INTRODUCTION

Nephrocalcinosis is described as deposits of mineral salts within kidney tubules and collecting ducts (Bruno, 1996). Renal calcification may occur at molecular, microscopic or macroscopic levels in the kidney, such as tubular cells, interstitial tissue or within the tubular lumen that may lead to progressive amounts of renal damages (Sayer et al., 2004). In a previous study, we found that the renal deposits in Atlantic salmon mainly consisted of amorphous carbonate apatite (amCAP), a calcium-dominated mineral (Klykken et al., 2022).

The aetiology of nephrocalcinosis in fish is not known (Klosterhoff et al., 2015), but there is a consensus that the condition is most likely related to the intensive production conditions in aquaculture (Applegate et al., 2016; Béland et al., 2020; Bjerknes et al., 1994; Cavrois-Rogacki et al., 2021; Gillespie & Evans, 1979; Klosterhoff et al., 2015; Lewisch et al., 2013; Smart et al., 1979).

The kidney is one of three primary organs (kidney, gills and intestine) involved in osmoregulation in salmon (Talbot & Thorpe, 1992). The renal function of the anadromous salmon undergoes major transformations as the fish prepare to migrate from freshwater (FW) to seawater (SW) environments (Takvam et al., 2021). In the hyperosmotic environment of FW, one of the main tasks of the glomerular kidneys is to excrete excess water while reabsorbing solutes (McDonald, 2007). In contrast, to counteract the potential dehydration in SW, salmon drink ambient water containing large concentrations of divalent ions. The primary function of the kidney in SW is,
therefore, to excrete divalent ions in strongly reduced isotonic urine (Beyenbach, 2000).

Severe nephrocalcinosis with extensive damage to the kidney is likely to result in impaired renal function. This can have dramatic consequences for osmoregulatory capability like water export in FW and divalent ion excretion in SW, posing a threat to health and welfare of the salmon. In the aquaculture industry, there is a general consensus that the disease is linked to commercial production conditions, but little is known of the internal mechanisms of nephrocalcinosis. To explore these mechanisms, we applied whole transcriptome profiling of salmon kidneys with severe nephrocalcinosis and compared the gene expression patterns with healthy salmon kidneys.

Transcriptome profiling is a powerful tool used to expose expression patterns (Rani & Sharma, 2017). Variations in the transcriptome can be observed for physiological conditions, developmental stages and the external environment of an organism which makes transcriptome profiling a robust tool for examining the relationship between the genotype and phenotype (Chandhini & Kumar, 2019). In aquaculture, transcriptomics has previously been utilized for examining immunity, diseases and nutrition (Martin et al., 2016; Martin & Król, 2017; Sudhagar et al., 2018; Ye et al., 2018) as well as detection of molecular markers (Chandhini & Kumar, 2019).

We hypothesize that severe nephrocalcinosis results in a generally altered physiology with differential gene regulation responses. By studying the effects of nephrocalcinosis on fish physiology and gene expression patterns, we aim to provide new insight into the mechanisms of the disease.

### MATERIALS AND METHODS

#### 2.1 | Data sampling

From a previous study with 420 farmed Atlantic salmon, which were analysed for nephrocalcinosis (Klykken et al., 2022), 16 were chosen according to the criteria of being either healthy (Ctrl, \(N = 8\)) or severely affected by nephrocalcinosis (NC, \(N = 8\)). The fish were smolts of similar sizes with no other pathological findings. The fish were collected from two different commercial salmon nurseries with production conditions complying with the Norwegian legislation (Akvakulturdriftsforskriften, 2008). The fish were fed until sampling, and they were killed with an overdose of Benzoak VET (200–400mg/L) followed by a sharp blow to the head according to Norwegian legislation (Akvakulturdriftsforskriften, 2008). Size measurements of individual fish included round body weight (W) in g (±1 g), fork length (L) (±0.5 cm), and condition factor: \(CF = 100(W/L^3)\).

