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RESEARCH ARTICLE

Prevalence of epitheliocystis in freshwater Atlantic salmon reared in flow-through and recirculation aquaculture systems

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Abstract

Epitheliocystis, an intracellular bacterial infection in the gills and skin epithelium, has been frequently reported in Atlantic salmon (*Salmo salar*) during freshwater production in a number of countries. This study describes the prevalence and intensity of a natural epitheliocystis infection present in the gills of two strains of Atlantic salmon reared in either a flow-through (FT) or a recirculation aquaculture system (RAS) in Ireland. Repeated sampling of gills prior to and throughout seawater transfer, histology and quantitative real-time PCR were used to determine infection prevalence and intensity. Despite no clinical gill disease, and minor histopathological changes, epitheliocystis lesions were identified in histology at all time points. Specific PCR confirmed the presence of *Candidatus* Clavichlamydia salmonicola in both strains and its number of copies was correlated with intensity of epitheliocystis lesions. A significant interaction between hatchery system and fish strain on the prevalence and intensity of gill epitheliocystis was found both using histological and molecular methods. Specifically, fish from FT had higher prevalence and intensity than RAS reared fish and within FT, the Irish cohort were more affected than Icelandic.

KEYWORDS
Candidatus Clavichlamydia salmonicola, genetic lines, recirculation aquaculture systems, *Salmo salar*

1 | INTRODUCTION

Epitheliocystis is a bacterial disease affecting the epithelium of the gill or skin in a range of freshwater and marine teleost fish which is characterized by intracellular inclusions containing gram-negative bacteria (Nowak & Lapatra, 2006). This bacterial infection causes hypertrophy of epithelial cells and subsequent displacement of the cell cytoplasm and nucleus (Nowak & Lapatra, 2006). Although the causative agent has not been cultured and Koch's postulates have not been fulfilled for epitheliocystis, there are several reports that identified the causative agent based on phylogenetic analysis of DNA sequence data during natural infections (Katharios et al., 2015; Qi et al., 2016; Taylor-Brown et al., 2017) and to date, more than ten *Candidatus* species from gamma, beta and *Chlamydiae* bacterial phyla have been associated with the infection (Blandford et al., 2018). From those, Ca. Clavichlamydia salmonicola, Ca. Piscichlamydia salmonis, Ca. Branchiomonas cysticola, Ca. Similichlamydia sp. and Ca. Syngnamydia salmonis

have been identified during natural epitheliocystis infection in wild and farmed salmonids including Atlantic salmon (*Salmo salar*) (Karlsen et al., 2008; Mitchell et al., 2013), Brown trout (*Salmo trutta*) (Guevara Soto, Vaughan, et al., 2016; Steinum et al., 2010), lake trout (*Salvelinus namaycush*) (Contador et al., 2016) and Pacific salmon (*Oncorhynchus tshawytscha*), Chum salmon (*Oncorhynchus keta*), Coho salmon (*Oncorhynchus kisutch*) and Pink salmon(*Oncorhynchus gorbuscha*) (Kent et al., 1998).

While clinical epitheliocystis is rarely reported in wild fish, the infection causes respiratory distress in farmed juvenile fish (Nowak & Lapatra, 2006). Indeed, naturally infected juvenile lake trout (*Salvelinus namaycush*) showed clinical gill disease and mortalities up to 100% in hatchery (Bradley et al., 1988). However, there is limited information regarding the mechanisms of pathophysiology of epitheliocystis (Blandford et al., 2018; Lai et al., 2013; Taylor-Brown et al., 2017). Usually, gill epitheliocystis has less impact (non-proliferative and unnoticeable effect on the fish) in older fish, particularly in cultured salmon (Nowak & Clark, 1999). Epitheliocystis has been one of the conditions contributing to multifactorial gill disease conditions in farmed salmon smolts such as complex gill disease (proliferative gill disease) and mixed gill infections (Boerlage et al., 2020; Gjessing et al., 2017). Compromised gill health is known to diminish productivity and welfare in salmon farming (Koppang et al., 2015; Sommerset et al., 2020), for that reason, these gill conditions have a high economic impact on the salmon industry (Rodger, 2007).

Age of the host appears to be a factor in determining host susceptibility to epitheliocystis, but other host attributes like genetics could play a significant role on infection resistance in fish of the same species (Buchmann, 2022; Yáñez et al., 2014). Indeed, there are several examples of genetic strains of Atlantic salmon and rainbow trout driving disease resistance particularly against bacterial infections like bacterial cold water disease (Leeds et al., 2010), lactococcosis (Bulfon et al., 2020), furunculosis (Gjedrem & Gjøen, 1995), enteric red mouth (Zuo et al., 2020), vibriosis (Karami et al., 2020) and salmonid rickettsia septicaemia (Dettleff et al., 2015). Dettleff et al. (2015) showed disease resistance of Atlantic salmon at family level against *Piscirickettsia salmonis*, causative agent of salmonid rickettsia septicaemia, in fact certain salmon families with high mean mortality had larger bacterial load while the immune response of resistant families (with low mortality) was efficient in keeping bacteria under the detection threshold. Unfortunately, most likely due to the lack of experimental infections, there are no reports of fish genetic resistance to epitheliocystis.

Investigations of the infection origin or natural reservoir have identified infected wild brown trout (*Salmo trutta*) and wild Atlantic salmon (*Salmo salar*) as reservoir of Ca. Clavichlamydia salmonicola in freshwater (Guevara Soto, Vidondo, et al., 2016; Karlsen et al., 2008; Schmidt-Posthaus et al., 2012). In Atlantic salmon the infection acquired during freshwater production stage subsides after seawater transfer (Mitchell et al., 2010) and transmission among susceptible fish within freshwater production is horizontal (Wiik-Nielsen et al., 2017). This is particularly relevant to the salmon industry and the current trend to implement recirculation aquaculture systems (RAS).

