Received: 13 October 2022 | Revised: 13 December 2022 | Accepted: 16 December 2022

DOI: 10.1111/jfd.13745

Journal of Fish Diseases WILEY

RESEARCH ARTICLE

Vertical transmission of *Renibacterium salmoninarum* in cutthroat trout (*Oncorhynchus clarkii*)

Tawni B. Riepe[1] | Eric R. Fetherman[2] | Brad Neuschwanger[3] | Tracy Davis[3] | Andrew Perkins[3] | Dana L. Winkelman[4]

[1]Colorado Cooperative Fish and Wildlife Research Unit, Colorado Parks and Wildlife, Aquatic Wildlife Research Section, Colorado, Fort Collins, USA

[2]Colorado Parks and Wildlife, Aquatic Wildlife Research Section, Fort Collins, Colorado, USA

[3]Colorado Parks and Wildlife, Bellvue Fish Research Hatchery, Bellvue, Colorado, USA

[4]U.S. Geological Survey, Colorado Cooperative Fish and Wildlife Research Unit, Fort Collins, Colorado, USA

Correspondence
Tawni B. Riepe, Colorado Cooperative Fish and Wildlife Research Unit, Colorado Parks and Wildlife, 317 W Prospect Road Fort Collins, Colorado, USA.
Email: tawni.riepe@state.co.us

Funding information
Colorado Parks and Wildlife; Species Conservation Trust Fund

Abstract

Vertical transmission of *Renibacterium salmoninarum* has been well-documented in anadromous salmonids but not in hatchery-reared inland trout. We assessed whether the bacterium is vertically transmitted in cutthroat trout (*Oncorhynchus clarkii*) from a Colorado, USA hatchery, and assessed the rate of transmission from male and female brood fish. Adult brood fish were killed, tested for *R. salmoninarum* in kidney, liver, spleen, ovarian fluid, blood and mucus samples, then stripped of gametes to create 32 families with four infection treatments (MNFN, MNFP, MPFN, MPFP; M: male, F: female, P: positive, N: negative). Progeny from each treatment was sampled at 6 and 12 months to test for the presence of *R. salmoninarum* with an enzyme-linked immunosorbent assay and quantitative polymerase chain reaction. Our study indicated that vertical transmission was high and occurred among 60% of families across all infection treatments. However, the average proportion of infected progeny from individual families was low, ranging from 1% (MNFP, MPFN and MPFP treatments) up to 21% (MPFP treatment). Hatcheries rearing inland salmonids would be well suited to limit vertical transmission through practices such as lethal culling because any amount of transmission can perpetuate the infection throughout fish on a hatchery.

KEYWORDS
bacterial kidney disease, Colorado, cutthroat trout, *Renibacterium salmoninarum*, vertical transmission

1 | INTRODUCTION

Pathogens established in aquaculture facilities are often difficult to eliminate without complete depopulation of the host species and decontamination of the unit (Behringer et al., 2020). As such, disease outbreaks can cause high economic and conservation losses (Shinn et al., 2015). Bacterial diseases are often one of the leading causes for high mortality events in hatchery facilities (Sudheesh et al., 2012). The bacteria *Aeromonas salmonicida*, causing furunculosis, has caused losses of up to 80% (Austin et al., 2007), and bacterial coldwater disease, caused by *Flavobacterium psychrophilum*, of up to 90% among reared salmonids (Barnes & Brown, 2011; Nilsen et al., 2011). In extreme disease outbreaks, mortality of up to 100% has been observed in rainbow trout (*Oncorhynchus mykiss*) due to *Pseudomonas fluorescens* (Pękala-Safińska, 2018). The number of pathogenic bacteria that are being isolated from reared fish has been steadily increasing (Harvel et al., 1999). Thus, understanding the transmission of

bacterial pathogens among hosts is important to reduce losses in aquaculture facilities from disease outbreaks.

Renibacterium salmoninarum is the bacteria responsible for bacterial kidney disease in salmonid species and can contribute to high mortality among reared salmonids. For instance, infections have caused 40% mortality among Atlantic salmon and 80% mortality among Pacific salmonids in aquaculture facilities (Evenden et al., 1993; Wiens, 2011). The bacteria can be transmitted through both vertical and horizontal transmission (Balfry et al., 1996; Evelyn et al., 1986b). Horizontal transmission occurs from direct contact with infected fish or contaminated water, or through the ingestion of contaminated faecal matter (Balfry et al., 1996). Vertical transmission typically occurs from the maternal fish to the progeny through an intra-ovum infection (Evelyn et al., 1986b). *Renibacterium salmoninarum* is primarily detected from haematopoietic tissues but has also been detected in the ovarian fluid of adult spawning fish, fertilized eggs, progeny and milt (Brown et al., 1994; Fetherman et al., 2020; Hamel, 2005; Larenas et al., 2003). Infections of eggs may occur early during egg development (oogenesis) or acquired from the surrounding ovarian fluids, such that the bacteria passively enter through the micropyle of the egg (Evelyn, Prosperi-Porta, & Ketcheson, 1984; Potts & Rudy Jr, 1969). Pseudo-vertical transmission may also be possible, where the bacteria are in the water and enter the egg during the water-hardening process of fertilization (Kumagai et al., 2000). *Renibacterium salmoninarum* has also been detected from spermatozoa, but the significance of bacteria present on the spermatozoa for successful vertical transmission of *R. salmoninarum* is not clear (Daly & Stevenson, 1989). Thus, successful infection of *R. salmoninarum* from vertical transmission is thought to be primarily driven by the female.

Currently, there is no treatment to eliminate *R. salmoninarum* from the environment or from an infected population. Therefore, methods have been developed to limit transmission. Attempts to decrease the prevalence and limit transmission have included depopulation of fish from an infected hatchery unit, culling to reduce potentially infected eggs and injections of erythromycin to reduce the probability of vertical transmission from adult broodstock to progeny (Fetherman et al., 2020). Depopulation and culling of brood fish seem to be the most effective methods to reduce transmission (Munson et al., 2010; Colorado Parks and Wildlife unpublished data). Culling involves spawning brood fish and sampling tissues to test for *R. salmoninarum*. If the brood fish test positive for an infection, their eggs are discarded. However, this method resulted in a loss of approximately 2800 cutthroat trout (*Oncorhynchus clarkii*) broodstock from a Colorado hatchery between 2017 and 2019 (Colorado Parks and Wildlife unpublished data). Many hatcheries in Colorado maintain rare lineages of cutthroat trout and whirling disease-resistant rainbow trout broodstocks, which produce millions of eggs for production. Therefore, lethal culling or depopulation to regulate the presence of *R. salmoninarum* may not be appropriate. Although erythromycin injections in the adult spawning fish seemed promising as an intervention, they did not prevent transmission from occurring (Fetherman et al., 2020) and are a costly and time-consuming method to use among all infected fish in a hatchery.