#### 2.2 | Histopathology

Tissues from the mid-kidney were sampled from all individuals for histopathological analysis of nephrocalcinosis. The kidney tissues were fixed in 4% formaldehyde solution, embedded in paraffin wax and routinely processed (Suvarna et al., 2019). All sections were stained by haematoxylin and eosin. A selection of sections in paraffin was stained with von Kossa stain for visualization of calcium deposits.

| Severity | 1 | 2 | 3 |
|----------|---|---|---|
| Category 1 | Presence of deposits | Sparse amounts in collecting ducts and ureters close to absence in tubules and affects <10% of the excretory system | Moderate amounts in collecting ducts and ureters sparse amounts in tubules and affects between 10% and 50% of the excretory system | Extensive quantities in ureters, collecting ducts and tubules and affecting more than 50% of the excretory system |
| Category 2 | Epithelial degeneration and/or necrosis | Affects <10% of tubules and collecting ducts | Affects between 10% and 50% of tubules and collecting ducts | Affects more than 50% of tubules and collecting ducts |
| Category 3 | Pathological changes in the glomeruli | Dilatation of the glomerular space (urine stagnation) and fibrosis/thickening of the parietal Bowman’s capsule, changes in <10% of the glomeruli | Dilatation/thickening of the parietal Bowman’s capsule, peri-glomerular fibrosis—changes in between 10% and 50% of glomeruli | Dilatation, thickening of the parietal Bowman’s capsule, peri-glomerular fibrosis, changes in over 50% of glomeruli |
| Category 4 | Pathological changes in the interstitial tissue | Affects <10% of interstitial tissue | Affects between 10% and 50% of interstitial tissue | Affects more than 50% of interstitial tissue |

Note: The scores are weighted based on the effect the various changes are believed to have on the development of the disease and the time it will take to heal the condition.
deposits (Rungby et al., 1993). The histopathological diagnosis of nephrocalcinosis was defined as the presence of amorphous (structureless), basophilic deposits in tubules, collecting ducts and excretory ducts. The severity of deposits and tissue damage was evaluated according to the score given in Klykken et al. (2022) (Table 1). Total nephrocalcinosis score is calculated as:

$$\sum_{n=1}^{4} C_n \times S_n$$

Where $C$ is the category, $S$ is the severity and $n$ is the category number.

In the nephrocalcinosis score, overall scores 1 to 10 were generally considered mild changes, scores 11 to 20 were considered moderate changes, and scores greater than 20 were regarded as severe changes.

### 2.3 Blood collection and determination of plasma chemistry

Vacutainer tubes (Becton-Dickinson) with lithium heparin as anticoagulant were used to collect blood from the caudal vein, immediately after euthanasia. After thorough mixing, the samples were centrifuged at 13,500 rpm for 5 min (VWR Mikrostar 12, 12 x 1.5/2.0 ml) and the plasma was transferred to Eppendorf tubes and kept frozen (minimum −20°C) until analysis.
The following parameters were measured at the laboratory of Aqua Kompetanse using an automated dry chemistry analyser (CatalystOne, IDEXX Laboratories, Westbrook, ME, [Boes et al., 2018]): alkaline phosphatase (ALKP), aspartate aminotransferase (AST), creatinine (CREA), calcium (Ca), sodium (Na), potassium (K), chloride (Cl), magnesium (Mg), inorganic phosphate (PHOS), glucose (GLU) and lactate (LAC). Each of the assays used a standard kit developed for the automated analyser (CatalystOne, IDEXX Laboratories, Westbrook, ME).

The plasma occasionally showed activities for inorganic phosphate exceeding the instrument ranges and sporadically for other parameters: in these cases, a physiological saline solution (0.9% NaCl) was used to dilute the samples (1:4).

2.4 Gene expression patterns by mRNA sequencing

Mid-kidney tissue was isolated and immediately transferred into tubes containing RNAlater © (Life technologies) and stored in 4 °C for 24 h before storage at −20 °C until analysis.

The samples were processed according to (Levmo et al., 2021). Total RNA was extracted from the mid-kidney of 8 fish with severe nephrocalcinosi s and 8 healthy fish, a total of 16 samples. mRNA extraction was done using a RNeasy Plus Universal Mini kit (Qiagen) according to the manufacturer’s instruction. RNA concentrations were measured using a Nanodrop 8000 (Thermo Scientific). RNA quality was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies). All 16 samples passed the quality check (RIN >7).

cDNA libraries were prepared using TruSeq Stranded mRNA Sample prep HS protocol (Illumina) according to the manufacturer’s instruction, with selection for 500bp fragments. The cDNA libraries were sent to Genomics Core Facility at the Norwegian University of Science and Technology (NTNU) for sequencing. Libraries were quantified by qPCR using the KAPA SYBR FAST library quantification kit developed for the automated analyser (CatalystOne, IDEXX Laboratories, Westbrook, ME).

