Received: 17 June 2023 | Revised: 22 September 2023 | Accepted: 25 September 2023

DOI: 10.1111/jfd.13870

RESEARCH ARTICLE

Multiple *Aeromonas* strains isolated from Atlantic salmon (*Salmo salar*) displaying red skin disease signs in Scandinavian rivers

Erwan Lagadec | Even Bysveen Mjølnerød | Øyvind Marius Jensen | Heidrun Plarre | Are Nylund

Fish Disease Research Group, Department of Biological Sciences, University of Bergen, Bergen, Norway

Correspondence
Erwan Lagadec, Fish Disease Research Group, Department of Biological Sciences, University of Bergen, Bergen, Norway.
Email: erwan.lagadec@uib.no

Funding information
Miljødirektoratet; SalmonCamera

Abstract

Since 2014, Atlantic salmon (*Salmo salar* L.) displaying clinical signs of red skin disease (RSD), including haemorrhagic and ulcerative skin lesions, have been repeatedly observed in Swedish rivers. Although the disease has since been reported in other countries, including Norway, Denmark, Ireland and the UK, no pathogen has so far been conclusively associated with RSD. In this study, the presence of 17 fish pathogens was investigated through qPCR in 18 returning Atlantic salmon with clinical signs of the disease in rivers in Sweden and Norway between 2019 and 2021. Several potential pathogens were repeatedly detected, including a protozoan (*Ichthyobodo* spp.), an oomycete (*Saprolegnia* spp.) and several bacteria (*Yersinia ruckeri*, *Candidatus* Branchiomonas cysticola, *Aeromonas* spp.). Cultivation on different media from ulcers and internal organs revealed high concentrations of rod-shaped bacteria typical of *Aeromonadaceae*. Multilocus phylogenetic analysis of different clones and single gene phylogenies of sequences obtained from the fish revealed concurrent isolation of several bacterial strains belonging to the species *A. bestiarum*, *A. piscicola* and *A. sobria*. While these bacterial infections may be secondary, these findings are significant for future studies on RSD and should guide the investigation of future outbreaks. However, the involvement of *Aeromonas* spp. as putative primary etiological agents of the disease cannot be ruled out and needs to be assessed by challenge experiments.

KEYWORDS
Aeromonas, Atlantic salmon, genotyping, phylogeny, red skin disease

1 | INTRODUCTION

Numerous moribund wild Atlantic salmon (*Salmo salar* L., 1758) displaying haemorrhagic, ulcerative and necrotic abdominal skin lesions were caught in Enningdal river in the southeast of Norway during the summer of 2019. Fish displaying similar clinical signs of disease were reported shortly after from multiple rivers in Norway and have since been re-occurring the following years. Atlantic salmon suffering from severe abdominal skin lesions have previously been reported from rivers of several northern European countries, predominantly surrounding the Baltic Sea (ICES, 2018; Weichert et al., 2021).

The rapid increase in reports of diseased salmon in Norwegian rivers culminated in a proposal of a novel disease termed ‘red skin

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2023 The Authors. *Journal of Fish Diseases* published by John Wiley & Sons Ltd.

disease' (RSD) during a workshop hosted by the Norwegian Institute for Nature Research (NINA) in November 2019 (ICES, 2020). No primary etiological agent has thus far been established despite the wide array of diagnostic measures (histopathology, virology, bacteriology, molecular analyses) taken. The diagnostic criteria for RSD are, therefore, solely based on the characteristic haemorrhagic and ulcerative lesions on the abdomen of salmon returning to the affected rivers.

Recent microbiological and molecular analyses from diseased fish from Norwegian and Swedish rivers suggest, however, one or more bacterial agents possibly involved in the development of RSD. The present study reports the findings of several species of *Aeromonas* isolated from salmon displaying clinical and pathological signs of RSD caught in Norwegian and Swedish rivers in the period 2019–2021. The phylogenetic relationship between *Aeromonas* spp. isolated from the affected fish and previously characterized *Aeromonas* species associated with fish disease, was reconstructed through Multilocus phylogenetic analysis (MLPA).

2 | MATERIALS AND METHODS

2.1 | Fish sampling

Eighteen moribund adult Atlantic salmon showing clinical signs of 'red skin disease' (e.g. haemorrhages along the underside and the flanks of the fish, ulcerative skin) were caught by fishermen in rivers in Norway and Sweden, in the spring of 2019 and 2020, and in October 2021. Three fish caught in Norway in May 2019, May 2020 and in October 2021 and displaying massive ulceration of the skin with openings in the abdominal cavity were sent fresh to the Fish Diseases Research Group (FDRG) laboratory at the University of Bergen (UiB) and aseptically sampled by certified fish-health biologists. Organs were kept on ice during the sampling and subsequently stored at −80°C. Fifteen Atlantic salmon were sampled on site by fish-health biologists who accompanied fishermen and organs were fixed in 70% ethanol. Details of the sampling and pictures of the fish are given in Table 1 and Figure 1, respectively.

2.2 | Bacterial isolation and identification through *16S rRNA* sequencing

Bacteria were cultured from ulcers, gills and kidneys from fresh fish on Marine Agar (MA) (Difco 2216) and on Tryptic Soya Agar (Sigma-Aldrich) supplemented with and without 5% defibrinated sheep blood (Thermo Scientific). Bacteria were cultured for a minimum of 3 days at 16°C. The haemolytic activity of the colonies was readily observed by looking at the blood-supplemented plates through a light source. A selection of dominant and minority bacterial colonies were further sub-cultured, and clones stored in 50:50 Biofreeze freezing medium (Biochrom™) and Marine broth (Difco 2216) or Tryptic Soy broth in liquid nitrogen. Bacterial DNA was extracted with DNeasy® Blood & Tissue Kit (Qiagen) according to the manufacturer's instructions. All DNA was subsequently stored at −20°C.

PCR and sequencing of the 16S *rRNA* gene were performed using the primers 27F and 1518R (Giovannoni et al., 1996) as described in Frisch et al. (2018). PCR products (1491 bp) were run on a 1% agarose electrophoresis gel stained with GelRed™ (Biotium, USA). Positive PCR products were subsequently purified using Exo-CleanUp FAST (VWR) in a Veriti thermal cycler (Applied Biosystems) at 37°C for 5 min and 80°C for 10 min before being sequenced at the Sequencing Facility at the University of Bergen (http://www.uib.no/seqlab) using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sanger DNA sequencing was performed on a capillary-based Applied Biosystem 3730XL Analyser. Consensus sequences were obtained using VectorNTI 9.0.0 software (Invitrogen) and a BLAST search (Altschul et al., 1990) was performed for preliminary bacterial identification.

2.3 | RNA extraction and qPCR

Organs were dissected aseptically on ice in the laboratory. Approximately 1 mm^3 of tissue was resuspended in 1 mL of Trizol (Gibco BRL) and homogenized in a TissueLyser (QIAGEN) for 3 min at 30 Hz using 3 mm tungsten beads. RNA was extracted as described by Gunnarsson et al. (2017). We evaluated the quality of RNA extraction by using a qPCR assay that targets the Atlantic salmon elongation factor 1α (Olsvik et al., 2005). The presence of a range of relevant fish pathogens was tested by using 17 qPCR assays already published or developed in our laboratory (see details in Table 2). Efficiency of each assay was calculated from the slope of calibration curve according to the equation $E = 10^{[-1/\text{slope}]} - 1$ as described by (Bustin et al., 2009). All qPCRs were run using the AgPath-ID™ One-Step Reverse-Transcriptase qPCR kit (Applied Biosystems) in a total of 12.5 μL with primers (10 μM) and probe (10 μM) on a QuantStudio 3 qPCR System Cycler (Applied Biosystems). Cycling conditions were 45°C for 10 min and 95°C for 10 min (reverse-transcriptase step), followed by 45 cycles of 95°C for 15 s and 60°C for 45 s. RNA extraction controls (lacking sample) and qPCR negative samples (with no template) were included in each qPCR.

2.4 | Multilocus phylogenetic analysis

As observed in other bacterial genera, 16S rRNA sequences of *Aeromonas* are highly conserved. As a result, it can be challenging to distinguish between closely related species, such as *A. bestiarum*/*A. piscicola*/*A. salmonicida* (Martinez-Murcia et al., 1992; Martínez-Murcia et al., 1999, 2005). Since a multilocus phylogenetic analysis (MLPA) based on housekeeping gene sequences would provide a more accurate delineation, all isolated clones of *Aeromonas* spp. were included in a previously developed MLPA (Martinez-Murcia et al., 2011), using primers targeting the six housekeeping genes *atpD* (501 bp), *dnaJ* (809 bp), *gyrA* (709 bp), *gyrB* (563 bp), *recA*

13652761, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfd.13870 by UNIVERSITY OF BERGEN, Wiley Online Library on [23/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 1 Details of the 19 wild salmon included in this study.