RAS, compared to the traditional flowthrough system, offers multiple advantages when it is managed properly, like reduced water usage, low environmental impact, increased productivity and improved control over water quality, nutrition and biosecurity (Martins et al., 2010). In fact, farming in RAS offers a barrier against waterborne pathogens (Summerfelt et al., 2009). The strongest biosecurity barrier in RAS against pathogens entry is the control over the water input from the water source (or make-up water) (Yanong, 2012). If any pathogens enter the system they can be controlled via serial filtration and disinfection with UV, water ozonation or other chemicals (e.g., peracetic acid) or biocontrol (Blancheton et al., 2013; Liu et al., 2018; Rurangwa & Verdegem, 2015).

Salmon farming industry in Ireland produced ~12,000 tons of fish in 2019 (FAO, 2021). A large proportion of salmon production in hatcheries is based on traditional flowthrough systems, although there is some interest to implement the use of RAS in the freshwater production of salmon as a sustainable production system that can improve productivity, biosecurity, welfare and underpin the future development of Irish salmon aquaculture. To this end, the Salmson project aimed to assess RAS for the freshwater production of organically certified salmon smolts at the Marine Institute nursery facilities in Newport, Ireland.

There are reports of epitheliocystis in freshwater farmed and wild salmonids caused by Ca. Clavichlamydia salmonicola in Ireland (Mitchell et al., 2010), but to the best of our knowledge, there has not been a comparison of epitheliocystis in two genetic lines of the same fish species or the effect different hatchery systems on the infection. This study compared natural epitheliocystis infection in two genetic lines of Atlantic salmon reared in two collocated hatchery systems (flow-through and RAS) and subsequently during seawater transfer.

2 | MATERIALS AND METHODS

2.1 | Fish source

The fish used for this study were sourced as eyed eggs from two commercial companies, a mixed-sex Irish strain (Fanad, Mowi Ireland) and an all-female Icelandic strain (Stofnfiskur, Benchmark Genetics Iceland HF). The eggs incubated in a common CompHatch system (Alvestad Marin, Oslo) using flow through natural water at the Marine Institute Ireland Newport Research Facility (Co. Mayo). The fish hatched in February 2020 and were transferred in May 2020, one cohort of each strain was transferred to a separate tank of two co-located production systems (flowthrough or RAS) for first feeding and raised to smolt stage using standard hatchery protocols.

2.2 | Freshwater rearing

A common water intake from Lough Furnace in the Burrishoole catchment at seasonal ambient temperature of between 4.2 and

20°C (Dillane et al., 2019) drawn water in to primary filtration. Subsequently, filtered water supplied the flowthrough (FT) and the RAS hatchery. In the flowthrough (FT) hatchery, fish were held in two 6.5 m^{-3} circular tanks, with 2000 Irish strain fish in one tank and 2000 Icelandic strain fish in the other (25 kg^{-1} m^{-3} at the time of transfer). The RAS (total volume 44,000 L^{-1}) run on a maximum turnover of 10% water exchange per day, and temperature was maintained between 12 and 16°C. There were two square 5.5 m^{-3} tanks in the RAS containing approximately 2000 fish in each (30 kg^{-1} m^{-3} at the time of transfer) included in this study. Water samples for nutrient analysis in the RAS system were collected on a weekly basis and tested for pH, ammonia, nitrite and nitrate. Both flowthrough and RAS fish were fed with commercial salmon diets (Cargill/EWOS Ltd. Scotland) to apparent satiation using automatic feeders (Arvo-Tec, Finland), feeding rates were automated based on biomass data, incoming water temperature and dissolved oxygen. The fish were subjected to commercial protocol of vaccination using multivalent Aquavac PD 7 (MSD Aqua Health) during the hatchery rearing period.

To aid identification of the fish after transfer to the marine site, the adipose fins were removed from all Icelandic fish using sterile equipment and on September 21, 2020 tanks were exposed to constant 24:0 L:D photoperiod for eight weeks until seawater transfer.

2.3 | Marine transfer and rearing

Fish were caught with hand nets and placed into 2 m^{-3} transport tanks with a maximum density of 150 kg^{-1} m^{-3} for transport to the marine site in Lehanagh pool, Cashel, Co. Galway. Dissolved oxygen was maintained at >95% saturation during transport. Two circular marine pens of 15 m^{-1} diameter, with a net depth of 10 m^{-1} were used in this study, each pen contained either RAS or FT reared fish with a 50:50 mix of Irish and Icelandic fish in each pen. Fish were fed twice daily until satiation.

2.4 | Sampling

Twenty Icelandic fish (ten RAS Icelandic and ten FT Icelandic) and twenty Irish fish (ten Irish RAS and ten Irish FT) were collected from each tank during freshwater rearing or fish pen during seawater transfer at seven time points: 3 weeks (−3w), two weeks (−2w), and one week (−1w) pre-transfer, immediately after marine transfer (T) and one week (1w), two weeks (2w) and five weeks (5w) after seawater transfer. Fish in the seawater pens were identified by adipose fin presence (Irish) or absence (Icelandic).

Fish were quickly and carefully anaesthetized and killed with 200 mg^{-1} L^{-1} of MS-222. A separate container was used for each holding unit with water taken from the intake supply and water was changed between each sample unit, fish type and system type.

Samples of gill mucosal microbiota were taken by swabbing both hemibranchs on both sides of the first and second right side arches (R1 and R2), ensuring rotation of the cotton sterile swab and preserved in 1 ml AL lysis buffer (Qiagen). Samples were stored at −20°C prior to DNA extraction.