Sequence reads were de-multiplexed and converted from BCL to fastq file format using bcl2fastq2 conversion software V2.20.0422 (Illumina, Inc.). Reads were mapped to the Atlantic Salmon reference genome (ICSASG_v2. 6-10-2016) using HISAT2 v2.1.0 with default parameters (Kim et al., 2015). Gene expression levels were based on read counts per gene and were quantified from mapping results using feature Counts v1.6.5 (Liao et al., 2014). Gene regions on the ICSASG_v2 reference genome were defined using RefSeq annotation data and metadata. A count table of read counts per gene for each sample was used as the foundation for comparative analysis of gene expression. The table was imported into R version 4.0.5 (Team, 2017) where all subsequent downstream analyses were completed, including differential expression analysis, pathway enrichment and functional annotation. Sample variance and clustering, including outlier detection, was assessed by generating density plots, PCA plots, heat maps (Figure S1) and dendrograms using base R tools. Differential expression (DE) analysis was completed using the DESeq2 package (Love et al., 2014). Identification of DE genes by DESeq2 was done using the following statistical steps: normalization completed by multiplying read counts by per-sample size factors, which were generated by dividing the read counts per sample by the geometric mean for each gene across all samples. Then, gene-wise dispersions were estimated and shrunk. These shrunk estimates are fit to a negative binomial generalized linear model (GLM), estimating expression strength per gene, by group. A Wald test was used to test the significance of expression strength between groups, generating p values for each gene. Finally, the false discovery rate (FDR) of p values was estimated using the Benjamini and Hochberg method (Benjamini &

### TABLE 2 Body weight, length, condition factor and blood chemistry parameters of nephrocalcinosis-affected (NC) salmon compared with healthy fish (Ctrl)

| Parameter             | NC    | Ctrl |
|-----------------------|-------|------|
| Number of fish        | 8     | 8    |
| Body weight (g)       | 279 ± 52 | 249 ± 12 |
| Length (cm)           | 29 ± 2 | 28 ± 1 |
| Condition factor      | 1.2 ± 0.1 | 1.1 ± 0.1 |
| **Enzymes**           |       |      |
| Alkaline phosphatase  | 370.8 ± 192.5* | 669.1 ± 103.6 |
| (U/L)                 |       |      |
| Aspartate aminotransferase (U/L) | 476.8 ± 204.1* | 247.4 ± 89.2 |
| **Creatinine (U/L)**  | 84.9 ± 76.8 | 37.8 ± 16.3 |
| **Electrolytes**      |       |      |
| Calcium (mmol/L)      | 3.7 ± 0.7* | 2.7 ± 0.2 |
| Magnesium (mmol/L)    | 5.2 ± 1.2* | 1.0 ± 0.2 |
| Phosphate (mmol/L)    | 7.1 ± 1.1* | 4.5 ± 0.8 |
| Sodium (mmol/L)       | 160.1 ± 12.0 | 162.1 ± 1.9 |
| Potassium (mmol/L)    | 2.3 ± 0.5 | 3.1 ± 1.0 |
| Chloride (mmol/L)     | 126.5 ± 12.1 | 127.5 ± 3.9 |
| **Metabolites**       |       |      |
| Glucose (mmol/L)      | 9.4 ± 1.8* | 6.5 ± 0.8 |
| Lactate (mmol/L)      | 8.2 ± 1.2* | 3.4 ± 0.8 |

Note: Significant differences between the groups are indicated with * (p ≤ 0.05).
Significantly DE genes were identified as having an FDR adjusted \( p \) value of <.05.

### 2.5 | Statistical analysis

All statistical analyses were performed using R software 4.0.5 (R Core Team, 2017). Normality was tested with Shapiro–Wilks test, and non-normal distributed data were transformed using square root, cube root or Tukey’s ladder. Magnesium concentration in plasma was not normally distributed. These samples were analysed using Wilcoxon rank-sum test. Blood chemistry parameters are presented as mean ± standard deviation. \( p \) values of ≤0.05 were considered statistically significant.

### 3 | RESULTS

#### 3.1 | Histopathology of kidney with nephrocalcinosis

There were extensive amounts of mineral deposits in ureters and bladder of fish affected by nephrocalcinosis (NC) (Figure 1). The kidneys of the affected fish were swollen and the tissue appeared pale compared with controls. All fish in the NC group (\( n = 8 \)) were diagnosed with severe nephrocalcinosis with a score ranging from 26 to 30 (Table 1).