Fish ID	Sampling date	Country	River system	Ulcers severity[a]	Conservation	Sampled organs	Source organ for phylogenetic analyses (number of sequences)		
							MLSA	*atpD*	*gyrB*
19SE_1	June 2019	Sweden	Enningdal	Moderate	70% Ethanol	Ulcer		Ulcer (4)	Ulcer (1)
19SE_3	June 2019	Sweden	Enningdal	Moderate	70% Ethanol	Ulcer			
19SE_4	June 2019	Sweden	Enningdal	Moderate	70% Ethanol	Ulcer		Ulcer (5)	Ulcer (2)
19SE_5	June 2019	Sweden	Enningdal	Moderate	70% Ethanol	Ulcer			
19SO_1	June 2019	Sweden	Örekilsälven	Moderate	70% Ethanol	Ulcer, Gill, Kidney, Spleen, Heart		Gill (6)	Gill (10) Heart (3)
19SO_3	June 2019	Sweden	Örekilsälven	Moderate	70% Ethanol	Ulcer		Ulcer (1)	Ulcer (8)
19N_1	May 2019	Norway	Enningdal	Severe	Sent fresh	Ulcer, Gill, Kidney, Spleen, Heart, Liver, Liver, Ps, CNS	Ulcer (2) Gill (1)	Ulcer (2) Gill (1)	Ulcer (2) Gill (1)
20N_11	May 2020	Norway	Enningdal	Severe	Sent fresh	Ulcer, Gill, Kidney, Liver, Ps, CNS, ovaries	Ulcer (5) Gill (1) Kidney (3)	Ulcer (5) Gill (1) Kidney (4)	Ulcer (5) Gill (1) Kidney (4)
20N_15	May 2020	Norway	Enningdal	Moderate	70% Ethanol	Ulcer, Gill, Kidney, Heart			
20N_16	June 2020	Norway	Enningdal	Moderate	70% Ethanol	Ulcer, Gill, Kidney, Heart			
20N_17	June 2020	Norway	Enningdal	Moderate	70% Ethanol	Ulcer, Gill, Kidney, Heart		Heart (6)	Heart (4)
20N_18	June 2020	Norway	Enningdal	Moderate	70% Ethanol	Ulcer, Gill, Kidney, Heart			
20N_19	June 2020	Norway	Enningdal	Moderate	70% Ethanol	Ulcer, Gill, Kidney, Heart		Gill (5) Heart (8)	Gill (7) Heart (7)
20N_25	June 2020	Norway	Enningdal	Moderate	70% Ethanol	Ulcer, Gill, Kidney, Heart			Gill (7)
20N_27	June 2020	Norway	Enningdal	Moderate	70% Ethanol	Ulcer, Gill, Kidney, Heart			
20N_34	June 2020	Norway	Enningdal	Moderate	70% Ethanol	Ulcer, Gill, Kidney		Gill (7)	Gill (6)
20N_35	June 2020	Norway	Enningdal	Moderate	70% Ethanol	Ulcer, Gill, Kidney, Heart			
21N_1	October 2021	Norway	Arna	Mild	Sent fresh	Gill, Kidney, Heart, Liver	Gill (4)	Gill (6)	Gill (6)

Abbreviations: CNS, central nervous system; Ps, pseudobranch.

[a]According to the Severity Field Guide from The Salmon Alliance (https://missingsalmonalliance.org/news/red-skin-disease-in-wild-atlantic-salmon-a-severity-field-guide).

13652761, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfd.13870 by UNIVERSITY OF BERGEN, Wiley Online Library on [23/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

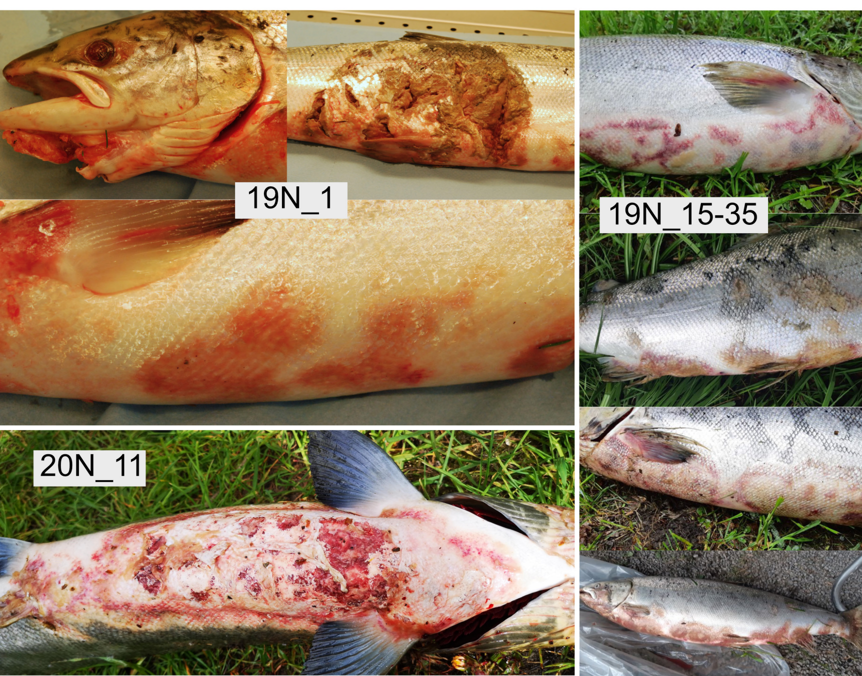

FIGURE 1 Details on symptoms. Haemorrhage and ulcer and details from 19N_1, 19N15-35 and 20N_11. Image credits: Photo by Heidrun Plarre (19N_1), Ragnar Itland (20N_11), and Trygve Poppe and Tor Alte Mor (19N15-35).

(526 bp) and *rpoD* (667 bp). The gene *dnaX* was not included, due to poor sequencing results. As the MLPA results from a concatenation of the six housekeeping genes, only pure cloned strains could be included.

The analysis included 16 clones isolated from fresh tissue (clones 1, 2 (ulcer) and 3 (gill) from fish 19N_1; clones 1, 2, 3 (kidney), 7 (gill), 8, 9, 10, 11 and 12 (ulcer) from fish 20N_11, clones 1, 2, 20 and 24 (gill) from fish 21N_1), and 42 sequences obtained from GenBank: 22 type strains in the genus *Aeromonas* (*A. allosaccharophila*^{T}, *A. aquariorum*^{T}, *A. bestiarum*^{T}, *A. bivalvium*^{T}, *A. cavernicola*^{T}, *A. caviae*^{T}, *A. diversa*^{T}, *A. encheleia*^{T}, *A. enteropelogenes*^{T}, *A. eucrenophila*^{T}, *A. hydrophila*^{T}, *A. jandaei*^{T}, *A. media*^{T}, *A. molluscorum*^{T}, *A. piscicola*^{T}, *A. popoffii*^{T}, *A. salmonicida*^{T}, *A. schubertii*^{T}, *A. simiae*^{T}, *A. sobria*^{T}, *A. tecta*^{T}, *A. veronii*^{T}) and 20 strains belonging to five *Aeromonas* species closely related to the strains isolated in this study: *A. bestiarum* ($n=4$), *A. piscicola* ($n=4$), *A. popoffii* ($n=4$), *A. salmonicida* ($n=5$) and *A. sobria* ($n=3$).

Sequence alignments were constructed for all six loci, separately, using AlignX in the VectorNTI 9.0.0 software package (Invitrogen). The sequences were trimmed and adjusted to correct reading frames in GeneDoc (Nicholas & Nicholas, 1997). Concatenation of the six housekeeping genes was performed using Kakusan4 (Tanabe, 2011). The substitution rate, codon position and best fit substitution model for the individual loci were calculated with Kakusan4. The Bayesian phylogenetic analysis was performed in MrBayes 3.2 (Ronquist & Huelsenbeck, 2003) with a Markov Chain Monte Carlo (MCMC) analysis. The run included 20,000,000 generations and trees were sampled every 1000 generations. The initial 10,000 trees were discarded as a conservative 'burn-in' in TreeAnnotator and the final tree was visualized and rooted to midpoint in FigTree v1.4.3 (http://tree.bio.ed.ac.uk/). GenBank accession numbers of the sequences from this study are provided in Table S1.

2.5 | Single gene phylogenetic analyses

Single locus phylogenetic analyses were conducted to include the *Aeromonas* sequences obtained from samples that could not be cultivated and were, therefore, not included in the MLPA (sequences obtained from tissues and organs kept in EtOH). Organ of origin for each sequence included in these single locus phylogenetic analyses are provided in Table 1.