The second gill arch from the left-hand side (L2) was dissected within 30 s after death and fixed in 10% neutralized buffered formalin (NBF) for freshwater samples and seawater Davidson's fixative for seawater samples for 24 h, subsequently, samples were transferred to 70% ethanol and processed using standard histology protocols.

Water samples for each fish cohort and hatchery type were taken in duplicate at each time point from either freshwater tank or marine fish pens. Water samples of 2 L were filtered using Sterivex™ 0.22 µm GP sterile vented filter units (EMD Millipore, Burlington MA) and a peristaltic pump at 60 ml^{-1} min^{-1} nominal flowrate and 3 bar maximum pressure. Excess water was then flushed out of each filter using a sterile syringe and the filter chamber was filled with RNALater® (Invitrogen), capped using Luerlock plugs and stored at 4°C for 24 h., then the units were stored at −80°C until DNA extraction.

2.5 | Animal ethics statement

The experimental protocols in this study were conducted in accordance with Irish Medicines Board acts 1995 and 2006 Authorisation of Breeder/Supplier/User establishment Pursuant to part 6 of the European Union (Protection of Animals used for scientific purposes) Regulation 2012 (S.I. No. 543 of 2012), Authorisation number AE19121 Case no: 7028937.

2.6 | Histology

Gill samples were processed using an automatic tissue processor (Tissue-Tek II) at the Aquatic Animal Health laboratory (University of Tasmania, Launceston, Tasmania). Processed samples were embedded in paraffin and subsequently sectioned using a microtome (Microm, Heidelberg) to produce two 5 µm-thick sections. Sections were stained with haematoxylin–eosin (HE). HE stained slides were used for histopathology examination using a light microscope (Leitz Labourlux D, Leica Microsistems, DE) at ×200 magnification. Only well-oriented filaments identified as filaments with secondary lamellae of similar length on both sides were analysed. Fish showing one or more cysts in histology sections were identified as infected fish, the point prevalence was calculated as the percentage of fish infected on each time point and the intensity of infection was determined for each fish by quantifying the mean number of cysts per filament. Slides were randomized to blind the assessor and prevent bias.

2.7 | DNA extraction and plasmid production

DNA was extracted from gill swabs and sterivex filter units using DNeasy® Animal Tissue/Swabs (QIAGEN, Hilden, Germany) and DNeasy Power-Water Sterivex Kit (QIAGEN, Hilden, Germany), respectively, as per manufacturer's protocol. DNA quality and quantity were determined using a NanoDrop

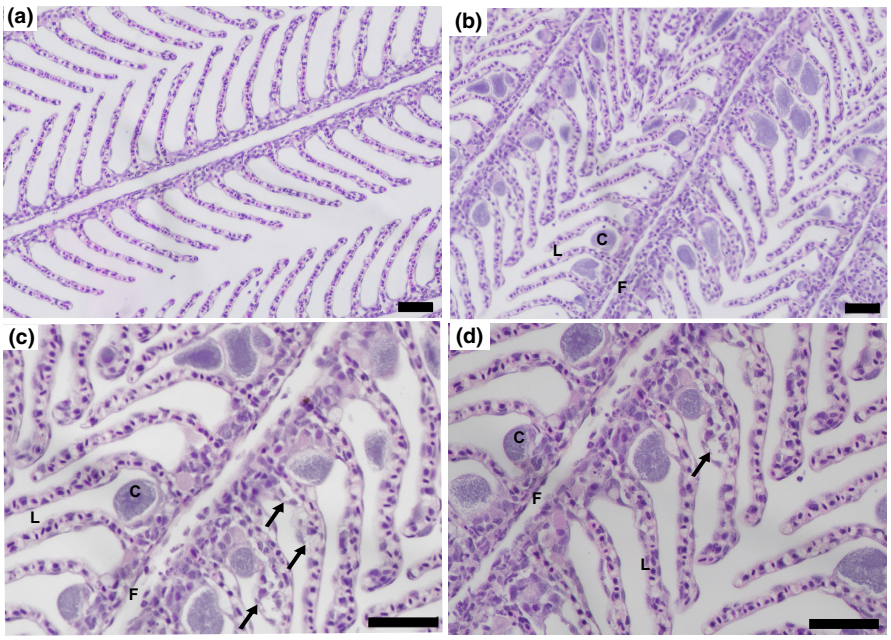

FIGURE 1 Histopathology (a) unaffected gill, (b) location of cysts along the lamellae, higher magnification showing slight subepithelial oedema with infiltration of leucocytes close to cysts (arrows) in RAS system (c) and FT system (d). L = lamella, F = filament, c = cyst, H&E, bar = 50 μm.

ND-5000 spectrophotometer (Nanodrop Technologies, DE, US). Initially, two samples with high count of cysts based on histology were selected and PCR was used to produce a 178 bp fragment from the 16S rRNA gene of Ca. Clavichlamydia salmonicola with primers ClavoRTf 5′-GGAACGATRACTTCGGTTGTTG-3′ and ClavoRTr 5′-CATAGACTCTTCATCAACCACG-3′ (Mitchell et al., 2010). The 50 μl PCR reaction contained 5 μl PCR buffer 10x, 1.5 μl $MgSO_4$, 1 μl DNTPs, 1 μl of each primer, 0.2 μl Platinum® Taq DNA Polymerase High fidelity (Invitrogen, CA, USA) and 4 μl of DNA template (~9 ng μl concentration). The end-point reaction was carried out in a Master cycler nexus (Eppendorf AG, Hamburg, DE) under the following conditions: 95°C for 2 min, 35 cycles at 95°C for 30 s, 55°C for 30 s and 72°C for 30 s and finalizing by 72°C for 5 min. CLavoRT fragments were confirmed via agarose gel and purified using QIAquick® PCR purification kit (Quiagen, Hilden, Germany) according to the manufacturer's instructions. Cloning was done using pGEM®-T easy vector system (Promega, WI, USA), the resulting plasmids from individual clones were purified following the manufacturer's instruction from PureYield™ Plasmid miniprep system (Promega, WI, USA), subsequently the plasmids were sent to Ramaciotti Centre for genomics (University of New South Wales, Sydney, Australia) for sanger sequencing.