The NC group displayed extensive damage to the tubular wall, with complete loss of epithelium with fibrosis of the basal membrane and dilatation and fibrosis in the lumen-containing deposits (Figure 2b–g).
Figure 4: Mirrored bar plot of the number of upregulated and downregulated DE genes in each significantly (padj < 0.05) enriched KEGG pathway Ctrl Vs NC. Bars are shaded by adjusted p values per pathway.
Acute interstitial inflammation and chronic interstitial fibrosis was also observed (Figure 2f). The surrounding interstitial tissue showed granulomatous inflammation with the presence of Langhans giant cells (Figure 2h) and chronic glomerulitis was also seen (Figure 2b). Von Kossa showed black staining of the tissue, indicating calcium salts in the deposits associated with nephrocalcinosis (Figure 2i).

3.2 | Blood chemistry

The blood chemistry parameters differed significantly between Ctrl and NC (Table 2). The NC group had significantly higher concentrations of aspartate aminotransferase (AST), calcium, magnesium, phosphate, glucose and lactate, whereas alkaline phosphatase (ALKP) was significantly lower. The variance in the NC group was considerably higher than in the Ctrl.

3.3 | Gene expression patterns

A total of 10133 genes displayed differential expression patterns in fish with severe nephrocalcinosis (NC) compared with healthy fish (Ctrl). 6970 of the DE genes were upregulated (log2fold change [LFC] >1) and 3163 were downregulated (LFC < -1).

The gene expression patterns of the two groups, Ctrl and NC, showed good separation as illustrated in the heatmap and PCA plot (Figure 3). The KEGG pathway enrichment analysis was used to identify pathways significantly regulated (enriched DEGs) (Figure 4 shows top 30). There were 31 significantly enriched KEGG pathways. Among these were 22 metabolic pathways, 4 pathways involved in environmental information processing, 2 pathways connected to cellular processes and 3 pathways belonging to organismal systems.

One of the most significant changes in fish affected with nephrocalcinosis was the general and apparently massive downregulation of many metabolic pathways related to ATP production, glycolysis and Krebs cycle activity. The oxidative phosphorylation and Krebs cycle appeared to be particularly affected with 153 and 30 genes, respectively, significantly downregulated (Figures 5 and 6, respectively). Many other processes feeding into these pathways were also downregulated such as peroxisomes, fatty acid and amino acid degradation/metabolism. Other notable pathways downregulated included the P450 xenobiotics. In addition, the glutathione metabolism pathway was mostly downregulated, with exception of ribonucleoside-diphosphate reductase which was upregulated.

**FIGURE 5** Pathway map sasa00190—oxidative phosphorylation. The protein EC number within each box is given in either green, red or white, indicating upregulation, downregulation or no change, respectively, with increased colour intensity indicating increased log2fold change.
pathway is important for DNA and RNA repair, and the upregulation suggests increased repair requirement in the tissue.

Most gene pathways upregulated in fish with nephrocalcinosis were related to cell cycle and maintenance. All three Wnt signalling pathways were upregulated (93 genes) including the canonical Wnt pathway, the non-canonical planar cell polarity pathway and the non-canonical Wnt/calcium pathway. Adherence junctions were also strongly upregulated (61 genes) including transmembrane nectin and cadherin (Figure 7).

Upregulated genes downstream of nectin included many genes involved in cytoskeleton and cell shape regulation (PAR3, Src, Ponsin, ZO-1, alpha-actinin and IRSp53). Likewise, numerous cell adhesion molecules were also strongly upregulated in fish affected with nephrocalcinosis. These include many leucocyte proteins including major histocompatibility complex II (MHCII), CD22 (B cells), B7H3 and poliovirus receptor-related 2 (PVRL2) and 3. It was interesting to note that some members of the adaptive immune system like CD2 found on T and NK cells were downregulated. CD99 was upregulated, indicating the migration of immune cells to inflammation in the kidney tissue (Figure 8). There was also a notable upregulation of genes encoding for the junctional adhesion proteins B and C (junctional adhesion molecules 2 and 3, respectively, JAM2, JAM3), but not JAM1. Interestingly, the expression of another set of tight junctional proteins, Claudins (Claudin 2, 3, 10 and 14), was downregulated.

The signal transduction pathways were upregulated, as were the signalling molecules and interaction pathways, and in particular, the ECM-receptor interaction pathway was highly upregulated (Figure 9). Genes involved in cellular communication were also primarily upregulated.

Genes in the cardiac muscle contraction pathway were primarily downregulated (60 downregulated and 28 upregulated, Figure 10). Tropomyosin (TPM) was greatly upregulated in the NC group, pointing to cell structure maintenance and repair since TPM and myosin are important for cytoskeleton and cell migration. The ubiquinol-cytocrome and cytochrome c subunits were also downregulated. These molecules are part of oxidative phosphorylation and metabolism, which were distinctly downregulated.