Approximately 1 mm^3 of each tissue was finely chopped on ice and resuspended in 180 µL of ATL lysis buffer (Qiagen) and 20 µL of proteinase K at 56°C overnight. DNA was subsequently extracted with DNeasy® Blood & Tissue Kit (Qiagen) according to the manufacturer's instructions.

As we obtained the highest number of sequences with *atpD* and *gyrB*, we conducted phylogenetic analyses using these two genes. PCR was performed as described in (Martinez-Murcia et al., 2011) using VWR Taq DNA Polymerase (VWR). PCR products were ligated into the vector pCR®4-TOPO® Vector (Invitrogen, USA) using TOPO TA Cloning® Kit for Sequencing (Invitrogen). Ligated DNAs were transformed into chemically competent cells (One Shot®TOP10; Invitrogen), and colonies that grew on selection plates were screened by PCR to verify the size of the inserts using M13F and M13R primers following the manufacturer's recommendations. PCR products were subsequently purified and sequenced at the Sequencing Facility at the University of Bergen as previously described. Both single locus phylogenetic analyses included the 42 strains obtained from GenBank (22 type strains within *Aeromonas* genus and 20 strains belonging to *A. bestiarum*, *A. piscicola*, *A. popoffii*, *A. salmonicida* and *A. sobria*) and 61 *atpD* sequences (*atpD* analysis), and 73 *gyrB* sequences (*gyrB* analysis) obtained during this study. Phylogenetic analyses were conducted independently for both genes in MEGA X (Kumar et al., 2018) using the Maximum Likelihood method and a

13652761, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfd.13870 by UNIVERSITY OF BERGEN, Wiley Online Library on [23/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 2 Details of 18 qPCR assays used in this study.

Target (assay name)	Primer	Sequence (5′–3′)	PCR efficiency[a]	Reference
Elongation factor 1α (EF1AA)	Forward	CCCCTCCAGGACGTTTACAAA	E = 1.09	Olsvik et al. (2005)
	Probe	FAM-ATCGGTGGTATTGGAAC-MGB		
	Reverse	CACACGGCCCACAGGTACA		
Piscine orthoreovirus (PRV)	Forward	CAATCGCAAGGTCTGATGCA	E = 0.95	Repstad (2011)
	Probe	FAM-CTGGCTCAACTCTC-MGB		
	Reverse	GGGTTCTGTGCTGGAGATGAG		
Salmonid alphavirus (SAV)	Forward	CCGGCCCTGAACCAGTT	E = 0.972	Hodneland and Endresen (2006)
	Probe	FAM-CTGGCCACCACTTCGA-MGB		
	Reverse	GTAGCCAAGTGGGAGAAAGCT		
Infectious salmon anaemia virus (ISAV)	Forward	TGGGATCATGTGTTTCCTGCTA	E = 0.946	Plarre et al. (2005)
	Probe	FAM-CACATGACCCCTCGTC-MGB		
	Reverse	GAAAATCCATGTTCTCAGATGCAA		
Infectious pancreatic necrosis virus (IPNV)	Forward	ACCCCAGGGTCTCCAGTC	E = 0.96	Watanabe et al. (2006)
	Probe	FAM-TCTTGGCCCCGTTCATT-MGB		
	Reverse	GGATGGGAGGTCGATCTCGTA		
Salmon gill poxvirus (SGPV)	Forward	CAGAGGTTTTTCATACGCCAGAA	E = 0.968	This study
	Probe	FAM-TTATACACCATCACATT TGTG-MGB		
	Reverse	GAGGTCACGGTGATGACAGAAC		
Piscine myocarditis virus (PMCV)	Forward	AGGGAACAGGAGGAAGCAGAA	E = 1.096	Repstad (2011)
	Probe	FAM-TGGTGGAGCGTTCAA-MGB		
	Reverse	CGTAATCCGACATCATTTTGTGA		
Paranucleospora theridion (NUC)	Forward	CGGACAGGGAGCATGGTATAG	E = 1.068	Nylund et al. (2010)
	Probe	FAM-TTGGCGAAGAATGAAA-MGB		
	Reverse	GGTCCAGGTTGGGTCTTGAG		
Ichthyobodo spp. (Costia)	Forward	ACGAACTTATGCGAAGGCA	E = 0.997	Isaksen et al. (2012)
	Probe	FAM-TCCACGACTGCAAACGA TGACG-MGB		
	Reverse	TGAGTATTCACTYCCGATCCAT		
Parvicapsula pseudobranchicola (Parvi)	Forward	TCGTAGTCGGATGACAAGAACGT	E = 0.972	Nylund et al. (2011)
	Probe	FAM-CCGTATTGCTGTCTTTGA-MGB		
	Reverse	AAACACCCCGCACTGCAT		
Tetracapsula bryosalmonae (PKX)	Forward	CAAGATCGCGCCCTATCAAT	E = 0.93	This study
	Probe	FAM-TGTTGTTAGGATATTTTCC-MGB		
	Reverse	CGTCACCCGTTACAACCTTGT		
Paramoeba perurans (Pperu)	Forward	GATAACCGTGGTAAATCTAG AGCTAAT	E = 0.97	Nylund et al. (2018)
	Probe	FAM-CTGGTTCTTTCGRGAGC-MGB		
	Reverse	TGGCATTGGCTTTTGAATCT		
Ca. Branchiomonas cysticola (Epit)	Forward	GAGTAATACATCGGAACGTG TCTAGTG	E = 0.982	Repstad (2011)
	Probe	FAM-ACTTAGCGAAAGTTAAGC-MGB		
	Reverse	CTTTCCTCTCCCAAGCTTATGC		
Piscichlamydia salmonis (Pch)	Forward	TCACCCCCAGGCTGCTT	E = 1.001	Nylund et al. (2008)
	Probe	FAM-CAAAACTGCTAGAC TAGAGT-MGB		
	Reverse	GAATTCCATTTCCCCCTC TTG		

(Continues)

13652761, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfd.13870 by UNIVERSITY OF BERGEN, Wiley Online Library on [23/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 2 (Continued)

Target (assay name)	Primer	Sequence (5′–3′)	PCR efficiency[a]	Reference
Renibacterium salmoninarum (BKD)	Forward	CAAGGCTTGACATGGATTAGAAAA	$E=0.94$	This study
	Probe	FAM-TGCAGAAATGTACTCCC-MGB		
	Reverse	CACCTGTGAACCAACCCAAAA		
Yersinia ruckeri (YR)	Forward	GCGAGGAGGAAGGGTTAAGTG	$E=0.988$	This study
	Probe	FAM-TAATAGCACTGAAC ATTGAC-MGB		
	Reverse	CGGTGCTTCTTCTGCGAGTAA		
Saprolegnia spp. (SP)	Forward	TCCGGTCGAGTTTATCTCTGTACT	$E=0.96$	This study
	Probe	FAM-ATGGCCCAARCATCCA-MGB		
	Reverse	AGCGCCCCCTCACAAAA		
Aeromonas spp. (AERO)	Forward	GGCGGACGGGTGAGTAATG	$E=0.94$	This study
	Probe	FAM-ATCTGCCCAGTCGAGG-MGB		
	Reverse	GCAGTCGTTTCCAACTGTTATCC		

[a]PCR efficiency $E=10^{[-1/\text{slope}]}-1$.

Tamura 3-parameter + G + I model (Tamura, 1992) and 1000 bootstraps replicates. The final trees were visualized in FigTree v1.4.3 (http://tree.bio.ed.ac.uk/). GenBank accession numbers of the sequences from this study are provided in Table S1.

3 | RESULTS

3.1 | Colony morphology and 16S rRNA sequencing

Plates showed mixed bacterial cultures dominated by grey circular convex colonies. Among these similar dominant colonies, some displayed strong β-haemolytic activity on agar supplemented with 5% defibrinated sheep blood, while others did not. Some of these bacteria turned slightly greenish after a day of incubation. Dominant colonies were preferably selected to 16S rRNA sequencing. We included also some non-haemolytic grey-green minority colonies.

In total, 48 colonies were subjected to DNA extraction and subsequent 16S rRNA sequencing. Based on this locus, among the dominant bacterial colonies, we identified 28 strains as *Aeromonas salmonicida* (displaying β-haemolytic activity) and 12 as *Aeromonas sobria* (without any β-haemolytic activity). The minority colonies were identified as *Serratia quinivorans* ($n=4$) and *Pseudomonas* sp. ($n=4$).