Aquaculture propagation of cutthroat trout is a necessary component of their management because large numbers of genetically diverse fish are needed to rapidly establish populations (Harig et al., 2000). Broodstock of cutthroat trout has been established at Colorado Parks and Wildlife (CPW) hatcheries to allow more rapid reintroduction through stocking. In 2017, hatcheries rearing isolated strains of the Greenback cutthroat trout (*O. clarkii stomias*) contributed 1.5 million eggs during the spawning season. However, one major constraint to maintaining the spawning production of the cutthroat trout is the spread of disease within a facility through vertical transmission. In 2016 *R. salmoninarum* was detected in cutthroat trout broodstock at the CPW Poudre Rearing Unit (PRU). Lethal culling of the adult male and female fish during the spawning season occurred in an attempt to decrease vertical transmission, but this did not result in the successful elimination of the pathogen among progeny. To better evaluate which method(s) are suitable to lower or eliminate *R. salmoninarum* prevalence among progeny, we attempted to estimate the rate of vertical transmission among cutthroat trout. Specifically, we artificially spawned and tested cutthroat trout at the PRU to produce families from parents with a known infection status, then tested the progeny to estimate the rate of vertical transmission of *R. salmoninarum* from infected broodstock to progeny.

2 | MATERIALS AND METHODS

During the 2019 cutthroat trout spawn at the CPW PRU, we screened 352 3-year-old female and 352 2-year-old male broodstock for *R. salmoninarum*. Adult fish were initially swabbed for a non-lethal mucus sample on both sides of the fish along the lateral lines (Riepe et al., 2021), weighed (g), measured (mm) and then killed using an overdose of tricaine methanesulfonate (MS-222: Western Chemicals). Adult females and males were dried with towels to prevent cross-contamination from the water source and stripped of gametes and spawned together in a clean, dry container. Each female adult was only spawned with one other male adult to create a family. The infection status of each adult was unknown prior to spawning thus we had an initial total of 352 families created from the adults to ensure an adequate representation of family treatments (discussed below). Ovarian fluid was collected prior to the addition of milt with a sterile syringe and placed in a 2 ml microcentrifuge tube. We did not collect milt due to the risk of not having enough milt for fertilization. After spawning, blood was collected through an intravenous puncture of the caudal vein and placed in a 2 ml microcentrifuge tube (Riepe, 2022). We also collected whole spleen, liver and kidney tissues through an abdominal incision from each fish. Each tissue sample was placed into individual whirl-pak-bags and labelled with a unique identifier for each individual fish. All samples were frozen on dry ice for transportation to the laboratory where they were stored at −20°C until sample processing.

2.1 | Egg fertilization and transportation

Immediately following milt extraction from the male brood fish, well water was added to the eggs and gently agitated for 2 min to induce egg fertilization. After fertilization, about half of the fertilized eggs from each pairing were subsampled and placed into individual egg incubation cups. The other half of the eggs were used for hatchery production purposes. Previously disinfected incubation cups were fabricated from 101.6 mm diameter schedule 40 PVC pipes that were 50.8 mm tall and fit with a 152.4 mm diameter lid with mesh screens to allow for water to flow through. Four incubation cups, labelled by family, were placed into a half-gallon water jug, filled with 50 ppm iodine mixed with water for surface disinfection and water hardening of eggs for 1 h. Although iodine is used to remove bacteria from the surface of the egg, it is not effective at reducing bacteria inside the egg (Evelyn et al., 1986b), and therefore, the use of iodine during water hardening did not affect the vertical transmission of *R. salmoninarum* in this study. Following water hardening, each jug was rinsed with well water, filled to the top and transported to the CPW Bellvue Fish Research Hatchery. Upon arrival, each jug was rinsed, filled with 100 ppm ovadine for additional surface disinfection of eggs for 10 min and rinsed again. Egg cups were placed into Heath stack incubator trays, with five egg cups per tray. Eggs were treated with 1667 ppm formalin at a flow of 5 gpm every other day until eggs were eyed to prevent fungal growth. Once eggs were eyed, unfertilized eggs were removed, and the egg cups were randomly assigned to tanks based on family treatment assignments.

2.2 | Adult tissue testing

Initial kidney tissue testing with quantitative polymerase chain reaction (qPCR) occurred within 72 h of collection to determine the infection status of broodstock for assigning eggs to appropriate treatments (discussed below). All other tissues were tested within 6 months of collection. We followed the American Fisheries Society (AFS)-Fish Health Blue Book (2016) recommended testing procedures for screening tissues for *R. salmoninarum* with qPCR. Tissues were homogenized in sterile whirl-pak-bags with rolling pins and serums mucus, blood and ovarian fluid vortexed in microcentrifuge tubes. To prepare mucus swabs, we incubated each swab in a 2 ml microcentrifuge tube for 3 h with 1.5 ml of 1X phosphate buffered solution (PBS) at room temperature. Duplicate samples of tissues (25 mg) or mucus, blood or ovarian fluid (200 µl) from the homogenized sample were collected for DNA extractions. We followed the protocol from Qiagen DNeasy Blood and Tissue Kit (Hilden, Germany) for tissues, blood and ovarian fluid and the Qiagen protocol for Gram-positive bacterial swabs for mucus samples, with the addition of an extra elution step to increase DNA concentration in all samples (Elliott et al., 2013). Following Chase et al. (2006), we used 5 µl of extracted DNA for each qPCR reaction with the forward primer (RS 1238 5′-GTGACCAACACCCAGATATCCA-3′), the reverse primer (RS 1307 5′-TCGCCAGACCACCATTTACC-3′) and a probe with 3′ MGBNFQ quencher (RS 1262, 5′-CACCAGATGGAGCAAC-3′). A TaqMan Gene Expression Master Mix (ThermoFisher) was used at 1X concentration. An Applied Biosystems Step One Plus system was used with an initial incubation time at 50°C for 2 min, 90°C for 10 min and 40 denaturing cycles at 95°C for 15 s, followed by 60 s of annealing at 60°C. Samples below a Cq value of 37.75 were considered positive for the presence of *R. salmoninarum* (Riepe, 2022). Analysis of qPCR output was compared with a previously developed standard curve to determine the number of bacteria from Cq values for each positive tissue sample (Riepe, 2022). This standard curve was based on 58 ten-fold serial dilutions of bacterial cells that were counted and used to determine the maximum Cq value used in this study (37.75), which is considered an acceptable value for *R. salmoninarum* detection with qPCR (Sandell & Jacobsen, 2011).