Genes involved in lipid metabolism were both up- and downregulated, with the majority of pathways having a predominance of

![Pathway map sasa00020—citrate cycle.](image-url)
downregulated genes with the exceptions of fatty acid degradation and glycerophospholipid metabolism, which had more upregulated genes. Arachidonic acid metabolism had several upregulated genes. The increase in the prostaglandin endoperoxide synthase and prostacyclin synthase suggests an increased synthesis of prostacyclin, which is an efficient inhibitor of platelet activation and a vasodilator. The activity of PLA2 appeared to increase, suggesting increased turnover of membrane lipids. There was also an increased phospholipid synthesis, particularly through the Kennedy pathway, and towards phosphatidylethanolamine, choline and other phospholipids. Interestingly, the activity of acetylcholinesterase was also increased pointing to increased neuronal signalling (see Figure S1–S16 in Appendix).

**4 | DISCUSSION**

This is the first transcriptome study profiling the response of Atlantic salmon (*Salmo salar*) with chronic and severe nephrocalcinosis (NC). The study showed numerous genes and KEGG pathways being differentially expressed in salmon with nephrocalcinosis compared with healthy salmon (Ctrl). In order to gather a sufficient number of samples from healthy fish and NC fish, kidney samples had to be collected from two separate nurseries. We are aware that this is a scientific element of uncertainty in the study, as we cannot know to which level the different environments may have affected the gene expression patterns in the two groups. Despite this, we consider the
study of value because we believe that the main differences in the transcriptome profiles are related to the pronounced tissue damage caused by nephrocalcinosis.

Histological examination of Atlantic salmon kidneys with severe NC revealed, extensive tissue damage similar to earlier observations (Harrison & Richards, 1979; Hicks et al., 1984; Klykken et al., 2022; Saraiva et al., 2016). The effects included changes in the glomeruli with dilatation of the glomerular space, fibrosis and thickening of the parietal layer of the Bowman’s capsule and varying degree of perglomerular fibrosis and glomerulitis. The damages to glomeruli have been suggested, in parts, to be a result of urine stagnation (Docherty et al., 2006) caused by mineral deposits formed during the development of NC (Harrison & Richards, 1979). There was also a significant granulomatous inflammation with the formation of giant cells in the NC group. Leukocyte transendothelial migration, which was upregulated in the NC group. Leukocyte transendothelial migration is a critical step in immune activation (Gao et al., 2017), and it plays an essential role in promotion of inflammatory responses (Boshra et al., 2006). These responses in the NC group are probably linked to the ongoing inflammation in the kidney tissue caused by nephrocalcinosis. This was also evident by the increased expression of several immune cell markers such as MHC II and CD22. The lower expression of CD2 and other markers of the adaptive immune system suggests that the innate system was mainly involved. It is worth noting that the cases of nephrocalcinosis investigated in this paper involve rupture of the basal membrane of one or more nephrons, causing interaction between the mineral deposits and interstitial immune tissue. Further studies should investigate whether the same level of immune regulation can be seen in fish with mineral deposits retained within the excretory system without the involvement of interstitial tissue. Besides nephrocalcinosis, mineral deposits are known to form within various internal organs of salmonids such as the gastrointestinal wall (Roberts & Rodger, 2012), pseudobranch, choroid plexus of the eye,
heart valves, compact layer or the cardiac ventricle, skeletal muscle and more often manifested as dystrophic calcification of parenchymatous cells in the resident tissue (Alf Dalum, personal observation). However, the causality of such soft-tissue mineralization, and a possible relation with nephrocalcinosis, has to our knowledge not been investigated. In our study, only renal tissue was subjected to histological investigation, and we cannot exclude a possible involvement of mineral deposits in other soft tissues.

Along with the inflammatory repair processes, transcriptomic data revealed significant upregulation of several repair and recruitment pathways such as membrane phospholipid reshuffling and synthesis (Kennedy pathway for DAG synthesis, PE, PC, PLA2, SCD-1 FATP1/4 and RXR), cell-to-cell communication, adherence and tight junctions, cytoskeletal functions and extracellular matrix (ECM), including many collagens, integrins, cadherins, laminins and thrombospondins essential for maintaining tissue structure and function (Webster et al., 2018). Upregulation of prostacyclin pathways can also be viewed as an attempt to inhibit blood clot at the damaged sites. The upregulation of some metabolic pathways (and claudin 11) appeared to be related to neuronal synthesis/maintenance (aspartyl-glutamate, citryl-glutamate and acetylcholine). It was interesting to note that in the cell adhesion pathway, many ‘tight’ sealing claudins like 1, 4, 5, 11 were upregulated while many ‘leaky’ pore-forming claudins (2, 10) were downregulated in NC fish, which could indicate
an attempt to seal the epithelial cell lining during nephrocalcinosis. In addition, Li et al. (2018) found that claudins may have vital functions in the immune responses in fish gill infections, which strengthens the observation that claudins are involved in immune responses in fish.