3.2 | qPCR results

We tested the presence of five bacteria using qPCR (see Table S2). No fish tested positive for *Renibacterium salmoninarum*. *Candidatus* Piscichlamydia salmonis and *Candidatus* Branchiomonas cysticola are two bacteria associated with epitheliocystis in Atlantic salmon in Norway. *Ca*. P. salmonis was present in six fish: one from Sweden (19SO_1), four caught in Enningdal river in 2020 (20N_16, 17, 19 and 25) and 21N_1 from Arna river. *Ca*. B. cysticola was detected in 13 of the included fish. Two fish from Sweden tested positive: 19SE_1 (ulcer, C_t-value 33.9) and 19SO_1 (heart, C_t-value 32.6 and gill, C_t-value 21.6). qPCR analysis was positive for every organ of 19N_1, with the lowest C_t-value being found in the gill (18.2). All fish from Enningdal river in 2020 tested positive (C_t-values ranged from 14.0 to 36.1). Gill sample from 21N_1, originated from Arna river tested positive, with a C_t-value of 18.6. The bacterium *Yersinia ruckeri* was detected in 15 fish. qPCR run on ulcer samples from Swedish fish produced positive results in three fish: 19SE_3 and 19SE_4 from Enningdal river system and 19SO_3 from Örekil river. All organs from the fresh fish sampled in 2019 (19N_1) and 2020 (20N_11) tested positive, with an average C_t-value of 28.5 and 18.1, respectively. All fish caught in Enningdal river in 2020 in Norway were also positive, with an average C_t-value of 32.3. Finally, *Yersinia ruckeri* was also detected in Arna river (21N_1 gill). All fish tested positive for *Aeromonas* spp. The six fish caught in Sweden in 2019 proved to be positive, with an average C_t-value of 22.2. Likewise, all organ and tissue samples from the fresh fish caught in Norway in 2019, 2020 and 2021 tested positive, with a mean C_t-value of 19.4. Finally, *Aeromonas* spp. was detected in the nine fish caught in 2020 in Enningdal river in Norway (mean C_t-value 28.6).

Samples were tested for six fish viruses. All tested negative for Salmonid alphavirus and Infectious salmon anaemia virus. Only one fish from Sweden (19SO_3) was positive for Infectious pancreatic necrosis virus, with a C_t-value of 35.8 (ulcer). Piscine orthoreovirus was detected from a gill of a fish caught in Sweden (19SO_1), with a similar C_t-value (35.7). Six fish tested positive for Piscine myocarditis virus19S_1 caught in Sweden, and five fish caught in Norway in 2020 from Enningdal river (20NE_16, 19, 25, 27 and 34). Salmon gill poxvirus was detected in 11 fish (C_t-values ranged from 23.7 to 38.7).

13652761, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfd.13870 by UNIVERSITY OF BERGEN, Wiley Online Library on [23/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

We tested the fish for the presence of six eukaryotic parasites. Every fish caught in Enningdal river in 2020 was positive for *Paramoeba perurans*, the causative agent of amoebic gill disease. The myxozoan parasite *Tetracapsuloides bryosalmonae* was detected in seven fish: a salmon caught in Sweden (19SO_1), five fish from Enningdal river (20NE_17, 18, 19, 27 and 35) and 21N_1 from Arna river. Among those, kidney was the organ that provided the lowest C_t-values (except 20N_19, for which only the heart tested positive). The myxosporean parasite *Parvicapsula pseudobranchicola* commonly infects farmed Atlantic salmon in northern Norway. Seven fish tested positive: 19N_1, 20NE_15, 17, 19, 27 and 35. Two fish from Sweden (19SE_1 and 19SO_1) tested positive for the microsporidian *Paranucleospora theridion*. It was also detected in the heart and muscle (C_t-values 33.1 and 36.6) from the Norwegian salmon 19N_1. All the nine fish caught in Enningdal river in 2020, and 21N_1 tested positive. Lastly, two parasites were massively detected. All the fish included in this study tested positive for the ectoparasitic flagellate *Ichthyobodo* sp. (C_t-values 6.6–32.2) and for the oomycete *Saprolegnia* sp. (C_t-values 8.6–32.7).

3.3 | Multilocus phylogenetic analysis

The Bayesian-inferred phylogenetic tree based on the concatenated nucleotide sequences of 16 clones isolated in this study and 42 strains from GenBank, is presented in Figure 2. The analysis grouped the 16 clones in three supported clades including strains belonging to three different *Aeromonas* species: *A. bestiarum*, *A. piscicola* and *A. sobria*. Two fish were co-infected by at least two *Aeromonas* species; 19N_1 caught in Norway in 2019 was infected by *A. sobria* and *A. piscicola* and 20N_11 caught in Norway in 2020 was infected by *A. sobria*, *A. piscicola* and *A. bestiarum*. We isolated several strains of *Aeromonas piscicola* from 21N_1, caught in Norway in 2021.

3.4 | Single gene phylogenetic analysis

Figure 3 presents two phylogenetic trees reconstructed by Maximum likelihood analysis of 103 (A: *atpD* locus) and 115 (B: *gyrB* locus) *Aeromonas* sequences. The arrangement of sequences within the trees is relatively consistent with that proposed by the Bayesian analysis. The main difference is the merging of two different clades in both trees: *Aeromonas salmonicida* and *A. bestiarum* strains clustered together in the *atpD* tree; *Aeromonas salmonicida* and *A. piscicola* in the *gyrB* tree. The sequences of the 16 clones included in the Bayesian analysis are situated in the same relative position, considering the abovementioned merging. Thus, indicating a strong congruence between the two analyses. The most salient result is the clustering of almost all sequences into the same three groups. Indeed, of the 61 sequences obtained in this analysis and included in the *atpD* phylogeny, only three are not found in one of these three clades. These three strains isolated from the gills of two Norwegian salmon caught in 2020 (20N_19 and 20N_34) cluster with *A. hydrophila* strains. Similarly, only six of the 73 strains included in the *gyrB* phylogeny are not related to *A. salmonicida/piscicola*, *A. bestiarum* or *A. sobria* strains. Four clones isolated from a gill of a salmon caught in 2020 in Norway (20N_34) and one clone isolated from an ulcer of a Swedish fish (19SO_1) group in a clade with *A. hydrophila* and *A. aquarium* type strains. An additional strain, isolated from the same Swedish fish (19SO_1), is closely related to *A. allosaccharophila*.

Eight fish non included in the MLSA provided sequences included in these single locus phylogenies (four from Norway, four from Sweden). Sequences from at least two *Aeromonas* species, including *A. sobria*, were detected in seven fish. *Aeromonas sobria* was the only bacterial species detected from 20N-25, caught in Norway in June 2020. However, it is worth noting that the other three fish caught during the same outbreak, also included in this phylogeny, provided sequences identified as *A. piscicola* (20N-17, 19 and -34) and *A. salmonicida/bestiarum* clade (20N-19 and -34).

4 | DISCUSSION

Salmon presenting RSD signs/syndrome have been repeatedly observed in Swedish river systems, with cases occurring annually since 2014. The disease has since been reported from several other countries, including Norway, Ireland, Denmark and the UK (Brockmark & Carlstrand, 2017; ICES, 2020; Weichert et al., 2021). Despite the apparent establishment of the disease in new countries, no etiological agent has so far been linked to RSD. In this study, we investigated the presence of potential fish pathogens that could be associated with the disease and characterized several strains of *Aeromonas* species that have been repeatedly detected and isolated from fish displaying RSD clinical signs caught in rivers in Norway and Sweden in the period 2019–2021. A notable finding is that most of the salmon included in this study were co-infected with at least two distinct species of *Aeromonas* (*A. piscicola* and *A. sobria*).

Two major lines of evidence suggest a strong correlation between bacteria belonging to the genus *Aeromonas* and RSD. Firstly, all samples were positive for *Aeromonas* spp. by qPCR detection (AERO assay, see Table 2). Moreover, detection of these bacteria in organs such as kidney, heart and spleen could suggest a systemic infection. Several other fish pathogens were also repeatedly detected by qPCR: a protozoan (*Ichthyobodo* sp.), an oomycete (*Saprolegnia* sp.) and two bacteria (*Yersinia ruckeri* and *Candidatus* Branchiomonas cysticola).