2.3 | Family treatment assignment

After we screened kidney tissues from brood fish for *R. salmoninarum* with qPCR, we assigned each family to a specific treatment based on *R. salmoninarum* infection status. The number of families for each treatment resulted in 8 'control' MNFN (M: male, F: female, N: negative) families that allowed us to evaluate whether *R. salmoninarum* transmission was occurring through contact with the water source; 8 MNFP (P: positive) families to determine vertical transmission from female brood fish; 5 MPFN families to determine vertical transmission from male brood fish; and 11 MPFP families to determine vertical transmission from both male and female brood fish. However, in a previous study, the detection probability of *R. salmoninarum* in kidney tissues was low, indicating that there is a high probability for false-negative results (Riepe, 2022). Therefore, we additionally tested individual liver and spleen tissues collected from the adults. This resulted in a post-hoc reassignment of family treatments based on whether the kidney, liver and/or spleen tissues from individual fish were positive. Final family treatment assignments resulted in 1 MNFN, 2 MNFP, 4 MPFN and 25 MPFP families. All families were randomly assigned to a single tank each (32 total).

2.4 | Rearing conditions

After eggs hatched, larvae took up to 2 weeks to swim up, after which we started feeding BioOregon size #0 feed. After 30 days of initial feeding, we subsampled 100 fish to remain in each tank for the duration of the experiment; other fish were killed and removed from the experiment. Fish were maintained in 75.7-L flow-through tanks with 13.0 ± 0.1°C well water with a flow rate of 7.6 L/min and aerated continuously using atmospheric air pumped through air stones. Every 2 weeks, all fish were weighed to determine a batch weight for each family and converted to average weight per individual based on the number of fish in each tank. Once the average weight of individual fish in a tank was equal to 0.4 g, we changed the feed to Rangen size #1 feed. Feed size and amount were increased throughout the experiment based on the average weight of fish, obtained

every 2 weeks, and following the manufacturer's recommendation for a 3% maintenance diet. Cleaning occurred every 2 days, and each family was assigned its own brush and suction tubing for cleaning to minimize the potential for cross-contamination.

2.5 | Progeny sampling

The timing of sampling progeny was based on the day we subsampled 100 fish for the experiment (hereafter referred to as post swim-up). At 6-month post swim-up, 50 fish were selected and killed to sample tissues for *R. salmoninarum* testing. The remaining fish were kept in the experiment for an additional 6 months. Fish were weighed, measured and killed with MS-222. An abdominal incision was made to collect spleen, liver and kidney tissues, which were pooled in one whirl-pak-bag and uniquely labelled by individual fish and treatment. Samples were immediately placed on dry ice for transportation to the laboratory. After 12-month post swim-up, we sampled the remaining fish in each tank and collected the same tissues as we did at 6-month post swim-up. Any mortalities over the course of the experiment were noted, but these fish were not tested for the presence of *R. salmoninarum* because fish tissues were typically decomposed.

All tissue samples were tested for *R. salmoninarum* with qPCR. DNA extraction and qPCR analysis were followed as outlined above. Pooled spleen, liver and kidney tissues from individual progeny were also screened with a double-sandwich enzyme-linked immunosorbent assay (ELISA). Homogenized tissues were prepared to a 1:4 (w/v) dilution with PBS, 0.05% (v/v) Tween-20 and 0.01% (w/v) thimerosal. Following an established ELISA protocol (Pascho et al., 1991), we used an affinity-purified *R. salmoninarum*—goat antibody as a coating antibody (KPL: Milford, MA, USA) and a horseradish-peroxidase (HRP) labelled *R. salmoninarum*—antibody as the conjugate (KPL: Milford, MA, USA). Each family was tested separately and replicates of the HRP conjugate, substrate-chromogen, cell culture water and tissues from known negative rainbow trout tissues were plated as assay controls. Four *R. salmoninarum* positive control dilutions (BacTrace, KPL: Milford, MA, USA) were prepared at 1:100, 1:1000, 1:2000 and 1:5000. A UV–Vis microplate spectrophotometer with a monochromator-based absorbance was used at 405 nm to determine optical density values (OD). Sensitivity of the ELISA assay was between 2 and 20 ng of *R. salmoninarum* (Pascho & Mulcahy, 1987). We used a conservative threshold of greater than 0.10 to determine positive samples (Elliott et al., 2013; Kowalski et al., 2022; Munson et al., 2010). Like other studies, we set criteria to characterize antigen load levels (Elliott et al., 2013): low (OD: 0.100–0.199), intermediate (OD: 0.200–0.999) and high (OD: > 1.000).

2.6 | Statistical analysis

We compared if the detection of *R. salmoninarum* differed among the four treatments using an analysis of variance (ANOVA) for unbalanced designs with both assays and the two age classes as predictor variables in addition to the treatments. Then, we evaluated whether the percent of families infected in a treatment (MNFP, MPFN, MPFP) differed as a function of treatment, family, assay type, age, weight and length of progeny with a generalized linear mixed model (GLMM) with a logit link. The model was fit in the lme4 package using the glmer function in R version 4.1.0. Treatment and average weights or lengths among each family were treated as fixed effects, and the age of progeny when sampled, assay type, family and assay x age interaction were treated as random effects. Weight and length were evaluated in separate models because they are known to be correlated. Models were compared using the Akaike Information Criteria (AICc) and the area under the curve (AUC) was used to validate the top model. The coefficients from the top model were used to calculate the proportion of positive families (p) for each assay and treatment combination using a logit link function:

$$p = \text{inv.logit}(\beta_0 + \beta_1 + \beta_2 \ldots \beta_N)$$

Lastly, we used Pearson's correlation test to determine whether the number of bacteria, based on the qPCR standard curve, present in either the adult female or male brood fish tissues influenced the number of positive progeny within an individual family screened by either assay. There were no detections of *R. salmoninarum* in the blood from male brood fish; thus, a correlation test was not completed. All tests were performed in R, and the significance was set at 0.05 (α).

3 | RESULTS

3.1 | Percent of families infected in each treatment

Infections of *R. salmoninarum* in 19 of the 32 families (60%) were detected. There were two of two families positive in the MNFP treatment, one out of four families positive in the MPFN treatment and 16 of 25 families positive in the MPFP treatment. *Renibacterium salmoninarum* positive detections differed by treatment (ANOVA: $F_{3,124} = 4.19, p < .05$) and the MNFP treatment was the only treatment significantly different from the control (MNFP: $p < .05$; MPFN: $p = .98$; MPFP: $p = .74$). Progeny age did not appear to influence infection status ($p = .69$).

The AIC analysis indicated that positive detections were a function of treatment, average length of progeny and assay type (Table 1). The AUC values for all models were >0.72 when evaluating model performance. The top model regression coefficients (Table 2) indicated that the proportion of positive families was lowest in the MPFN treatment. However, positive detections within this treatment indicate that males can contribute to vertical transmission. Model estimates indicated that treatments with positive females (MNFP, MPFP) had the highest proportion of family vertical transmission among infected adult broodstock and MNFP treatment had the greatest effect (Figure 1; Table 2).