2.8 | Quantification of Ca. Clavichlamydia salmonicola

Quantification of Ca. Clavichlamydia salmonicola was done using qPCR via standard curve of 10 X dilution series, following protocol and primers from Mitchell et al. (2010). Briefly, the ClavoRTf, ClavoRTfr primers and ClavoRTp Taqman® probe (5′-Fam-CGAA CTCCAGATGCCTGGAGCCG-BHQ-3′) (Thermo Fisher, USA) were used in a 20 μl real-time PCR reaction containing: 1 μl of each primer (10 μM), 0.5 μl of probe (10 μM), 10 μl of TaqMan Universal Master mix (Roche) and 2 μl of DNA template (~10 ng μl). qPCR was carried out in triplicate in a real-time PCR machine (System Quant Studio 5™, USA) under the following conditions: 50°C for 2 min, 95°C for 10 min followed by 50 cycles of 95°C for 30 s and 60°C for 1 min.

The detection limit for Ca. Clavichlamydia salmonicola was 1000 copies due to increased variation between replicates in the standard curve for low DNA concentration (Ct SD>0.5) (Forootan et al., 2017). Prevalence at time point was determined by the percentage of positive samples per time point and infection intensity reflects the number of copies based on qPCR. Standard curve for quantification of Ca. Clavichlamydia salmonicola had a slope −2.974, ɣ-intercept 39.7 and efficiency 116.8.

2.9 | Statistical analysis

Confidence intervals of prevalence data were calculated using confidence intervals for binomial probabilities (binconf function) from Hmisc package v 4.5.0 (Harrell Jr., 2021). The number of fish affected and infection intensity from histology and qPCR data were analysed using linear mixed-effects model (LMM) via maximum likelihood from the package lmerTest v 3.1.3 (Bates et al., 2020). LMM were used to detect significant interactions between fish cohorts, hatchery systems and time points. Chi-squared and Pearson correlation were applied to analyse the frequency and correlation of positive and negative samples detected with histology and qPCR.

3 | RESULTS

3.1 | Epitheliocystis pathology

No gross pathology was detected in gills throughout the sampling period. In histological sections, epitheliocystis lesions were characterized by intracellular basophilic inclusions in hypertrophic epithelial cells with marginalized nucleus. These cells were surrounded occasionally by mild subepithelial oedema with leucocyte

infiltration (Figure 1d). The inclusions were localized along the lamellae and along the gill filament (Figure 1b,c). Other minor histological changes identified in gill sections were focal and multifocal hyperplasia of epithelial cells, lamellar fusion involving 2 to 4 lamellae and subepithelial oedema. These lesions were not associated with epitheliocystis.

3.2 | Prevalence and infection intensity based on histology

Based on histopathology the prevalence of infection varied across both the strains, production systems and time course. The Irish strain was more affected with 100% of the Irish FT cohort at three weeks prior to transfer, and then a decrease in prevalence throughout the time points. In contrast only 11% of Irish fish held in RAS were affected at 3 weeks prior to transfer, however an increasing trend in prevalence was observed with 100% of fish affected at 2 weeks post transfer (Figure 2a). In general, the Icelandic strain was less affected in both FT and RAS systems, however a prevalence of up to 50% was observed at 2 weeks post transfer in the Icelandic cohort in the RAS system (Figure 2b).

Across the fish cohorts the number of cysts per filament ranged from 1 to 50 and the number of filaments affected per gill section ranged from 1 to 35. Although the Irish FT cohort had the highest number of cysts per filament particularly from three weeks to one week before transfer, subsequently, the number of cysts was reduced after transfer (Figure 3a).

Linear mixed-effects model (LMM) via maximum likelihood revealed that there was a statistically significant interaction effect between the fish strain and hatchery production system on the number of cysts per filament ($F_{1,244} = 6.47$, $p = .01$) (Figure 4b) and a significant interaction effect between hatchery type and sampling point ($F_{6,244} = 10.3054$, $p < .0001$). Additionally, there was a significant interaction effect between fish strain and hatchery system on the prevalence of epitheliocystis during freshwater ($F_{1,106} = 5.11$, $p = .025$). The number of fish affected with epitheliocystis depended on the hatchery rearing system and fish origin, fish reared in FT showed higher prevalence and intensity than fish reared in RAS and the Irish cohort had higher intensity and prevalence compared to Icelandic cohort (Figure 4a).

3.3 | Prevalence and intensity based on qPCR

The quantification of Ca. Clavichlamydia salmonicola in DNA from gill swabs based on qPCR showed that prevalence of epitheliocystis was consistently higher in Irish strain particularly in the Irish FT cohort where Ca. Clavichlamydia salmonicola was detected in 90% to 100% of samples at three weeks pretransfer, and the following time points until one week post transfer. In contrast, in the Irish RAS cohort the prevalence was 20% at three weeks prior to transfer with variable levels throughout the other time points but an increased prevalence was observed immediately after marine transfer (Figure 5a). Overall, the prevalence of Ca. Clavichlamydia salmonicola was lower in the Icelandic cohort across the time points, except at 2 weeks after transfer when the prevalence was 90% in fish from FT system (Figure 5b). Similarly, the prevalence of Ca. Clavichlamydia salmonicola was higher in the Icelandic RAS cohort at 2 weeks post transfer compared to other time points and decreased at 5 weeks after seawater input (Figure 5). The prevalence of epitheliocystis based on qPCR was significantly different between the hatcheries ($F_{1,244} = 7.83$, $p = .005$) and there was a significant interaction effect between fish strain and hatchery system on the prevalence of epitheliocystis based on qPCR during freshwater ($F_{1,97} = 10.71$, $p = .001$).