Ichthyobodo is a genus of parasitic flagellates that infect fish in both freshwater and seawater environments, causing severe disease outbreaks and significant losses for the aquaculture industry (Callahan et al., 2005; Lom & Dyková, 1992). In Norway, *I. necator* and *I. salmonis* have been associated with gill and skin diseases and mortalities of both farmed and wild salmon in freshwater (Isaksen et al., 2011). Signs include the appearance of grey patches on the skin and increased mucus production. However, it's worth noting that *Ichthyobodo* spp. are opportunistic parasites that tends to thrive in hosts that have weakened immune systems due to stressors or

13652761, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfd.13870 by UNIVERSITY OF BERGEN, Wiley Online Library on [23/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

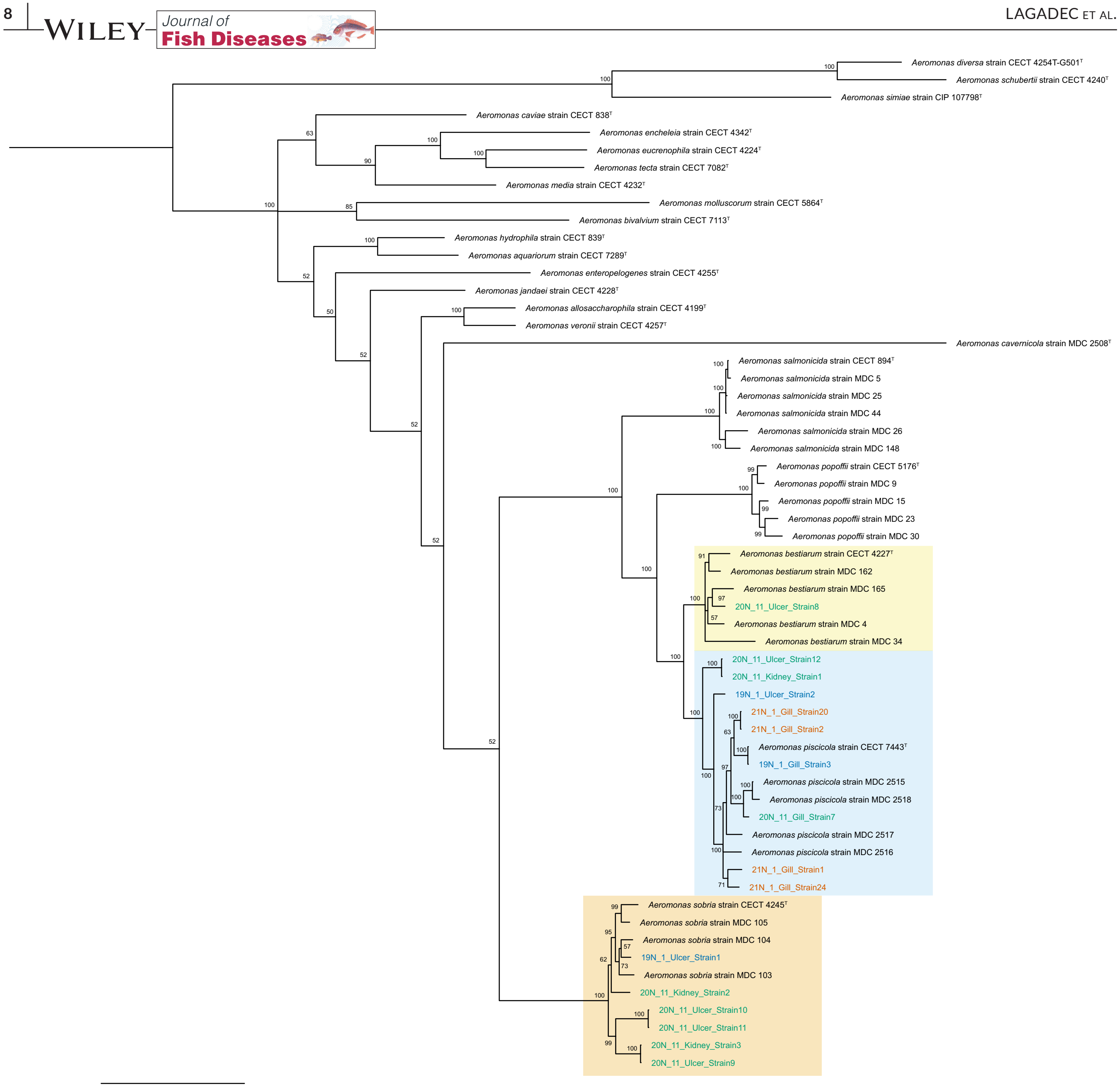

FIGURE 2 Bayesian analysis. Mid-rooted phylogenetic tree of 58 *Aeromonas* strains based on concatenated sequences of six genes (*atpd*, *dnaj*, *gyra*, *gyrb*, *reca* and *rpod*, total size: 3775 bp). Data set includes 42 *Aeromonas* strains from GenBank and 16 *Aeromonas* strains isolated during this study from three different fish caught in 2019 (in blue), 2020 (in green) and 2021 (in red). Posterior probabilities are indicated at each node. *Aeromonas bestiarum*, *Aeromonas piscicola* and *Aeromonas sobria* clades are highlighted in yellow, blue and orange, respectively. ID of each sequence indicates [Year (19: 2019; 20: 2020; 21: 2021); Origin (N: Norway; SE: Sweden Enningdal; SO:Sweden Örekilsälven)_Fish number_Strain number].

other infections such as SAV or *Aeromonas sobria* (Repstad, 2011; Yardimci & Turgay, 2021). *Saprolegnia* spp. are ubiquitous freshwater borne oomycetes that can have a significant impact on salmonid aquaculture (Hussein et al., 2001; Kitancharoen et al., 1997; Thoen et al., 2011). During a severe infection, the parasite can destroy the epithelial integrity, leading to cellular necrosis and tissue destruction. *Saprolegnia* spp. have already been isolated in association with *A. sobria* during an outbreak in rainbow trout fry (*Oncorhynchus mykiss*) (Yardimci & Turgay, 2021). However, the recurring presence of *Ichthyobodo* spp. and *Saprolegnia* spp. in freshwater, their distinct symptomatology, and the fact that they are typically considered secondary pathogens, suggest that their contribution to RSD is insignificant. We can nonetheless rule out their potential role in fish infection during the progress of the disease.

Yersinia ruckeri is a gram-negative bacterium that is responsible for causing enteric redmouth disease (ERM), a significant disease affecting various fish species in freshwater, particularly salmonids (Barnes, 2011; Kumar et al., 2015; Ross et al., 1966; Tobback

13652761, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfd.13870 by UNIVERSITY OF BERGEN, Wiley Online Library on [23/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