TABLE 1 Model selection results based on Akaike's information criterion corrected for small sample sizes (AICc) for factors influencing the proportion of positive detections. Models are ranked based on the AICc difference (ΔAICc) relative to the best model in the set. Akaike weights (Wt) quantify the probability that a particular model is the best model given the data and the model set, and only models with weight are shown. Parameter counts (*K*) for each model are represented. The area under the curve (AUC) was used to validate model performance

Model	AICc	ΔAICc	Wt	AUC	*K*
Treatment + Length + Assay	122.13	0	0.31	0.74	5
Treatment + Length + Family	123.42	1.29	0.16	0.72	5
Treatment + Length + Assay + Family	124.27	2.14	0.11	0.79	6
Treatment + Length + Assay:Age	124.37	2.24	0.10	0.73	6
Treatment + Weight + Assay	125.29	3.16	0.06	0.78	5
Length + Family	125.81	3.68	0.05	0.73	5
Treatment + Weight + Family	126.30	4.17	0.04	0.79	7
Treatment + Length + Assay:Age + Family	126.55	4.42	0.03	0.75	3
Treatment + Assay	127.22	5.09	0.02	0.77	4
Treatment + Assay + Weight + Family	127.37	5.24	0.02	0.83	6
Treatment + Weight + Assay:Age	127.54	5.41	0.02	0.78	6
Treatment + Family	128.50	6.50	0.01	0.79	4
Weight + Family	128.90	6.77	0.01	0.84	5
Treatment + Assay + Family	129.34	7.21	0.01	0.77	5
Treatment + Assay:Age	129.42	7.29	0.01	0.83	7
Treatment + Weight + Assay:Age + Family	129.65	7.52	0.01	0.82	3

TABLE 2 Model regression coefficients, 95% confidence intervals (CI) and standard errors (SE) for fixed effects, and variance, standard deviation (SD) and associated regression coefficients for the random effect assay (ELISA and qPCR) from the top model (Treatment + Length + Assay)

Type	Covariate	Coefficient	95% CI	SE
Fixed effects	Treatment: MPFN	−3.26	−6.45, −1.02	1.29
	Treatment: MPFP	−1.97	−3.67, −0.43	0.80
	Treatment: MNFP	2.33	<0.01, 4.91	1.22
	Length	−0.01	−0.03, < −0.01	0.01
	Variance	SD	Covariate	Coefficient
Random effect	0.20	0.45	ELISA	0.30
			qPCR	−0.28

3.2 | Percent of infected progeny in a family

All 32 families started with 100 fish, but due to natural mortality of fish (<8% across all tanks), the number of fish tested was not equal across all families. The average number of 6- and 12-month post swim-up fish sampled per family was 46 ± 4 fish and 46 ± 3 fish, respectively. The average weights for all 6-month post swim-up fish were 15.4 ± 2.0 g and 76.4 ± 9.4 g for 12-month post swim-up. The average lengths for 6-month post swim-up fish were 118.5 ± 5.6 mm and 191.1 ± 47.0 mm for 12-month post swim-up fish. Length was included in the top model and had a negative effect (though weak; Table 2), suggesting that the smaller the progeny the more likely it was to be infected. Weight was not included in the top model as an explanatory variable.

We observed a low infection prevalence of *R. salmoninarum* in individual progeny in each family. The highest proportion of progeny infected within a single family was 21% by ELISA in the MPFP treatment (Tank 17; Table 3). Eight of the families were noted as having low levels of detectable antigens with ELISA (MNFP: 1 family, MPFN: 1 family, MPFP: 6 families), six with intermediate levels (MNFP: 1 family, MPFP: 5 families) and one with high levels (MPFP). Although few families had intermediate to high OD levels or high number of bacteria in progeny (Table 3), we did not observe any signs of overt disease. The average number of bacteria from qPCR among positive progeny was low, ranging from 1.16 to 259.47 bacterial cells per individual (Table 3).

The number of bacteria in positive female brood fish affected the proportion of positive progeny in a family when kidney (kidney: $t_{15} = 2.13, p < .05$), liver (liver: $t_{15} = 2.09, p < .05$), spleen (spleen: $t_{15} = 2.07, p = .06$) or ovarian fluid ($t_{15} = 2.07, p = .06$) was positive. Positive detections in the adult female mucus or blood did not correlate with the proportion of positive progeny (mucus: $t_{15} = 0.94, p = .36$;

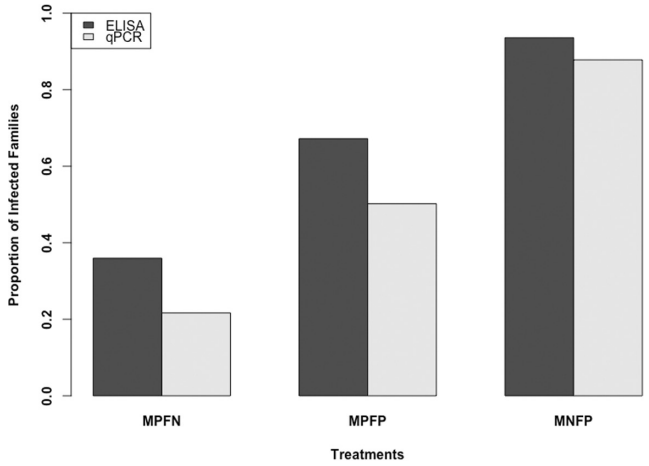

FIGURE 1 Calculated the proportion of families that are infected with *Renibacterium salmoninarum* for each assay and treatment combination by ELISA (dark) or qPCR (light) among the three positive treatments (M: male, F: female, N: negative, P: positive).

blood: $t_{15} = 1.40, p = .18$; Table 4). The number of bacteria in positive male brood fish did not correlate with any infections among progeny (kidney: $t_{14} = -0.49, p = .63$; liver: $t_{14} = -0.62, p = .55$; spleen: $t_{14} = -0.56, p = .58$; mucus: $t_{14} = 1.92, p = .08$; Table 4).

4 | DISCUSSION

A better understanding of the vertical transmission of *R. salmoninarum* is needed to effectively reduce infection among hatchery fish. Vertical transmission of *R. salmoninarum* has been demonstrated mostly among Pacific salmonids (Evelyn et al., 1986a), but there are limited studies that include inland salmonids. Successful vertical transmission has only been reported in inland rainbow trout (*O. mykiss*; Fetherman et al., 2020) and brook trout (*Salvelinus fontinalis*; Allison, 1958). In this study, we evaluated vertical transmission from an inland cutthroat trout broodstock to progeny and whether male or female brood fish contributed to an infection in progeny. The progeny in the 'control' treatment (MNFN) did not test positive for the detection of *R. salmoninarum* by either assay. Thus, we concluded any detectable infection in progeny among other families was a result of vertical transmission from infected brood fish. Our results demonstrated that while vertical transmission rates were high among families, the number of progeny infected with *R. salmoninarum* in an individual family was low. We also found evidence for transmission of *R. salmoninarum* from the male brood fish to progeny, suggesting that vertical transmission does not only occur from female brood fish in inland salmonid populations.