Based on the number of copies quantified through qPCR, the Ca. Clavichlamydia salmonicola intensity was over 60,000 copies in the

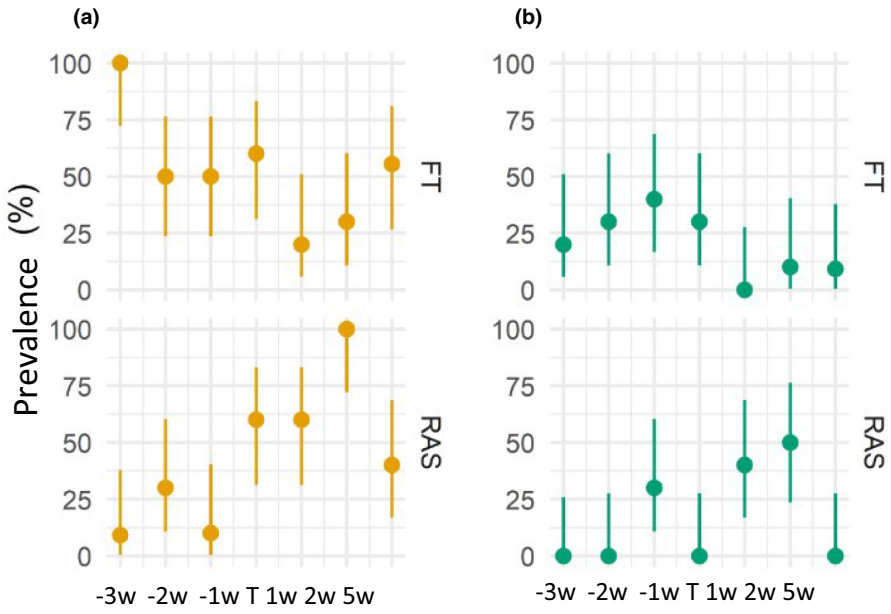

FIGURE 2 Prevalence of epitheliocystis based on histology in Irish (a) and Icelandic (b) fish cohorts cultured in RAS and FT from 3 weeks pre seawater transfer to 5 weeks post seawater transfer. Dots and error bars show prevalence percentage and confidence intervals, respectively.

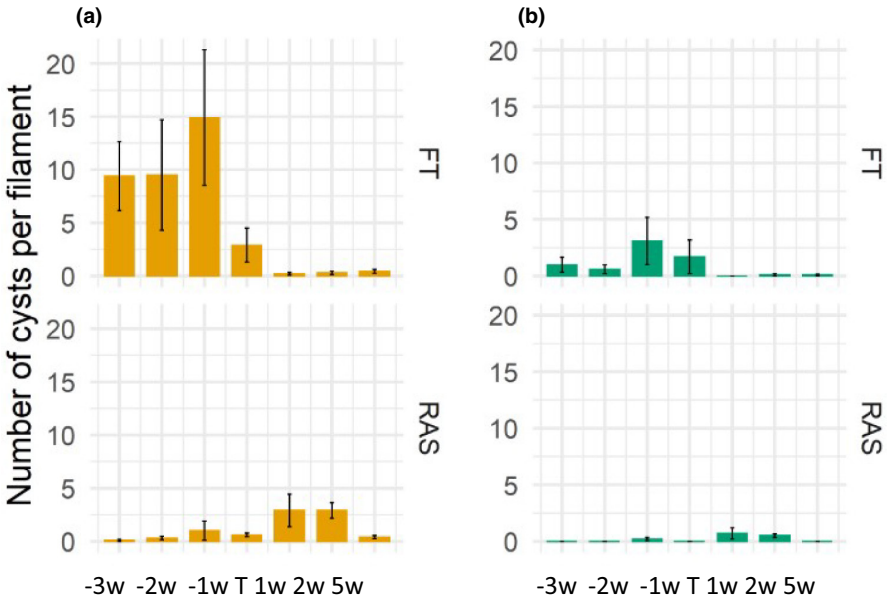

FIGURE 3 Intensity of epitheliocystis based on histology in Irish (a) and Icelandic (b) fish cohorts reared in RAS and FT, bars and error bars denote mean and standard error, respectively.