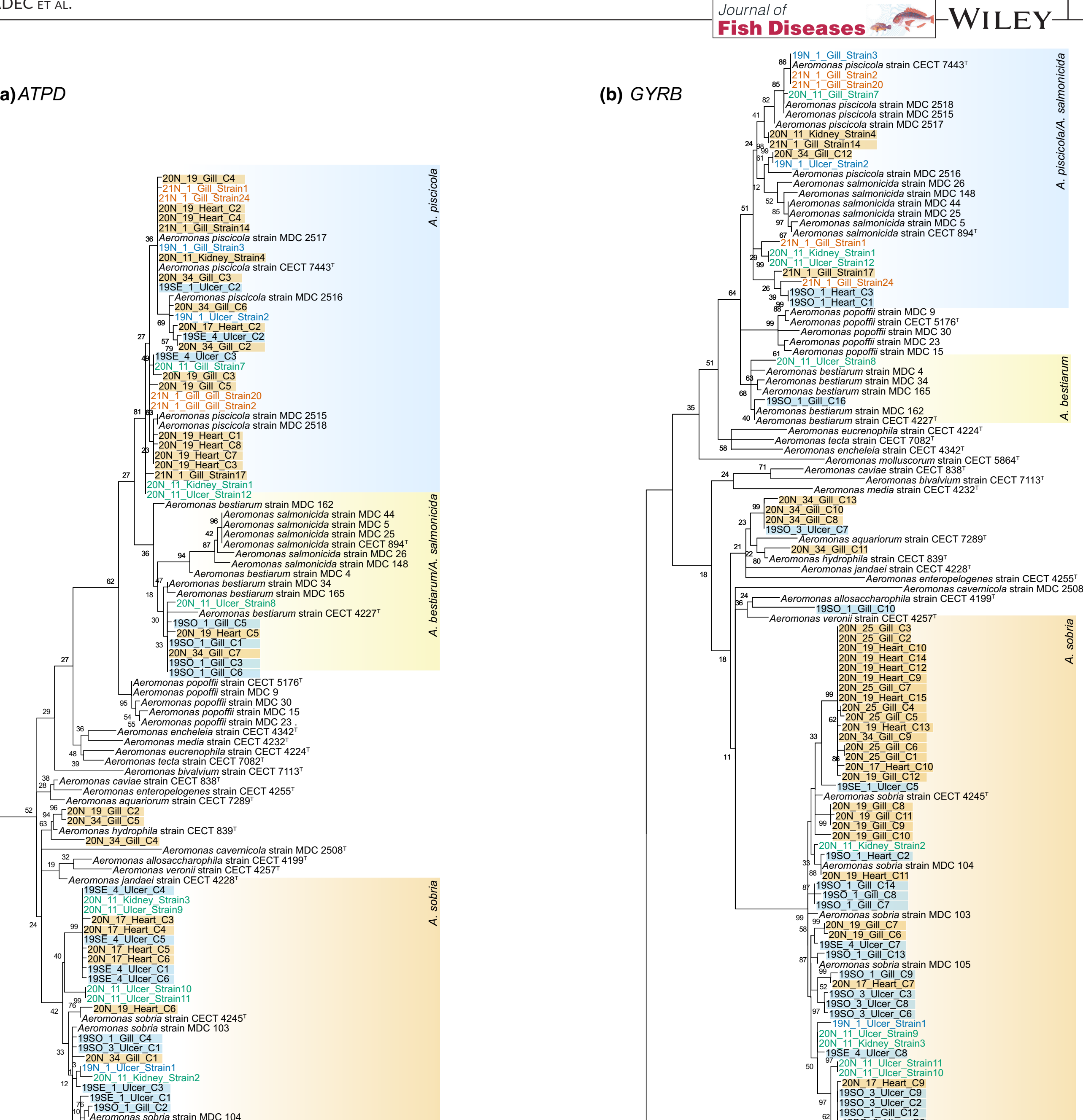

FIGURE 3 Mid-rooted Maximum likelihood phylogenetic trees. Phylogenetic relationship between 61 (a: *atpD* locus) and 74 (b: *gyrB* locus) sequences isolated during this study and 42 *Aeromonas* sequences from GenBank. Bootstrap values are indicated at each node. Font colours indicate the 16 clones isolated from the three Norwegian fish caught in 2019 (in blue), 2020 (in green) and 2021 (in red) and previously included in the Bayesian analysis. Highlight colours indicate sequences obtained from the Norwegian (orange) and Swedish (blue) fish organs fixed in 70% ethanol. Posterior probabilities are indicated at each node. *Aeromonas bestiarum*, *Aeromonas piscicola* and *Aeromonas sobria* clades are highlighted in yellow, blue and orange, respectively. ID of each sequence indicates [Year (19: 2019; 20: 2020; 21: 2021)_Origin (N: Norway; SE: Sweden Enningdal; SO:Sweden Örekilsälven)_Fish number_Organ source_Clone number].

et al., 2007). The bacterium primarily infects the fish via their digestive tract or gills, leading to inflammation of the mouth, eye and gills, as well as haemorrhages in internal organs and terminal septicaemia. In Norway, the disease is associated almost exclusively with Atlantic salmon with outbreaks occurring principally in western/central Norway and a few in the north (Sommerset et al., 2022). If the role of *Yersinia ruckeri* as a causative agent for RSD is not supported by the findings (e.g. distinct symptomatology observed, absence of

13652761, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfd.13870 by UNIVERSITY OF BERGEN, Wiley Online Library on [23/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

bacterial isolation in cultures, relatively low level of detection by qPCR analysis), the implication of the bacterium in the progression of the disease cannot be completely dismissed either. Indeed, *Y. ruckeri* was detected in 15 fish included in this study. The presence of *Y. ruckeri* in the cultures may have been hidden by the rapid growth of various *Aeromonas* strains. *Candidatus* Branchiomonas cysticola is an intracellular gram-negative bacterium causing epitheliocystis in Atlantic salmon (Gjessing et al., 2021; Mitchell et al., 2013; Nylund et al., 1998; Toenshoff et al., 2012). The bacterium infects gill epithelial cells, forming cysts that can lead to respiratory failure in affected fish. Since the bacterium is commonly detected in salmon and displays a distinct tissue tropism, it cannot be stated that it is associated with the development of RSD.

Secondly and more importantly, preliminary identification of the colonies morphology and subsequent 16S rRNA sequencing identified the dominant bacteria as *Aeromonaceae*. Phylogenetic reconstruction proved that most of the strains are closely related to *Aeromonas* species already known to be fish pathogens, namely *A. sobria*, *A. piscicola* and *A. bestiarum*. *Aeromonas sobria* is a significant fish pathogen that has been isolated from multiple fish species such as tilapia (*Oreochromis niloticus*), perch (*Perca fluviatilis*), gizzard shad (*Dorosoma cepedianum*) and from salmonids such as rainbow trout (*Oncorhynchus mykiss*) or Mexican golden trout (*Oncorhynchus chrysogaster*) (Austin et al., 1989; Fuentes-Valencia et al., 2022; Gauthier et al., 2017; Li & Cai, 2011; Toranzo et al., 1989; Wahli et al., 2005; Yardimci & Turgay, 2021).

Aeromonas bestiarum and *A. piscicola* both belong to the formerly known *A. hydrophila* complex that encompassed several other species such as *A. hydrophila*, *A. salmonicida* and *A. popoffii* (Ali et al., 1996; Joseph & Carnahan, 1994; Martínez-Murcia et al., 2005; Popoff et al., 1981). *Aeromonas bestiarum* is one of the causal agents of motile aeromonad septicaemia in fish. The bacterium has been isolated in commercial carp (*Cyprinus carpio*), tilapia (*Oreochromis niloticus*) and rainbow trout (*Oncorhynchus mykiss*) (Castro-Escarpulli et al., 2003; Kozinska & Guz, 2004; Pieters et al., 2008; Zepeda-Velázquez et al., 2017). *Aeromonas piscicola* was isolated and described in 2009 from moribund Atlantic salmon and rainbow trout in Spain (Beaz-Hidalgo et al., 2009).

Despite recent investigations (histopathology, pathogen screening) undertaken by fish-health authorities on several Atlantic salmon specimens displaying RSD, no infectious agent could be associated with the disease (ICES, 2018, 2020; Sommerset et al., 2021). As a result, a team decided to focus on biomarkers as well as biochemical and haematological parameters potentially associated with the disease (Weichert et al., 2021). Based on a large sampling of 87 returning Atlantic salmon caught in five different locations in Sweden in 2018, their results showed that RSD is associated with a significant osmotic haemodilution, an alteration of the carbohydrate metabolism and alteration of the immune system. Whole blood-associated parameters were particularly interesting. Indeed, neutrophil counting showed an initial neutrophilia and a possible end-stage neutropenia. Both suggest an immunological disease response, and possibly, a massive bacteraemia supported by this final decrease of neutrophils.

Nevertheless, several important points must be considered before associating *Aeromonas* spp. with RSD. Indeed, several results of this study tend to reassess the role of aeromonads as a precursor to the disease and instead lean towards a secondary infection. The high degree of genetic diversity observed within the analysed *Aeromonas* sequences could suggest opportunistic colonization directly from the natural aquatic environment. *Aeromonas* species are commonly found in freshwater ecosystems and have previously been acknowledged as secondary pathogens (Austin et al., 2007; Cipriano, 1984; Monfort & Baleux, 1990). As previously shown by Weichert et al. (2021), RSD impairs the host's immune system, thereby facilitating the establishment of secondary invaders. Furthermore, the lack of histological data does not allow for a definitive conclusion regarding the role of these bacteria in the development of this disease. If the detection of *Aeromonas* spp. in internal organs could suggest a systemic infection, the absence of distinct internal clinical indicators precludes a definitive conclusion of a systemic bacterial infection. Moreover, the fresh fish displayed massive ulceration of the skin with openings of the abdominal cavity, which may have led to passive contamination of internal organs. Similarly, the other fish having been sampled in the field, contamination of internal organs cannot be ruled out. A possible contamination of internal organs is, for instance, supported by the detection of *Ichthyobodo* spp. within the internal organs. Despite the limited number of studies on RSD, it is also surprising that the association between *Aeromonas* spp. and RSD has not been conclusively established.

To assess the role of *Aeromonas* in RSD, a thorough study needs to be undertaken, with a focus on *Aeromonas* strains in association with Atlantic salmon presenting RSD signs. Subsequently, conducting challenge experiments on Atlantic salmon infected with single or multiple species of *Aeromonas* isolated from this study will allow for a more accurate assessment of the definitive contribution of these bacteria in the development of RSD.

AUTHOR CONTRIBUTIONS

Erwan Lagadec: Conceptualization; investigation; writing – original draft; methodology; validation; visualization; writing – review and editing; software; formal analysis; data curation. **Even Bysveen Mjølnerød:** Writing – original draft; investigation; writing – review and editing. **Øyvind Marius Jensen:** Data curation; software; writing – original draft; writing – review and editing. **Heidrun Plarre:** Investigation; writing – original draft; writing – review and editing; data curation; formal analysis. **Are Nylund:** Conceptualization; investigation; funding acquisition; writing – original draft; methodology; validation; writing – review and editing; project administration; supervision; resources.