A notable observation in NC fish transcriptomics was the massive downregulation of most NADH/ATP energy-generating pathways (TCA-cycle and respiratory chain) including many pathways like amino acid metabolism and fatty acid β-oxidation that would normally feed carbon into the citric acid cycle. This suggests a malfunctional kidney with limited capacity for maintenance and repair. The increase in plasma AST and divalent ions, normally excreted over the kidney (magnesium, calcium and phosphate) (Beyenbach, 2000; Li et al., 2011; Nieves-Puigdoller et al., 2007; Singh et al., 2002; Wagner & Congleton, 2004), supports this notion. Another possible sign of kidney malfunction could be seen in the increased plasma lactate in NC fish. In clinical medicine, hyperlactatemia is indicative for kidney disease (Phypers & Pierce, 2006) and could be the consequence of impaired ATP production and perfusion. It is also interesting to note that Santis et al. (2015) found the same marked downregulation of metabolic pathways in their study of inflammation in the intestine caused by soybean protein. They also suggested that impaired metabolism could be a consequence of tissue malfunction.

5 | CONCLUSION

The transcriptome profiles of salmon affected by severe nephrocalcinosis revealed an upregulation of inflammation and repair processes together with a massive shut down of metabolism. These responses were most likely related to the severe tissue damage observed on histology resulting in kidney failure. The results of this study support that nephrocalcinosis is a major welfare challenge and most likely make the salmon more sensitive to additional stressors. The reduced renal function may have fatal consequences for the fish in the nurseries and should be further investigated.

ACKNOWLEDGEMENTS

We wish to thank Liss Lunde (Aqua Kompetanse AS) for her assistance with the data collection. We are also grateful to the operations data on KEGG graph

FIGURE 10 Pathway map sasa04260—cardiac muscle contraction. The protein EC number within each box is given in either green, red or white, indicating upregulation, downregulation or no change, respectively, with increased colour intensity indicating increased log2fold change.
managers and staff at the facilities for their contribution in the project.

**FUNDING INFORMATION**
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 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**
All data are stored at Aqua Kompetanse AS according to internal guidelines and are available on request.

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**REFERENCES**

Akvakulturdriftsforskriften (2008). Forskrift om drift av akvakulturanlegg for-2008-06-17-822.

Applegate, J. R., Lewbart, G. A., Daniels, H., Gill, A., & Stoskopf, M. K. (2016). Calcium urolithiasis in a breeding population of southern flounder (Paralichthys lethostigma) housed in a low salinity environment. *Veterinary Quarterly*, 36, 50–54.

Béland, K., Wong, E., St-Cyr, J., & Lair, S. (2020). High occurrence rate of xanthomatosis and nephrocalcinosis in aquarium-housed Atlantic wolffish *Anarhichas lupus* and spotted wolffish *A. minor*. *Diseases of Aquatic Organisms*, 139, 223–232.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, 57, 289–300.

Beyenbach, K. W. (2000). Renal handling of magnesium in fish: From whole animal to brush border membrane vesicles. *Frontiers in Bioscience*, 5, D712–D719.

Bjerkes, V., Lydersen, E., Golmen, L., Hobæk, A., and Holtet, L. (1994). Nefrokalsinose hos regnbueaure i oppdrettsanlegg ved trengereid. *NIVA-RAPPORT. ISBN-82-577-2487-4, 22p.*

Boes, K. M., Sink, C. A., Camus, M. S., & Werre, S. R. (2018). Evaluation of an in-clinic dry chemistry analyzer for canine, equine, and feline plasma samples. *Journal of Veterinary Diagnostic Investigation*, 30, 902–910.

Boshra, H., Li, J., & Sunyer, J. (2006). Recent advances on the complement system of teleost fish. *Fish Shellfish Immunology*, 20, 239–262.

Bruno, D. W. (1996). Nephrocalcinosis. *Aquaculture Information Series*, 16, 1–5.

Cavrois-Rogacki, T., Drabikova, L., Migaud, H., & Davie, A. (2021). Deformities prevalence in farmed ballan wrasse (labrus bergylta) in relation to hatchery origin and life stage. *Aquaculture*, 533, 736212.