Previous studies regarding the vertical transmission of *R. salmoninarum* have reported low vertical transmission rates to individual progeny (5%–15%; Evelyn, Ketcheson, & Prosperi-Porta, 1984; Evelyn, Prosperi-Porta, & Ketcheson, 1984; Evelyn et al., 1986a). Our study also supports these conclusions, with a low number of individual progeny infected (1%–21%). However, our results also indicate that there are high rates of vertical transmission occurring on a hatchery unit when considering the overall number of families that had infected progeny. This is especially evident in the MNFP treatment where both tanks (100% of families) had successful transmission from the adults to progeny. It is important to note that any level of vertical transmission is a concern for hatcheries rearing *R. salmoninarum*-infected broodstock because the bacteria may also be perpetuated in the population through vertical or horizontal transmission over time.

The adult brood fish were tested for *R.* salmoninarum initially with kidney tissues to determine family treatments. However, after additionally testing liver and spleen tissues, the number of families in each treatment dramatically changed. Initially, we assigned eight control families where *R. salmoninarum* was not detected in either the male or female adult brood fish kidney tissues. After testing the other tissues, seven of the eight control families were re-assigned into positive treatments. Four of the six re-assigned tanks had successful vertical transmission occur, leading to infections in the progeny. Missing the infection among the adults is problematic and indicates we have high false-negative results when testing the kidney tissues.

To limit vertical transmission in Colorado, kidney tissues are tested from the adult brood fish in lethal culling practices and associated eggs are destroyed from the infected spawning pair. If the adults do not test positive for the bacteria, the coinciding eggs are eyed, hatched and transported around the state for stocking efforts. If eggs are not discarded because infected brood fish are not accurately identified, we increase the risk of inadvertently spreading the pathogen. Kidney tissue is the suggested tissue to test for the presence of *R. salmoninarum* (AFS-FHS, 2016) and is therefore why it is used to test hatchery fish in Colorado. Hence, we anticipated low false-negative results using kidney tissues coupled with the specific and sensitive qPCR assay (Elliott et al., 2013). However, results from another study conducted after this one (Riepe, 2022) indicated that there is high uncertainty when testing only kidney tissues with qPCR, which may explain why our initial treatments changed after testing liver and spleen tissues. Our study suggests that we may not want to rely solely on testing adult kidney tissue if the goal is to disrupt the vertical transmission of *R. salmoninarum* by detecting it in the adult brood fish. Therefore, testing other tissues or using multiple tests on the kidney tissue could be considered (Riepe *unpublished data*).

Renibacterium salmoninarum can be transmitted by both vertical and horizontal transmission, but whether vertical transmission occurs from male brood fish is debated (Balfry et al., 1996; Evelyn et al., 1986b; Klontz, 1983). Daly and Stevenson (1989) noted the presence of *R. salmoninarum* on the tail region of the spermatozoa but never the head region. Therefore, they suggest the bacteria may not enter the micropyle of the egg during fertilization because the tail, and attached bacteria, is lost upon contact with the egg. In our study, one family from a positive male brood fish and negative female brood fish resulted in an *R. salmoninarum* positive tank by ELISA. Our model estimates suggest positive male brood fish may account

TABLE 3 Detection of *Renibacterium salmoninarum* among progeny (%; number in parentheses) in each treatment (M: male, F: female, N: negative, P: positive) and family by ELISA and qPCR. Assay measurements include optical density values (OD) for ELISA (ELISA level classifications: low (OD: 0.100–0.199), intermediate (OD: 0.200–0.999) and high (OD: > 1.000)) and the number of bacteria (bacteria/mL^{-1}) in positive fish from qPCR

Treatment	Family	Assay	Positive progeny	Assay measurement	ELISA level
MNFN	Tank 7	ELISA	0	–	–
		qPCR	0	–	–
MNFP	Tank 27	ELISA	9.4% (8)	0.32 ± 0.03	Intermediate
		qPCR	8.2% (7)	178.41 ± 443.57	–
	Tank 28	ELISA	1.1% (1)	0.10 ± 0.01	Low
		qPCR	0	–	–
	Tank 8	ELISA	0	–	–
		qPCR	0	–	–
MPFN	Tank 21	ELISA	0	–	–
		qPCR	0	–	–
	Tank 22	ELISA	0	–	–
		qPCR	0	–	–
	Tank 24	ELISA	1.1% (1)	0.11 ± 0.01	Low
		qPCR	0	0	–
MPFP	Tank 1	ELISA	1.2% (1)	0.11 ± 0.01	Low
		qPCR	0	–	–
	Tank 2	ELISA	15.1% (13)	0.30 ± 0.02	Intermediate
		qPCR	1.2% (1)	44.80	–
	Tank 3	ELISA	0	–	–
		qPCR	0	–	–
	Tank 4	ELISA	0	–	–
		qPCR	1.1% (1)	259.47	–
	Tank 5	ELISA	0	–	–
		qPCR	0	–	–
	Tank 6	ELISA	1.1% (1)	3.07 ± 4.24	High
		qPCR	0	–	–
	Tank 9	ELISA	0	–	–
		qPCR	2.2% (2)	1.61 ± 0.69	–
	Tank 10	ELISA	2.2% (2)	0.29 ± 31	Intermediate
		qPCR	0	–	–
	Tank 11	ELISA	0	–	–
		qPCR	0	–	–
	Tank 12	ELISA	0	–	–
		qPCR	0	–	–
	Tank 13	ELISA	0	–	–
		qPCR	0	–	–
	Tank 14	ELISA	0	–	–
		qPCR	0	–	–
	Tank 15	ELISA	2.2% (2)	0.12 ± 0.06	Low
		qPCR	0	–	–
	Tank 16	ELISA	0	–	–
		qPCR	1.0% (1)	2.07	–
	Tank 17	ELISA	21.1% (20)	0.31 ± 0.03	Intermediate
		qPCR	3.2% (3)	3.36 ± 2.14	–

(Continues)

TABLE 3 (Continued)

Treatment	Family	Assay	Positive progeny	Assay measurement	ELISA level
	Tank 18	ELISA	0	–	–
		qPCR	1.1% (1)	3.12	–
	Tank 19	ELISA	4.3% (4)	0.13 ± 0.01	Low
		qPCR	0	–	–
	Tank 20	ELISA	0	–	–
		qPCR	0	–	–
	Tank 23	ELISA	3.1% (3)	0.22 ± 0.18	Intermediate
		qPCR	0	–	–
	Tank 25	ELISA	1.1% (1)	0.11 ± 0.05	Low
		qPCR	0	–	–
	Tank 26	ELISA	1.1% (1)	0.39 ± 0.45	Intermediate
		qPCR	0	–	–
	Tank 29	ELISA	0	–	–
		qPCR	0	–	–
	Tank 30	ELISA	0	–	–
		qPCR	0	–	–
	Tank 31	ELISA	1.0% (1)	0.18 ± 0.16	Low
		qPCR	0	–	–
	Tank 32	ELISA	2.3% (2)	0.11 ± 0.01	Low
		qPCR	0	–	–

for 21–36% of transmission from spawning pairs in an infected broodstock. The ELISA optical density of the fish tissue sample was 0.10, suggesting a low-level infection (Faisal & Eissa, 2009; Meyers et al., 1993; Pascho et al., 1998). This infection may indicate an initial recovery stage of the fish (Faisal & Eissa, 2009). Interestingly, we did not determine any correlation between the number of bacteria detected in the male brood fish and the number of progeny infected that were spawned from infected males. Nevertheless, our finding supports the supposition that male brood fish can contribute to vertical transmission. Thus, it may still be important to test the milt or internal tissues of the male brood fish where infections are prevalent in the population or a hatchery to reduce any chance of vertical transmission.