ACKNOWLEDGEMENTS

We deeply thank Ragnar Itland and all the fishermen who shared their catch with our laboratory. Special thanks to Trygve Poppe and Tor Alte Mor for their support in providing fish samples from Sweden. Special thanks to our colleague Christiane Trösse for her help in the lab work. Additionally, we would like to express our appreciation

13652761, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfd.13870 by UNIVERSITY OF BERGEN, Wiley Online Library on [23/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

to the three anonymous reviewers for their contributions. Their insightful comments on the initial draft significantly enhanced the quality of this manuscript.

FUNDING INFORMATION

This study was financed by the Norwegian Environment Agency (project: 808036) (http://www.miljodirektoratet.no/en/) and SalmonCamera (http://salmoncamera.com/). The grant was received by A. Nylund.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

DATA AVAILABILITY STATEMENT

All data are available as part of this publication and in the Tables S1 and S2.

ORCID

Erwan Lagadec https://orcid.org/0000-0001-5064-8145
Even Bysveen Mjølnerød https://orcid.org/0000-0002-1649-6560
Heidrun Plarre https://orcid.org/0009-0004-7900-2101
Are Nylund https://orcid.org/0000-0002-1932-6604

REFERENCES

Ali, A., Carnahar, A. M., Altwegg, M., Lüthy-Hottenstein, J., & Joseph, S. W. (1996). *Aeromonas bestiarum* sp. nov. (formerly genomospecies DNA group 2 a. hydrophila). A new species isolated from non-human sources. *Medical Microbiology Letters*, *5*, 156–165.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410.

Austin, B., Austin, D. A., & Munn, C. (2007). *Bacterial fish pathogens: Disease of farmed and wild fish*. Springer.

Austin, D. A., McIntosh, D., & Austin, B. (1989). Taxonomy of fish associated *Aeromonas* spp., with the description of *Aeromonas salmonicida* subsp. *smithia* subsp. nov. *Systematic and Applied Microbiology*, *11*(3), 277–290.

Barnes, A. C. (2011). Enteric redmouth disease (ERM) (*Yersinia ruckeri*). *Fish Diseases and Disorders*, *3*, 484–511.

Beaz-Hidalgo, R., Alperi, A., Figueras, M. J., & Romalde, J. L. (2009). *Aeromonas piscicola* sp. nov., isolated from diseased fish. *Systematic and Applied Microbiology*, *32*(7), 471–479.

Brockmark, S., & Carlstrand, H. (2017). Sjuklighet och dödlighet i svenska laxälvar under 2014–2016.

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J., & Wittwer, C. T. (2009). The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry*, *55*(4), 611–622.

Callahan, H. A., Litaker, R. W., & Noga, E. J. (2005). Genetic relationships among members of the Ichthyobodo necator complex: Implications for the management of aquaculture stocks. *Journal of Fish Diseases*, *28*(2), 111–118.

Castro-Escarpulli, G., Figueras, M. J., Aguilera-Arreola, G., Soler, L., Fernández-Rendón, E., Aparicio, G. O., Guarro, J., & Chacón, M. R. (2003). Characterisation of *Aeromonas* spp. isolated from frozen fish intended for human consumption in Mexico. *International Journal of Food Microbiology*, *84*(1), 41–49.

Cipriano, R. C. (1984). *Aeromonas hydrophila and motile aeromonad septicemias of fish*. US Department of the Interior, Fish and Wildlife Service.

Frisch, K., Småge, S. B., Brevik, O. J., Duesund, H., & Nylund, A. (2018). Genotyping of *Tenacibaculum maritimum* isolates from farmed Atlantic salmon in Western Canada. *Journal of Fish Diseases*, *41*(1), 131–137.

Fuentes-Valencia, M. A., Osornio-Esquivel, J. L., Martínez Palacios, C. A., Contreras-Ávila, J. L., Barriga-Tovar, E., la Mora, G. I., Arellano-Torres, A., Baizabal-Aguirre, V. M., Bravo-Patiño, A., Cajero-Juárez, M., & Valdez Alarcón, J. J. (2022). Bacterial and parasite co-infection in Mexican golden trout (*Oncorhynchus chrysogaster*) by *Aeromonas bestiarum*, *Aeromonas sobria*, *Plesiomonas shigelloides* and *Ichthyobodo necator*. *BMC Veterinary Research*, *18*(1), 137.

Gauthier, J., Vincent, A. T., Charette, S. J., & Derome, N. (2017). Strong genomic and phenotypic heterogeneity in the *Aeromonas sobria* species complex. *Frontiers in Microbiology*, *8*, 2434.

Giovannoni, S. J., Rappé, M. S., Vergin, K. L., & Adair, N. L. (1996). 16S rRNA genes reveal stratified open ocean bacterioplankton populations related to the green non-sulfur bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(15), 7979–7984.

Gjessing, M. C., Spilsberg, B., Steinum, T. M., Amundsen, M., Austbø, L., Hansen, H., Colquhoun, D., & Olsen, A. B. (2021). Multi-agent in situ hybridization confirms Ca. Branchiomonas cysticola as a major contributor in complex gill disease in Atlantic salmon. *Fish and Shellfish Immunology Reports*, *2*, 100026.

Gunnarsson, G. S., Blindheim, S., Karlsbakk, E., Plarre, H., Imsland, A. K., Handeland, S., Sveier, H., & Nylund, A. (2017). *Desmozoon lepeophtherii* (microsporidian) infections and pancreas disease (PD) outbreaks in farmed Atlantic salmon (*Salmo salar* L.). *Aquaculture*, *468*, 141–148.

Hodneland, K., & Endresen, C. (2006). Sensitive and specific detection of salmonid alphavirus using real-time PCR (TaqMan). *Journal of Virological Methods*, *131*(2), 184–192.

Hussein, M. M., Hatai, K., & Nomura, T. (2001). Saprolegniosis in salmonids and their eggs in Japan. *Journal of Wildlife Diseases*, *37*(1), 204–207.

ICES. (2018). *Report of the Working Group on North Atlantic Salmon (WGNAS)*. ICES Expert Group Reports.

ICES. (2020). Working Group on North Atlantic Salmon (WGNAS). https://doi.org/10.17895/ICES.PUB.5973

Isaksen, T. E., Karlsbakk, E., Repstad, O., & Nylund, A. (2012). Molecular tools for the detection and identification of *Ichthyobodo* spp. (Kinetoplastida), important fish parasites. *Parasitology International*, *61*(4), 675–683.

Isaksen, T. E., Karlsbakk, E., Watanabe, K., & Nylund, A. (2011). *Ichthyobodo salmonis* sp. n. (Ichthyobodonidae, Kinetoplastida), an euryhaline ectoparasite infecting Atlantic salmon (*Salmo salar* L.). *Parasitology*, *138*(9), 1164–1175.

Joseph, S. W., & Carnahan, A. (1994). The isolation, identification, and systematics of the motile Aeromonas species. *Annual Review of Fish Diseases*, *4*, 315–343.

Kitancharoen, N., Hatai, K., & Yamamoto, A. (1997). Aquatic fungi developing on eggs of salmonids. *Journal of Aquatic Animal Health*, *9*(4), 314–316.

Kozinska, A., & Guz, L. (2004). The effect of various Aeromonas bestiarum vaccines on non-specific immune parameters and protection of carp (*Cyprinus carpio* L.). *Fish & Shellfish Immunology*, *16*(3), 437–445.

Kumar, G., Menanteau-Ledouble, S., Saleh, M., & El-Matbouli, M. (2015). Yersinia ruckeri, the causative agent of enteric redmouth disease in fish. *Veterinary Research*, *46*(1), 103.

13652761, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfd.13870 by UNIVERSITY OF BERGEN, Wiley Online Library on [23/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549.

Li, Y., & Cai, S. H. (2011). Identification and pathogenicity of *Aeromonas sobria* on tail-rot disease in juvenile tilapia *Oreochromis niloticus*. *Current Microbiology*, *62*(2), 623–627.

Lom, J., & Dyková, I. (1992). *Protozoan parasites of fishes*. Elsevier Science Publishers.

Martínez-Murcia, A. J., Antón, A. I., & Rodríguez-Valera, F. (1999). Patterns of sequence variation in two regions of the 16S rRNA multigene family of Escherichia coli. *International Journal of Systematic Bacteriology*, *49*(Pt 2), 601–610.

Martinez-Murcia, A. J., Benlloch, S., & Collins, M. D. (1992). Phylogenetic interrelationships of members of the genera Aeromonas and Plesiomonas as determined by 16S ribosomal DNA sequencing: Lack of congruence with results of DNA-DNA hybridizations. *International Journal of Systematic Bacteriology*, *42*(3), 412–421.