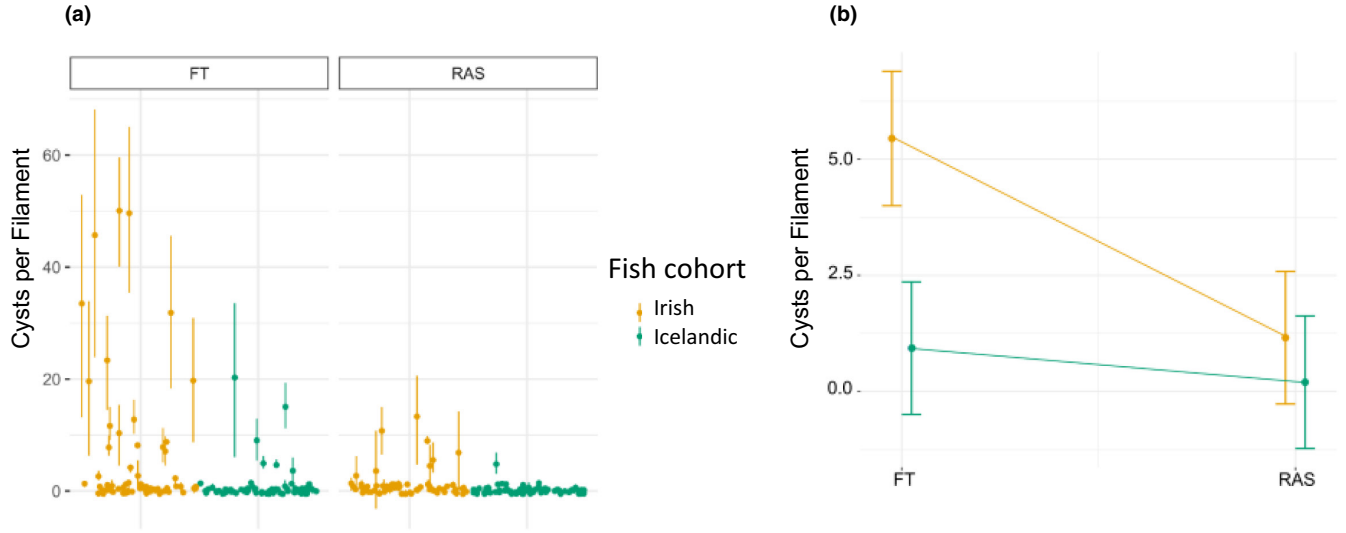

FIGURE 4 Epitheliocystis intensity based on histology. (a) Comparison of number of cysts per filament between fish cohort and hatchery system (dots show mean and line range shows standard deviation). (b) Linear mixed effect model interaction of predicted value of cysts per filament with hatchery system and fish cohort, dots show mean and error bars show confidence intervals.

Irish FT cohort, decreased to 10,000 during the freshwater phase (2 weeks to 1 week pretransfer) and less than 1000 by 5 weeks after transfer. Infection intensity in the FT Icelandic cohort decreased from 6000 at 2 weeks pretransfer to 3000 after transfer, although an increase in the number of copies was observed 2 weeks after seawater input. Levels of infection in the RAS cohorts were below 5000 copies which decreased after sea transfer then increased to 5000 copies at 2 weeks post transfer (Figure 6).

The linear mixed-effects model (LMM) via maximum likelihood revealed that there was a statistically significant interaction effect between the fish strain and hatchery type on the number of DNA copies ($F_{1,240}$ = 8.43, p = .004) (Figure 7). A significant interaction effect was found between hatchery type and sampling time on the prevalence of epitheliocystis based on qPCR ($F_{6,244}$ = 6.9657, p < .0001).

Pearson's chi-squared test showed that there was an association (χ^2 = 47.87, p < .0001) between the number of samples positive based on histology and on qPCR. There was moderate positive correlation between detection with histology and detection with qPCR (r = .43, t = 7.74, p < .0001). Additionally, there was a moderate positive correlation between the number of cysts per gill filament and the number of copies (r = .41, p < .0001). Among all samples positive to epitheliocystis based on histology, 74.7% were also positive using qPCR and 70% of samples negative to epitheliocystis via histology were also negative based on qPCR analysis (Figure 8). Ca. Clavichlamydia salmonicola in water was detected only in two

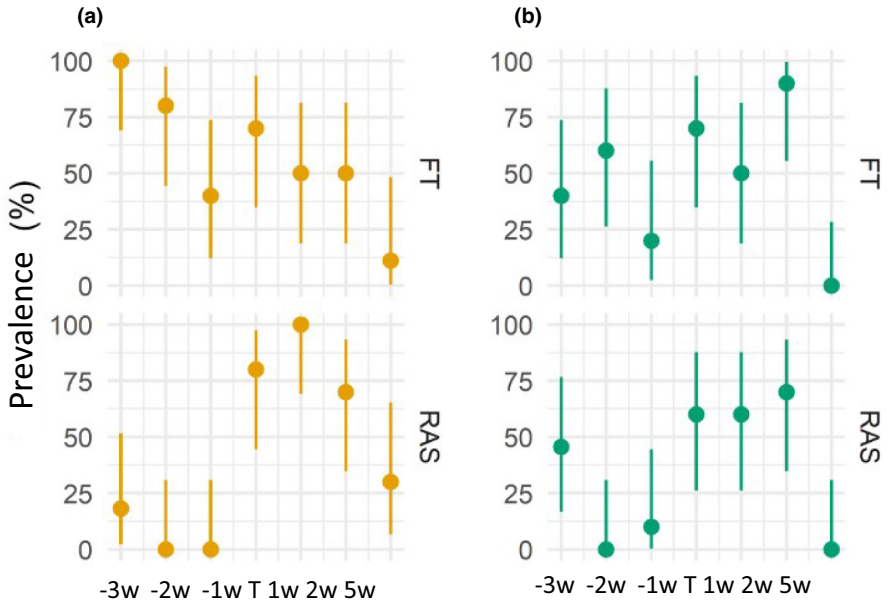

FIGURE 5 Prevalence of epitheliocystis based on qPCR in Irish (a) and Icelandic (b) fish strains over 7 time points around seawater transfer, dots show percentage of prevalence and error bars show binomial confidence intervals.