Renibacterium salmoninarum has been known to be localized in female reproductive tissues leading to successful transmission and a high prevalence of infection among eggs or progeny (Brown et al., 1994). Other studies have also observed high numbers of *R. salmoninarum* in progeny when ovarian fluid or other tissues were infected with high numbers of bacteria in the adult brood fish (Evelyn et al., 1986a). Similarly, in our study maternal infection intensity in internal tissues (liver, kidney, spleen) and ovarian fluid influenced the number of positive progeny in a family. Although the number of bacteria in the kidney tissue influenced the number of positive progeny, we have previously found kidney tissues tested by qPCR can lead to high false-negative results (Riepe, 2022). Thus, testing a combination of kidney, liver and ovarian fluid will increase the detection probabilities of *R. salmoninarum* among brood fish. It also appears that even when bacterial numbers in adult fish are low and ovarian fluid is negative, an infection can still be transmitted to the progeny, as evidenced by most of the MPFP families. This must be taken into consideration when using qPCR results to select eggs free of infection as ovarian fluid may not be a good measure of vertical transmission risk when infection intensity is low.

The positive detections from ELISA and qPCR did not always agree when a fish was determined positive by one of the assays. The double-sandwich antibody ELISA method detects the soluble antigen fractions of *R. salmoninarum* in the tissue samples and cannot distinguish between a current or previous infection, whereas qPCR detects the genomic DNA in the sample, thus a current infection or presence of live bacteria (Elliott et al., 2013; Faisal & Eissa, 2009; Pascho et al., 1998). Generally, the detection of *R. salmoninarum* was highest among progeny when using the ELISA assay, with few fish that were also found to be positive by qPCR. There were also few instances where detections of the bacteria were only found by qPCR and not by ELISA. Faisal and Eissa (2009) describe similar infection patterns from naturally infected salmonid species and suggest the disagreement between the assays may reflect different stages of infection.

An initial stage of infection often results in low levels of bacteria within the fish tissues and is likely only detectable by qPCR (Faisal & Eissa, 2009). Four families in our study were positive by only qPCR, indicating a low infection level as an infection was initially progressing. The next stage of infection may represent an infection that has progressed in the fish, resulting in an increased number of bacteria, and therefore, detection by both ELISA and qPCR can occur. Two of the families resulted in the high number of fish positive by ELISA and a couple of those fish were also positive by qPCR. Although this

TABLE 4 Bacterial counts (bacterial cells/mL^{-1}) from positive adult brood fish tissues and serums used in family treatment assignments (M: male, F: female, N: negative, P: positive). Bacteria numbers were estimated by a qPCR standard curve. Proportion of total progeny infected is also included. Data are only shown for the positive families in each treatment

Treatment	Family	Adult sex	Mucus	Blood	Ovarian fluid	Spleen	Liver	Kidney	Progeny
MNFP	Tank 27	Male	0	0	–	0	0	0	0.13
		Female	0	877.87	0	43.96	562.88	893.94	
	Tank 28	Male	0	0	–	0	0	0	0.01
		Female	0	0	1.38	20.89	169.28	193.03	
MPFN	Tank 24	Male	0	0	–	38.08	65.31	1444.24	0.01
		Female	0	0	0	0	0	0	
MPFP	Tank 1	Male	0	0	–	2.28	4.81	0	0.01
		Female	1.66	0	1.92	1.79	10.99	0	
	Tank 2	Male	20.17	0	–	0	6.86	1.20	0.15
		Female	11.30	0	50610.80	80618.45	55947.73	26638.76	
	Tank 4	Male	0	0	–	14.12	1.36	1.49	0.01
		Female	1.56	0	0	0	2.02	1.80	
	Tank 6	Male	3.28	0	–	0	2.76	3.03	0.01
		Female	1.73	0	0	5.60	2.14	0	
	Tank 9	Male	0	0	–	0	4.30	7.83	0.02
		Female	0	0	0	0	3.05	37.59	
	Tank 10	Male	1.67	0	–	3.01	6.46	4.47	0.01
		Female	1.91	0	1.75	0	5.28	4.05	
	Tank 15	Male	1.16	0	–	0	1.31	40.77	0.01
		Female	0	0	0	0	1.34	19.01	
	Tank 16	Male	3.03	0	–	0	1.14	1.40	0.01
		Female	11.60	0	0	0	2.79	0	
	Tank 17	Male	0	0	–	0	0	2.28	0.21
		Female	0	0	0	0	2.73	8.84	
	Tank 18	Male	0	0	–	0	1.55	0	0.01
		Female	0	0	1.38	20.89	169.28	193.03	
	Tank 19	Male	0	0	–	0	1.91	2.96	0.04
		Female	0	0	3.58	1.62	2.06	6.64	
	Tank 23	Male	0	0	–	116.53	13.55	11.74	0.03
		Female	0	0	2.63	26.03	11.36	11.34	
	Tank 26	Male	0	0	–	0	1.81	16.97	0.01
		Female	2.64	0	0	0	1.97	24.85	
	Tank 31	Male	2.35	0	–	118.92	127.60	82.82	0.01
		Female	4.21	0	0	107.83	20.57	27.73	
	Tank 32	Male	2.55	0	–	0	0	9.77	0.02
		Female	6.44	0	0	0	0	1.86	

type of infection pattern is suggestive of active infection (Faisal & Eissa, 2009), the lack of positive qPCR results may indicate an initial stage of recovery where the bacteria is rarely present in fish, but the detectable soluble antigen by ELISA remains in the tissues. When detections of *R. salmoninarum* only occur with ELISA, Faisal and Eissa (2009) suggest that this is indicative of an advanced stage of recovery in which we are detecting small traces of the bacteria or antigen that remain in the tissues. Across all positive detections, more progeny was positive by ELISA, and OD values were categorized as low to intermediate, which may indicate that most of the fish were already in that late stage of recovery. Lastly, the progeny in families that resulted in no infections may have been refractory from infection, and therefore, vertical transmission was unsuccessful or clearance of the infection occurred prior to sampling, and qPCR or ELISA did not detect the bacteria or antigen. To determine what is occurring, an experiment understanding the susceptibility of

cutthroat trout and how the fish respond to an infection from vertical transmission may be helpful to carry out in the future.

