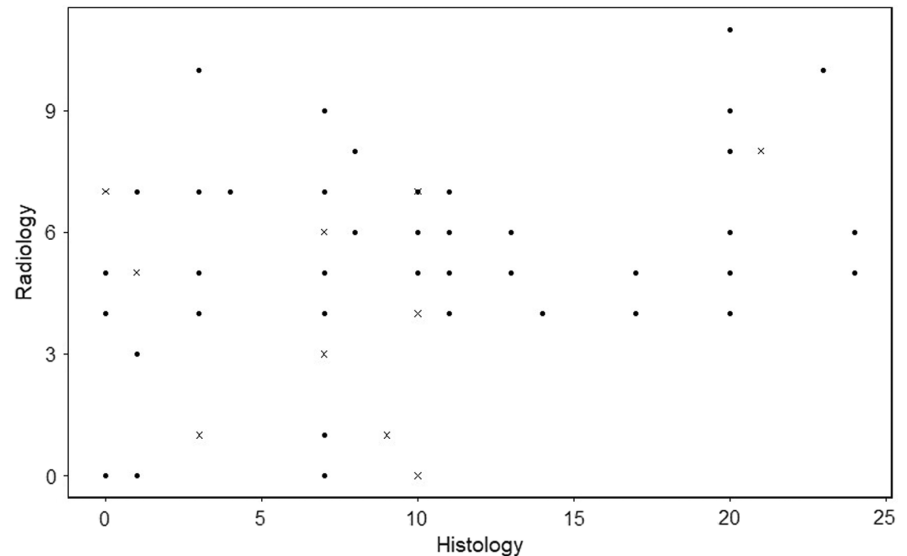
Regarding the future use of radiology to assess nephrocalcinosis, one should be aware of the limitations of the method, since soft tissue changes not including mineralization cannot be seen on radiographs. In general, it is not possible to evaluate the degree of soft tissue lesions associated with deposits based on radiology alone.

Another major limitation is the fact that the radiology cannot differentiate between different types of pathologies associated with mineral deposits in kidney tissue, meaning that conditions such as chronic granulomatous inflammation with secondary central calcification cannot be differentiated from deposits due to nephrocalcinosis. Histopathology will, therefore, still be crucial for the evaluation of soft tissue changes and for definitive confirmation of diagnosis.

The method should be further developed to establish cut-off categories, similar to those of the histological model. To do so, a larger sample size should be assessed, also including several individuals without nephrocalcinosis as 'control', to evaluate the proportion of false and true negatives. We believe that this will increase the sensitivity and specificity of radiology.

In conclusion, we are confident that radiology is a suitable diagnostic tool for assessing mineral deposits (amount and distributions) associated with nephrocalcinosis in farmed Atlantic salmon, with minimal risk to the operator when appropriate measures are taken. The methodology is significantly more cost-effective than histology

**FIGURE 3** Scatterplot of radiological (y-axis) assessment and histological assessment (x-axis). Each point represents a single individual. Those marked as 'x' indicate radiographs with background disturbances.  $df = 61$ ,  $p$ -value = .0084,  $r$ -square = 0.33.



with faster response time and reduces drastically the number of fish that need to be euthanized.

We recommend further developing radiology for the assessment of nephrocalcinosis in fish. This non-lethal method will make it possible to assess the disease progression on individual and group level over time, as well as exploring modes of prevention and treatments, greatly increasing the possibility of discovering its aetiology.

#### ACKNOWLEDGEMENTS

The authors wish to thank the staff of the facilities where sampling was carried out for their valuable assistance. Thanks also to Susanne Tofte for her help with sampling. The present study was financed by the Norwegian Research Council as part of Christine Klykken's Ph.D. thesis (project no. 304498) and the Norwegian Seafood Research Fund (FHF) as a part of the project NEFROSMOLT (FHF-901587).

#### CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest to disclose.

#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**How to cite this article:** Klykken, C., Dalum, A. S., Reed, A. K., Attramadal, K., Olsen, R. E., & Boissonnot, L. (2022). Radiological detection of nephrocalcinosis in farmed Atlantic salmon *Salmo salar* L.. *Journal of Fish Diseases*, 00, 1–6. <https://doi.org/10.1111/jfd.13704>