Chandhini, S., & Kumar, V. J. R. (2019). Transcriptionists in aquaculture: Current status and applications. *Reviews in Aquaculture*, 11, 1379–1397.

Damjanov, I. (2009). Inflammation and repair. In I. Damjanov (Ed.), *Pathology Secrets (Third Edition)* (pp. 19–37). Mosby.

Docherty, N. G., O’Sullivan, O. E., Healy, D. A., Fitzpatrick, J. M., & Watson, R. W. G. (2006). Evidence that inhibition of tubular cell apoptosis protects against renal damage and development of fibrosis following ureteric obstruction. *American Journal of Physiology - Renal Physiology*, 290, 4–13.

Gao, F.-X., Wang, Y., Zhang, Q.-Y., Mou, C.-Y., Li, Z., Deng, Y.-S., Zhou, L., & Gui, J.-F. (2017). Distinct herpesvirus resistances and immune responses of three gynogenetic clones of gibel carp revealed by comprehensive transcriptomes. *BMC Genomics*, 18, 561.

Gillespie, D. C., & Evans, R. E. (1979). Composition of granules from kidneys of rainbow trout (*Salmo gairdneri*) with nephrocalcinosis. *Journal of the Fisheries Research Board of Canada*, 36, 683–685.

Harrison, J. G., & Richards, R. H. (1979). The pathology and histopathology of nephrocalcinosis in rainbow trout salmo gairdneri richardson in fresh water. *Journal of Fish Diseases*, 2, 1–12.

Hicks, B. D., Hilton, J. W., & Ferguson, H. W. (1984). Influence of dietary selenium on the occurrence of nephrocalcinosis in the rainbow trout, *Salmo gairdneri* richardson. *Journal of Fish Diseases*, 7, 379–389.

Kim, D., Langmead, B., & Salzberg, S. L. (2015). Hisat: A fast spliced aligner with low memory requirements. *Nature Methods*, 12, 357–360.

Klosterhoff, M., Pedrosa, V., Sampaio, L. A., Ramos, L., Tesser, M. B., & Romano, L. A. (2015). Nephrocalcinosis and kidney stones in *Rachycantron canadum*. *Bulletin of the European Association of Fish Pathologists*, 35, 138–147.

Klykken, C., Reed, A., Dalum, A., Olsen, R., Moe, M., Attamadkal, K., & Boissonnot, L. (2022). Physiological changes observed in farmed atlantic salmon (*Salmo salar* L.) with nephrocalcinosis. *Aquaculture*, 554, 738104.

Kumar, S., Prasad, T., Narayan, P., & Muruganandhan, J. (2013). Granuloma with langhans giant cells: An overview. *Journal of Oral and Maxillofacial Pathology*, 17, 420–423.

Lewisch, E., Kucera, M., Tappert, R., Tessadri, R., Tappert, M., & Kanz, F. (2013). Occurrence of nephrolithiasis in a population of largesnout seahorse, *Hippocampus reidi* ginsburg, and analysis of a nephrolith. *Journal of Fish Diseases*, 36, 163–167.

Li, Z., Liu, X., Cheng, J., He, Y., Wang, X., Wang, Z., Qi, J., Yu, H., & Zhang, Q. (2018). Transcriptome profiling provides gene resources for understanding gill immune responses in japanese flounder (Paralichthys olivaceus) challenged with Edwardsiella tarda. *Fish Shellfish Immunology*, 72, 593–603.

Li, Z.-H., Velisek, J., Grubic, R., Li, P., Kolarova, J., & Randak, T. (2011). Use of hematological and plasma biochemical parameters to assess the chronic effects of a fungicide propiconazole on a freshwater teleost. *Chemosphere*, 83, 572–578.

Liao, Y., Smyth, G. K., & Shi, W. (2014). Featurecounts: An efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*, 30, 923–930.

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DEseq2. *Genome Biology*, 15, 1–21.

Lavmø, S. D., Whatmore, P., Sundh, H., Sigholt, T., Madaro, A., Bardal, T., & Olsen, R. E. (2021). Effects of atlantic salmon (*Salmo salar*) fed low- and high hufa diets on growth and midgut intestinal health. *Aquaculture*, 539, 736653.

Martin, S. A., Dehler, C. E., & Kröl, E. (2016). Transcriptomic responses in the fish intestine. *Developmental Comparative Immunology*, 64, 103–117.