Martinez-Murcia, A. J., Monera, A., Saavedra, M. J., Oncina, R., Lopez-Alvarez, M., Lara, E., & Figueras, M. J. (2011). Multilocus phylogenetic analysis of the genus Aeromonas. *Systematic and Applied Microbiology*, *34*(3), 189–199.

Martínez-Murcia, A. J., Soler, L., Saavedra, M. J., Chacón, M. R., Guarro, J., Stackebrandt, E., & Figueras, M. J. (2005). Phenotypic, genotypic, and phylogenetic discrepancies to differentiate *Aeromonas salmonicida* from *Aeromonas bestiarum*. *International Microbiology*, *8*(4), 259–269.

Mitchell, S. O., Steinum, T. M., Toenshoff, E. R., Kvellestad, A., Falk, K., Horn, M., & Colquhoun, D. J. (2013). 'Candidatus Branchiomonas cysticola' is a common agent of epitheliocysts in seawater-farmed Atlantic salmon *Salmo salar* in Norway and Ireland. *Diseases of Aquatic Organisms*, *103*(1), 35–43.

Monfort, P., & Baleux, B. (1990). Dynamics of *Aeromonas hydrophila*, *Aeromonas sobria*, and *Aeromonas caviae* in a sewage treatment pond. *Applied and Environmental Microbiology*, *56*(7), 1999–2006.

Nicholas, K. B., & Nicholas, H. B. (1997). GeneDoc: a tool for editing and annotating multiple sequence alignments.

Nylund, A., Kvenseth, A., & Isdal, E. (1998). A morphological study of the epitheliocystis agent in farmed Atlantic salmon. *Journal of Aquatic Animal Health*, *10*(1), 43–55.

Nylund, A., Pistone, D., Trösse, C., Blindheim, S., Andersen, L., & Plarre, H. (2018). Genotyping of *Candidatus Syngnamydia* salmonis (chlamydiales; Simkaniaceae) co-cultured in *Paramoeba perurans* (amoebozoa; Paramoebidae). *Archives of Microbiology*, *200*(6), 859–867.

Nylund, A., Watanabe, K., Nylund, S., Karlsen, M., Saether, P. A., Arnesen, C. E., & Karlsbakk, E. (2008). Morphogenesis of salmonid gill poxvirus associated with proliferative gill disease in farmed Atlantic salmon (*Salmo salar*) in Norway. *Archives of Virology*, *153*(7), 1299–1309.

Nylund, S., Andersen, L., Saevareid, I., Plarre, H., Watanabe, K., Arnesen, C. E., Karlsbakk, E., & Nylund, A. (2011). Diseases of farmed Atlantic salmon *Salmo salar* associated with infections by the microsporidian *Paranucleospora theridion*. *Diseases of Aquatic Organisms*, *94*(1), 41–57.

Nylund, S., Nylund, A., Watanabe, K., Arnesen, C. E., & Karlsbakk, E. (2010). *Paranucleospora theridion* n. gen., n. sp. (microsporidia, Enterocytozoonidae) with a life cycle in the Salmon louse (*Lepeophtheirus salmonis*, Copepoda) and Atlantic Salmon (*Salmo salar*). *The Journal of Eukaryotic Microbiology*, *57*(2), 95–114.

Olsvik, P. A., Lie, K. K., Jordal, A.-E. O., Nilsen, T. O., & Hordvik, I. (2005). Evaluation of potential reference genes in real-time RT-PCR studies of Atlantic salmon. *BMC Molecular Biology*, *6*(1), 21.

Pieters, N., Brunt, J., Austin, B., & Lyndon, A. R. (2008). Efficacy of in-feed probiotics against *Aeromonas bestiarum* and *Ichthyophthirius multifiliis* skin infections in rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Journal of Applied Microbiology*, *105*(3), 723–732.

Plarre, H., Devold, M., Snow, M., & Nylund, A. (2005). Prevalence of infectious salmon anaemia virus (ISAV) in wild salmonids in western Norway. *Diseases of Aquatic Organisms*, *66*(1), 71–79.

Popoff, M. Y., Coynault, C., Kiredjian, M., & Lemelin, M. (1981). Polynucleotide sequence relatedness among motile Aeromonas species. *Current Microbiology*, *5*(2), 109–114.

Repstad, O. (2011). *Pathogen dynamics in farmed Atlantic salmon (Salmo salar) with the diagnosis pancreas disease (PD) (in Norwegian)*. MSc thesis (p. 86). University of Bergen.

Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, *19*(12), 1572–1574.

Ross, A. J., Rucker, R. R., & Ewing, W. H. (1966). Description of a bacterium associated with redmouth disease of rainbow trout (*Salmo gairdneri*). *Canadian Journal of Microbiology*, *12*(4), 763–770.

Sommerset, I., Bang Jensen, B., Wiik-Nielsen, J., Bornø, G., & Oliveira, V. H. S. (2022). *Norwegian fish health report 2021, Norwegian veterinary institute report*. Norwegian Veterinary Institute.

Sommerset, I., Bornø, B., Haukaas, A., & Brun, E. (Eds.). (2021). *The health situation in Norwegian aquaculture 2020*. Norwegian Veterinary Institute.

Tamura, K. (1992). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content biases. *Molecular Biology and Evolution*, *9*(4), 678–687.

Tanabe, A. S. (2011). Kakusan4 and Aminosan: Two programs for comparing nonpartitioned, proportional and separate models for combined molecular phylogenetic analyses of multilocus sequence data. *Molecular Ecology Resources*, *11*(5), 914–921.

Thoen, E., Evensen, O., & Skaar, I. (2011). Pathogenicity of *Saprolegnia* spp. to Atlantic salmon, *Salmo salar* L., eggs. *Journal of Fish Diseases*, *34*(8), 601–608.

Tobback, E., Decostere, A., Hermans, K., Haesebrouck, F., & Chiers, K. (2007). Yersinia ruckeri infections in salmonid fish. *Journal of Fish Diseases*, *30*(5), 257–268.

Toenshoff, E. R., Kvellestad, A., Mitchell, S. O., Steinum, T., Falk, K., Colquhoun, D. J., & Horn, M. (2012). A novel Betaproteobacterial agent of gill Epitheliocystis in seawater farmed Atlantic Salmon (*Salmo salar*). *PLoS One*, *7*(3), e32696.

Toranzo, A. E., Baya, A. M., Romalde, J. L., & Hetrick, F. M. (1989). Association of Aeromonas sobria with mortalities of adult gizzard shad, *Dorosoma cepedianum* Lesueur. *Journal of Fish Diseases*, *12*(5), 439–448.

Wahli, T., Burr, S. E., Pugovkin, D., Mueller, O., & Frey, J. (2005). Aeromonas sobria, a causative agent of disease in farmed perch, *Perca fluviatilis* L. *Journal of Fish Diseases*, *28*(3), 141–150.

Watanabe, K., Karlsen, M., Devold, M., Isdal, E., Litlabø, A., & Nylund, A. (2006). Virus-like particles associated with heart and skeletal muscle inflammation (HSMI). *Diseases of Aquatic Organisms*, *70*(3), 183–192.

Weichert, F. G., Axén, C., Förlin, L., Inostroza, P. A., Kammann, U., Welling, A., Sturve, J., & Asker, N. (2021). A multi-biomarker study on Atlantic salmon (*Salmo salar* L.) affected by the emerging red skin disease in the Baltic Sea. *Journal of Fish Diseases*, *44*(4), 429–440.

Yardimci, R., & Turgay, E. (2021). Diagnosis of Aeromonas sobria and *Saprolegnia* sp. co-infection in rainbow trout fry (*Oncorhynchus mykiss*). *Aquatic Research*, *4*, 65–72.

Zepeda-Velázquez, A. P., Vega-Sánchez, V., Ortega-Santana, C., Rubio-Godoy, M., de Oca-Mira, D. M., & Soriano-Vargas, E. (2017).

13652761, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfd.13870 by UNIVERSITY OF BERGEN, Wiley Online Library on [23/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Pathogenicity of Mexican isolates of *Aeromonas* sp. in immersion experimentally-infected rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792). *Acta Tropica*, *169*, 122–124.

SUPPORTING INFORMATION

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

How to cite this article: Lagadec, E., Mjølnerød, E. B., Jensen, Ø. M., Plarre, H., & Nylund, A. (2023). Multiple *Aeromonas* strains isolated from Atlantic salmon (*Salmo salar*) displaying red skin disease signs in Scandinavian rivers. *Journal of Fish Diseases*, *00*, 1–13. https://doi.org/10.1111/jfd.13870

13652761, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfd.13870 by UNIVERSITY OF BERGEN, Wiley Online Library on [23/10/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License