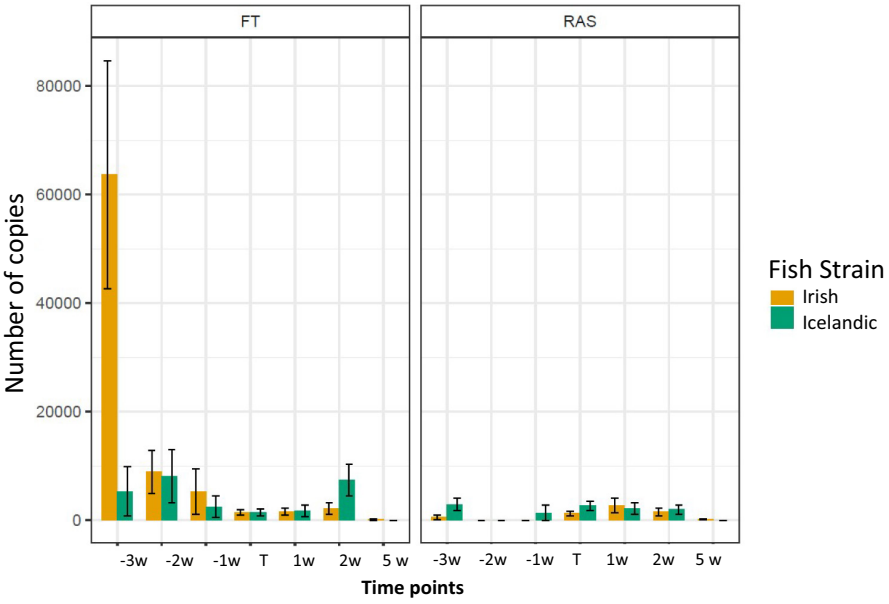

FIGURE 6 Infection intensity based on number of copies from qPCR in Irish and Icelandic fish cohorts from flowthrough and RAS system through seven sampling time points. Bars show mean number of copies and error bars show standard error.

samples, one from the RAS Irish cohort (331 copies) and from the FT Icelandic cohort (31,200 copies) taken in the freshwater hatcheries two weeks before seawater transfer.

4 | DISCUSSION

The eggs of the Atlantic salmon cohorts of this study were held in a common flowthrough incubation system and were separated to different rearing systems immediately post hatch and prior to fist feeding. Despite evidence of vertical transmission of other Chlamydia-like agent of epitheliocystis (Ca. Similichlamydia laticola) affecting barramundi (*Lates calcarifer*) (Stride et al., 2013), it is unknown whether Ca. Clavichlamydia salmonicola can enter Atlantic salmon eggs. As the eggs from both cohorts were incubated in a flowthrough system until hatching and then split into RAS or flowthrough systems for first feeding, it is likely that the animals were exposed to waterborne natural infection from post hatching stage.

The Atlantic salmon cohorts sampled in this study were clinically healthy and histological examination identified intracellular basophilic inclusions in gill epithelium previously described as epitheliocystis (Hoffman et al., 1969). A number of histopathological changes were found in gills, but only slight subepithelial oedema was observed close to the epitheliocystis lesions. The same pathology was reported previously during natural epitheliocystis infections in freshwater reared Brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*) (Karlsen et al., 2008; Mitchell et al., 2010).

Intensity and prevalence of epitheliocystis varied across the time in both fresh and marine water, with a drastic decrease observed after marine transfer for the cohorts from flowthrough hatcheries.

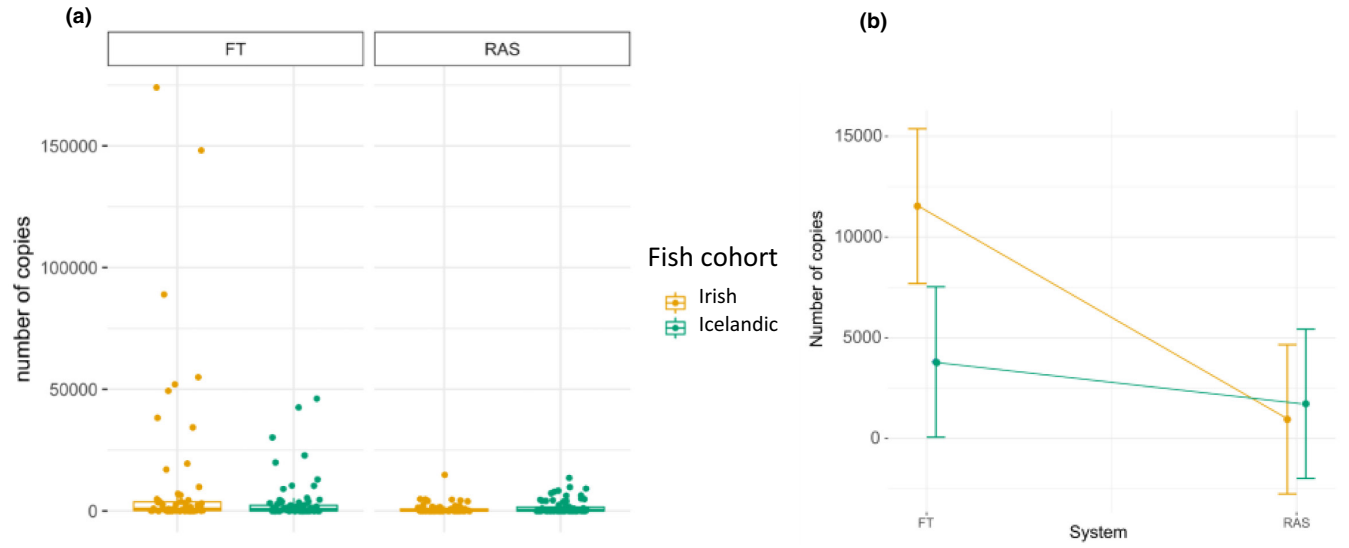

FIGURE 7 Number of copies of Ca. Clavichlamydia salmonicola based on qPCR. (a) Comparison of the number of copies of between fish cohort and hatchery system. (b) Linear mixed effect model interaction of hatchery system and fish cohort on the number of copies, dots show mean and error bars show confidence intervals for the prediction.