Detection of *R. salmoninarum* in progeny is often difficult because there may be a lack of space or resources to rear the fish until they are able to be tested. Therefore, strategies to limit transmission have relied heavily on the development of vaccines or chemotherapy injections, depopulation or culling of brood fish (Evelyn et al., 1986b; Fetherman et al., 2020; Riepe, 2022). Lethal culling has been used in Colorado to limit transmission by testing kidney tissues from adult broodstock. Our results suggest that infections among adult fish may be missed when only testing the kidney tissue, thus not successfully preventing all vertical transmission. Therefore, we suggest testing a combination of kidney, liver and ovarian fluid to assess whether the brood fish may transmit the bacteria to progeny. In addition, other studies have shown that testing a subsample of eggs for *R. salmoninarum* with a specific, sensitive and reliable method, such as qPCR or ELISA, may allow for increased detection without the high-volume loss of broodstock from lethal culling. Testing DNA extracted from eggs with qPCR has been shown to detect as few as two bacterial cells and therefore may be a feasible alternative in future *R. salmoninarum* management in hatcheries (Brown et al., 1994; Gudmundsdóttir et al., 2000). Continued testing of males either by lethal methods or testing milt to further limit transmission remains a viable strategy. The potential for vertical transmission from males, especially in inland salmonid populations, requires further study.

ACKNOWLEDGEMENTS

We would like to thank the Colorado Parks and Wildlife (CPW) Poudre Rearing Unit personnel, M. Hersh, J. Ingram, T. Meyer and N. Yates, for the large part they had in the adult fish tissue collection and fertilization of eggs. We also gratefully thank C. Altweis, B. Avila, C. Baum, J. Brandt, A. Chudkoo, A. Feuka, C. Garvey, K. Hall, R. Kanaziz, C. Lee, V. Milano, G. Moreno, K. Navarre, N. Salinas, G. Schisler and I. Thibedeau for adult and/or progeny fish tissue collection. We thank C. Webb, K. Huyvaert and J. Drennan for study design discussions and reviewing this manuscript. We would also like to thank D. Preston for reviewing this manuscript prior to submission.

FUNDING INFORMATION

This work was supported through Colorado Parks and Wildlife, Species Conservation Trust Fund Grant SCA20A.

CONFLICT OF INTEREST

Any use of trade, firm or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

DATA AVAILABILITY STATEMENT

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

INSTITUTIONAL REVIEW BOARD STATEMENT

The animal study protocol was approved by the Institutional Animal Care and Use Committee Review Board of Colorado State University (protocol number 721).

ORCID

Tawni B. Riepe https://orcid.org/0000-0002-0022-3568
Eric R. Fetherman https://orcid.org/0000-0003-4792-7148
Dana L. Winkelman https://orcid.org/0000-0002-5247-0114

REFERENCES

AFS-FHS (American Fisheries Society-Fish Health Section). (2016). *FHS Blue Book: Suggested procedures for the detection and identification of certain finfish and shellfish pathogens, 2020th ed*. Available: https://units.fisheries.org/fhs/fish-health-section-blue-book-2020/ (February 2021)

Allison, L. N. (1958). Multiple sulfa therapy of kidney disease among brook trout. *The Progressive Fish-Culturist*, *20*(2), 66–68.

Austin, B., Austin, D. A., & Munn, C. (2007). *Bacterial fish pathogens: Disease of farmed and wild fish* (Vol. *26*). Springer.

Balfry, S. K., Albright, L. J., & Evelyn, T. P. T. (1996). Horizontal transfer of *Renibacterium salmoninarum* among farmed salmonids via the fecal-oral route. *Diseases of Aquatic Organisms*, *25*, 63–69.

Barnes, M. E., & Brown, M. L. (2011). A review of *Flavobacterium psychrophilum* biology, clinical signs, and bacterial cold water disease prevention and treatment. *Open Fish Science Journal*, *4*, 40–48.

Behringer, D. C., Silliman, B. R., & Lafferty, K. D. (2020). *Marine disease ecology*. Oxford University Press.

Brown, L., Iwama, G., Evelyn, T., Nelson, W., & Levine, R. (1994). Use of the polymerase chain reaction (PCR) to detect DNA from *Renibacterium salmoninarum* within individual salmonid eggs. *Diseases of Aquatic Organisms*, *18*(3), 165–171.

Chase, D. M., Elliott, D. G., & Pascho, R. J. (2006). Detection and quantification *of Renibacterium salmoninarum* DNA in salmonid tissues by real-time quantitative polymerase chain reaction analysis. *Journal of Veterinary Diagnostic Investigation*, *18*(4), 375–380.

Daly, J. G., & Stevenson, R. M. (1989). Communications: Agglutination of salmonid spermatozoa by *Renibacterium salmoninarum*. *Journal of Aquatic Animal Health*, *1*(2), 163–164.

Elliott, D., Applegate, L., Murray, A., Purcell, M., & McKibben, C. (2013). Bench-top validation testing of selected immunological and molecular *Renibacterium salmoninarum* diagnostic assays by comparison with quantitative bacteriological culture. *Journal of Fish Diseases*, *36*(9), 779–809.

Evelyn, T. P. T., Ketcheson, J., & Prosperi-Porta, L. (1984). Further evidence for the presence of *Renibacterium salmoninarum* in salmonid eggs and for the failure of povidone-iodine to reduce the intra-ovum infection rate in water-hardened eggs. *Journal of Fish Diseases*, *7*(3), 173–182.

Evelyn, T. P. T., Prosperi-Porta, L., & Ketcheson, J. (1984). *The salmonid egg as a vector of the kidney disease bacterium, Renibacterium salmoninarum. Fish Diseases, Fourth COPRAQ Session, 111–117.*

Evelyn, T. P. T., Prosperi-Porta, L., & Ketcheson, J. (1986a). Experimental intra-ovum infection of salmonid eggs with *Renibacterium salmoninarum* and vertical transmission of the pathogen with such eggs despite their treatment with erythromycin. *Diseases of Aquatic Organisms*, *1*(3), 197–202.

Evelyn, T. P. T., Prosperi-Porta, L., & Ketcheson, J. (1986b). Persistence of the kidney-disease bacterium, *Renibacterium salmoninarum*, in coho salmon, *Oncorhynchus kisutch* (Walbaum), eggs treated during and after water-hardening with povidone-iodine. *Journal of Fish Diseases*, *9*(5), 461–464.

Evenden, A., Grayson, T., Gilpin, M., & Munn, C. (1993). *Renibacterium salmoninarum* and bacterial kidney disease—The unfinished jigsaw. *Annual Review of Fish Diseases*, *3*, 87–104.

Faisal, M., & Eissa, A. (2009). Diagnostic testing patterns of *Renibacterium salmoninarum* in spawning salmonid stocks in Michigan. *Journal of Wildlife Diseases*, *45*(2), 447–456.