Martin, S. A., & Kröl, E. (2017). Nutrigenomics and immune function in fish: New insights from omics technologies. *Developmental Comparative Immunology*, 75, 86–98.

McDonald, M. D. (2007). The renal contribution to salt and water balance. In B. Baldisserotto, J. M. Manceria, & B. G. Kapoor (Eds.), *Fish Osmoregulation* (pp. 322–345). CRC Press.

Nieves-Puigdoller, K., Björnsson, B. T., & McCormick, S. D. (2007). Effects of hexazinone and atrazine on the physiology and endocrinology of smolt development in atlantic salmon. *Aquatic Toxicology*, 84, 27–37.
O’Regan, A., & Berman, J. S. (2001). Osteopontin: A key cytokine in cell-mediated and granulomatous inflammation. International Journal of Experimental Pathology, 81, 373–390.

Patel, S., Alam, A., Pant, R., & Chattopadhyay, S. (2019). Wnt signaling and its significance within the tumor microenvironment: Novel therapeutic insights. Frontiers in Immunology, 10, 2872.

Phypers, B., & Pierce, J. T. (2006). Lactate physiology in health and disease. Continuing Education in Anaesthesia Critical Care Pain, 6, 128–132.

Rani, B., & Sharma, V. K. (2017). Transcriptome profiling: methods and applications - a review. Agricultural Reviews, 38, 271–281.

Roberts, R., & Rodger, H. (2012). The pathophysiology and systematic pathology of teleosts. Fisheries Pathology, 3, 62–143.

Sakai, H., Okafuji, I., Nishikomori, R., Abe, J., Izawa, K., Kambe, N., Yasumi, T., Nakahata, T., & Heike, T. (2012). The CD40-CD40L axis and IFN-γ play critical roles in langhans giant cell formation. International Immunology, 24, 5–15.

Santis, C. D., Bartie, K. L., Olsen, R. E., Taggart, J. B., & Tocher, D. R. (2015). Nutrigenomic profiling of transcriptional processes affected in liver and distal intestine in response to a soybean meal-induced nutritional stress in Atlantic salmon (Salmo salar). Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, 15, 1–11.

Saraiva, A., Costa, J., Eiras, J. C., & Cruz, C. (2016). Histological study as indicator of juveniles farmed turbot, Scophthalmus maximus L. health status. Aquaculture, 459, 210–215.

Sayer, J. A., Carr, G., & Simmons, N. L. (2004). Nephrocalcinosis: Molecular insights into calcium precipitation within the kidney. Clinical Science, 106, 549–561.

Shah, K. K., Pritt, B. S., & Alexander, M. P. (2017). Histopathologic review of granulomatous inflammation. Journal of Clinical Tuberculosis and Other Mycobacterial Diseases, 7, 1–12.

Shavit, L., Jaeger, P., & Unwin, R. J. (2015). What is nephrocalcinosis? Kidney International, 88, 35–43.

Singh, N., Das, V. K., & Srivastava, A. K. (2002). Insecticidies and ionic regulation in teleosts: A review. Zoologica Poloniae, 47, 21–36.

Smart, G. R., Knox, D., Harrison, J. G., Ralph, J. A., Richard, R. H., & Cowey, C. B. (1979). Nephrocalcinosis in rainbow trout Salmo gairdneri richardson; the effect of exposure to elevated CO₂ concentrations. Journal of Fish Diseases, 2, 279–289.

Sudhagar, A., Kumar, G., & El-Matbouli, M. (2018). Transcriptome analysis based on RNAseq in understanding pathogenic mechanisms of diseases and the immune system of fish: A comprehensive review. International Journal of Molecular Sciences, 19, 245.

Suvarna, K. S., Layton, C., & Bancroft, J. D. (Eds.). (2019). Bancroft’s Theory and Practice of Histological Techniques (8th ed.). Elsevier.

Takvam, M., Wood, C. M., Kryvi, H., & Nilsen, T. O. (2021). Ion transporters and osmoregulation in the kidney of teleost fishes as a function of salinity. Frontiers in Physiology, 12, 1–25.

Ye, H., Lin, Q., & Luo, H. (2018). Applications of transcriptomics and proteomics in understanding fish immunity. Fish Shellfish Immunology, 77, 319–327.

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Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Klykken, C., Boissonnot, L., Reed, A. K., Whatmore, P., Atttramadal, K., & Olsen, R. E. (2022). Gene expression patterns in Atlantic salmon (Salmo salar) with severe nephrocalcinosis. Journal of Fish Diseases, 45, 1645–1658. https://doi.org/10.1111/jfd.13687