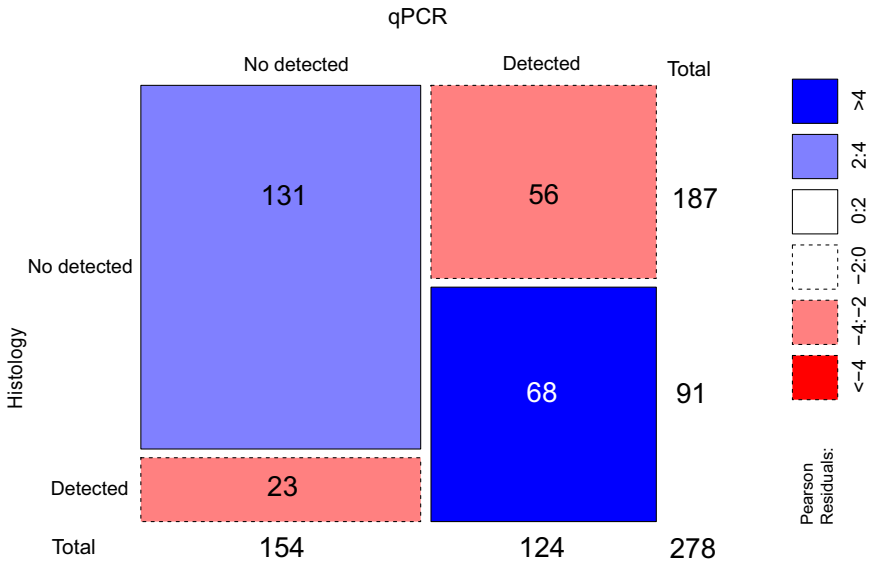

FIGURE 8 Mosaic plot of contingency table for 278 samples analysed using histology and qPCR to detect epitheliocystis. Mosaic colours are Pearson residuals.

Previously, a progressive decrease was reported in prevalence and intensity of epitheliocystis caused by Ca. Clavichlamydia salmonica as the fish transitioned from freshwater to marine rearing where the condition was present for 6 weeks and disappeared in the course of 10 weeks after seawater transfer (Mitchell et al., 2010). This aligns with the decreased prevalence and intensity of epitheliocystis once all fish cohorts were transferred to sea water and in the subsequent 5 weeks in our study.

This study showed that the prevalence and intensity depended on the hatchery system where the fish were reared and that epitheliocystis was highly prevalent in FT hatchery. This has been reported previously in Ireland where epitheliocystis has been found in wild and freshwater farmed salmonids, and a number of rivers used as water source of FT system are natural reservoir of the pathogen causing epitheliocystis (Mitchell et al., 2010). It is important to note that in this study, the intake water source was shared between the RAS and FT hatcheries, although the water intake in RAS was treated through serial filtration and UV, which would likely reduce the pathogen input to the system (Blancheton et al., 2013). Control over the water source, together with other biosecurity measures, limit the possibility of bacteria pathogens like *Yersinia ruckeri* of entering the RAS systems (Holan et al., 2020). Besides, the filtration and water treatment installed to reuse water in the loop system can decrease the levels of facultative pathogens like *Streptococcus iniae* (Aruety et al., 2016). The daily water exchange in RAS was 10% which is higher than the 5% reported in

modern RAS (Badiola et al., 2012) and could be a factor contributing to entry of Ca. Clavichlamydia salmonicola to the RAS system. Despite the daily water intake to RAS hatchery, fish reared in RAS showed low infection prevalence and intensity compared to FT reared fish. This can be attributed to the use of UV filters and other filters used as part of water management in the RAS hatchery. The implementation of modern RAS with <5% of water exchange and more stringent water treatment could increase the biosecurity in the hatchery restricting the entry of gill pathogens like those causing epitheliocystis.

Interestingly, a significant interaction effect between fish strain and hatchery system on epitheliocystis intensity was observed in this study. Fish genetics play a significant role in disease resistance (Buchmann, 2022; Yáñez et al., 2014), indeed distinct genetic lines of salmonids have shown differences in susceptibility to a number of bacterial infections like bacterial cold water disease (Leeds et al., 2010) or lactococcosis (Bulfon et al., 2020). Although epitheliocystis intensity was higher in the Irish compared to the Icelandic strain, these results should be interpreted with caution because the infection described in this study was natural with no control over pathogen inoculation. Hence, studies are required to elucidate the susceptibility of genetic lines of salmon to epitheliocystis when the infection can be reproduced.

In this study, an increase in epitheliocystis prevalence and intensity was observed in the two fish cohorts from RAS at week 2 post marine transfer. A possible explanation is that fish from RAS were in close contact with heavily infected FT cohorts during and post seawater transfer. Indeed, fish were transferred from the hatcheries to the same seawater pool into two adjacent pens where the Irish and the Icelandic cohorts were mixed. Horizontal transmission of epitheliocystis (caused by Ca. Branchiomonas cysticola and Ca. Piscichlamydia salmonis) was demonstrated previously in Atlantic salmon using water from tanks with infected fish as source of waterborne infection in naïve fish (Wiik-Nielsen et al., 2017), although further studies are required to clarify the transmission of Ca. Clavichlamydia salmonicola during the first week after transfer to sea water. Moreover, in this study, only Ca. Clavichlamydia salmonicola was examined given the level of prevalence reported in this area of Ireland (Mitchell et al., 2010); however, the possibility of other species should also be considered.

The prevalence based on histology and qPCR were comparable as we found a moderate correlation on the positive samples detected using both methods. The variability observed on the intensity data when comparing methods can be attributed to the limited gill area examined by histology. The histological analysis can only cover a limited area of the gill compared to the qPCR methodology which include samples taken by swabbing both sides of the gill arch, this has been observed by other authors when comparing histology and qPCR to quantify gill pathogens (Downes et al., 2017).

In summary, we found that the prevalence of natural infection of epitheliocystis in freshwater salmon depended on the rearing system in which two cohorts of Atlantic salmon smolts were produced and the fish strain, fish from RAS hatchery were less affected.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of financial or personal interest involved in this work.

DATA AVAILABILITY STATEMENT

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

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