Fetherman, E. R., Neuschwanger, B., Davis, T., Wells, C. L., & Kraft, A. (2020). Efficacy of erymicin 200 injections for reducing

Renibacterium salmoninarum and controlling vertical transmission in an inland rainbow trout brood stock. *Pathogens*, *9*(7), 547.

Gudmundsdóttir, H., Jónsdóttir, H., & Benediktsdóttir, E. (2000). Measures applied to control *Renibacterium salmoninarum* infection in Atlantic salmon: A retrospective study of two sea ranches in Iceland. *Aquaculture*, *186*(3–4), 193–203.

Hamel, O. S. (2005). Immunosuppression in progeny of Chinook salmon infected with *Renibacterium salmoninarum*: Re-analysis of a brood stock segregation experiment. *Diseases of Aquatic Organisms*, *65*(1), 29–41.

Harig, A. L., Fausch, K. D., & Young, M. K. (2000). Factors influencing success of greenback cutthroat trout translocations. *North American Journal of Fisheries Management*, *20*(4), 994–1004.

Harvel, C. D., Kim, K., Burkholder, J. M., Colwell, R. R., Epstein, P. R., Grimes, D. J., Hofmann, E. E., Lipp, E. K., Osterhaus, A. D. M. E., Overstreet, R. M., Porter, J. W., Smith, G. W., & Vasta, G. (1999). Emerging marine diseases–climate links and anthropogenic factors. *Science*, *285*(5433), 1505–1510.

Klontz, G. (1983). Bacterial kidney disease in salmonids: An overview. *Antigens of Fish Pathogens: Development and Production for Vaccines and Serodiagnostics Collection Fondation Marcel Merieux*, *Lyons*, *France*, *3*, 177–200.

Kowalski, D. A., Cordes, R. J., Riepe, T. B., Drennan, J. D., & Treble, A. J. (2022). Prevalence and distribution of *Renibacterium salmoninarum*, causative agent of bacterial kidney disease, in wild trout fisheries in Colorado. *Diseases of Aquatic Organisms*, *149*, 109–120.

Kumagai, A., Yamaoka, S., Takahashi, K., Fukuda, H., & Wakabayashi, H. (2000). Waterborne transmission of *Flavobacterium psychrophilum* in coho salmon eggs. *Fish Pathology*, *35*(1), 25–28.

Larenas, J., Bartholomew, J., Troncoso, O., Fernández, S., Ledezma, H., Sandoval, N., Vera, P., Contreras, J., & Smith, P. (2003). Experimental vertical transmission of *Piscirickettsia salmonis* and in vitro study of attachment and mode of entrance into the fish ovum. *Diseases of Aquatic Organisms*, *56*(1), 25–30.

Meyers, T., Short, S., Farrington, C., Lipson, K., Geiger, H., & Gates, R. (1993). Establishment of a negative-positive threshold optical density value for the enzyme-linked immunosorbent assay (ELISA) to detect soluble antigen of *Renibacterium salmoninarum* in Alaskan Pacific salmon. *Diseases of Aquatic Organisms*, *16*(3), 191–197.

Munson, A. D., Elliott, D. G., & Johnson, K. (2010). Management of bacterial kidney disease in Chinook salmon hatcheries based on broodstock testing by enzyme-linked immunosorbent assay: A multiyear study. *North American Journal of Fisheries Management*, *30*(4), 940–955.

Nilsen, H., Olsen, A., Vaagnes, Ø., Hellberg, H., Bottolfsen, K., Skjelstad, H., & Colquhoun, D. (2011). Systemic *Flavobacterium psychrophilum* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum), farmed in fresh and brackish water in Norway. *Journal of Fish Diseases*, *34*(5), 403–408.

Pascho, R., & Mulcahy, D. (1987). Enzyme-linked immunosorbent assay for a soluble antigen of *Renibacterium salmoninarum*, the causative agent of salmonid bacterial kidney disease. *Canadian Journal of Fisheries and Aquatic Sciences*, *44*(1), 183–191.

Pascho, R. J., Chase, D., & McKibben, C. L. (1998). Comparison of the membrane-filtration fluorescent antibody test, the enzyme-linked immunosorbent assay, and the polymerase chain reaction to detect *Renibacterium salmoninarum* in salmonid ovarian fluid. *Journal of Veterinary Diagnostic Investigation*, *10*(1), 60–66.

Pascho, R. J., Elliott, D. G., & Streufert, J. M. (1991). Brood stock segregation of spring Chinook salmon *Oncorhynchus tshawytscha* by use of the enzyme-linked immunosorbent assay (ELISA) and the fluorescent antibody technique (FAT) affects the prevalence and levels of *Renibacterium salmoninarum*. *Diseases of Aquatic Organisms*, *12*(1), 25–40.

Pękala-Safińska, A. (2018). Contemporary threats of bacterial infections in freshwater fish. *Journal of Veterinary Research*, *62*(3), 261–267.

Potts, W., & Rudy, P., Jr. (1969). Water balance in the eggs of the Atlantic salmon *Salmo salar*. *Journal of Experimental Biology*, *50*(1), 223–237.

Riepe, T. B. (2022). *Detection and transmission of Renibacterium salmoninarum in Colorado inland trout* [PhD Dissertation]. Colorado State University.

Riepe, T. B., Vincent, V., Milano, V., Fetherman, E. R., & Winkelman, D. L. (2021). Evidence for the use of mucus swabs to detect *Renibacterium salmoninarum* in brook trout. *Pathogens*, *10*(4), 460.

Sandell, T. A., & Jacobsen, K. C. (2011). Comparison and evaluation of *Renibacterium salmoninarum* quantitative PCR diagnostic assays using field samples of Chinook and coho salmon. *Diseases of Aquatic Organisms*, *93*(2), 129–139.

Shinn, A., Pratoomyot, J., Bron, J., Paladini, G., Brooker, E., & Brooker, A. (2015). Economic impacts of aquatic parasites on global finfish production. *Global Aquaculture Advocate*, *2015*, 58–61.

Sudheesh, P. S., Al-Ghabshi, A., Al-Mazrooei, N., & Al-Habsi, S. (2012). Comparative pathogenomics of bacteria causing infectious diseases in fish. *International Journal of Evolutionary Biology*, *2012*. https://doi.org/10.1155/2012/457264

Wiens, G. D. (2011). Bacterial kidney disease (*Renibacterium salmoninarum*). *Fish Diseases and Disorders*, *3*, 338–374.

How to cite this article: Riepe, T. B., Fetherman, E. R., Neuschwanger, B., Davis, T., Perkins, A., & Winkelman, D. L. (2023). Vertical transmission of *Renibacterium salmoninarum* in cutthroat trout (*Oncorhynchus clarkii*). *Journal of Fish Diseases*, *00*, 1–11. https://doi.org/10.1111/jfd.13745