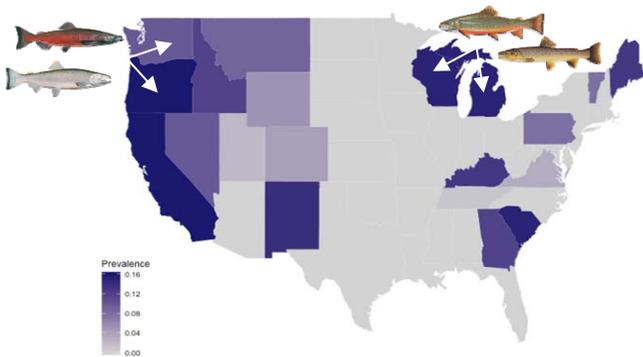


Renibacterium salmoninarum Transmission Research



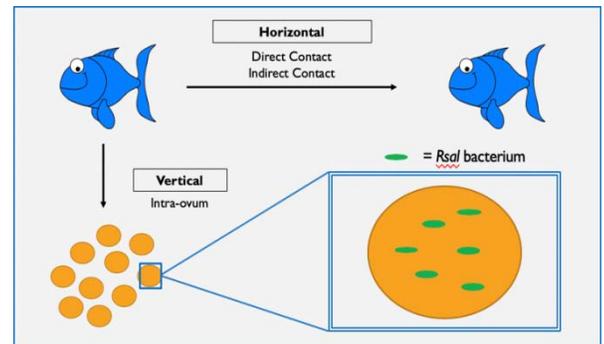
TRANSMISSION OF *RENIBACTERIUM SALMONINARUM* (BACTERIAL KIDNEY DISEASE) IN HATCHERY-REARED FISH



Prevalence of *Renibacterium salmoninarum* in the US. Highest detections occur in the Pacific Northwest and Midwest.

Renibacterium salmoninarum, the bacterium that causes bacterial kidney disease (BKD), was first described in Scotland in 1930, and first detected in the US in 1934. BKD is now found in trout and salmon throughout the US and worldwide, and can cause significant mortality in infected populations. In the US, the bacterium is most commonly detected in Coho and Chinook Salmon in the Pacific Northwest, and Brook Trout and Brown Trout in the Midwest. The transfer of infected eggs and the practice of pasteurizing fish by-products for fish feed (now discontinued) are suspected to have contributed to the worldwide spread of the pathogen.

R. salmoninarum can be transmitted in two ways, presenting challenges for prevention and management. Horizontal transmission of the bacteria is not completely understood. However, fish may become infected through direct contact with another infected fish, ingestion of contaminated fecal matter, or contact with a contaminated water source from which the bacteria enters through the gills, eyes, gut, and external injuries of the fish. Vertical transmission occurs during the spawn. Bacteria in the female find their way into the eggs during egg development. There is no method for safely disinfecting the inside of the eggs, and using iodine to disinfect the egg surface during hatchery spawns is not effective for preventing vertical transmission.

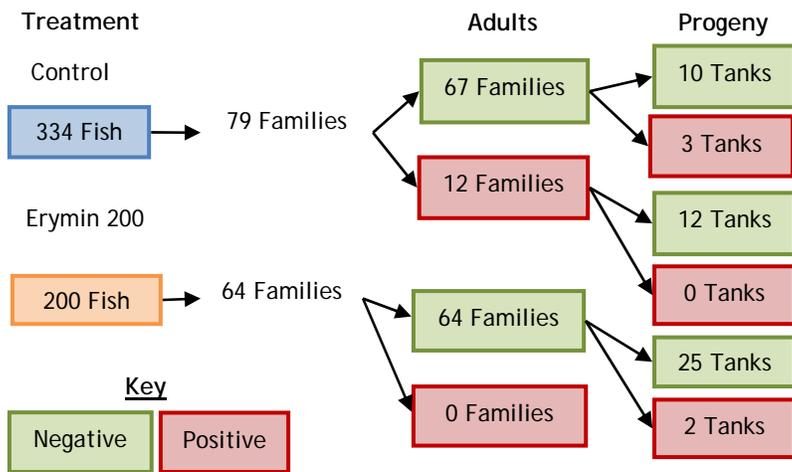


Horizontal and vertical transmission of *R. salmoninarum*.

R. salmoninarum is a regulated pathogen in Colorado. Current regulations prevent the transfer or stocking of infected fish. While infrequently found in Colorado since the 1960's, it has recently been detected in six state and federal hatcheries. In 2015, *R. salmoninarum* was detected in the CPW Glenwood Springs Hatchery. To eradicate the pathogen and prevent stocking infected fish, the hatchery was temporarily depopulated. *R. salmoninarum* was also detected in other CPW hatcheries rearing valuable brood stocks of whirling disease-resistant Rainbow Trout and Native Greenback Cutthroat Trout, and depopulation of these hatcheries was not an option. Recent management options have focused on methods to prevent vertical transmission during fish spawning, although other approaches are also being examined.

Management Options for Controlling Bacteria Transmission

Lethal spawning has been evaluated in Rainbow Trout and Cutthroat Trout brood stocks at the CPW Poudre Rearing Unit (PRU) and Fish Research Hatchery (FRH). Eggs are fertilized and collected as normal from brood fish during the spawn. After being spawned, adult fish are euthanized and tested for *R. salmoninarum*. Eggs from positive parents are then culled so that only eggs from negative parents are retained on the unit for stocking and management purposes. This technique has shown promise in preventing vertical transmission. However, more research was needed to understand the transmission rates of *R. salmoninarum*, develop management and regulatory protocols to decrease the prevalence of the pathogen within hatchery brood stocks, and limit the dissemination of positive progeny to other waters and hatcheries in Colorado.



Antibiotics administered orally or by injection have been shown to reduce bacterial concentrations in fish. Erythromycin (Erymin 200), an antibiotic approved for use in fish, was injected into Rainbow Trout at the CPW FRH to determine if its use could reduce *R. salmoninarum* levels in spawning fish and control vertical transmission to progeny. Male and female brood fish were injected with the antibiotic three times prior to spawning. The injections reduced bacteria levels in the injected fish to below detectable levels. However, progeny that tested positive for *R. salmoninarum* were produced by both treated

and control fish that had tested negative for the bacteria. Additionally, positive control adults produced negative progeny. Results also indicated that the male may play a role in vertical transmission in inland trout, a significant finding as only females were previously known to contribute to vertical transmission of the bacteria. This study revealed several unknowns regarding pathogen transmission that needed to be answered for effective management in Cutthroat Trout. CPW and Colorado State University are currently conducting two studies to better understand transmission rates in Native Greenback Cutthroat Trout.

Transmission Studies in Native Greenback Cutthroat Trout

Horizontal Transmission

Horizontal transmission of *R. salmoninarum* within a hatchery may pose a risk to fish throughout the unit, as well as feral fish in the river below a unit if the bacteria are present in the hatchery effluent. In summer 2019, two sentinel cages were deployed in six locations at PRU and at three time periods encompassing a range of optimal temperatures for bacterial growth (10-18°C). Each cage housed 10 Cutthroat Trout. After 30 days, fish were collected and tested for *R. salmoninarum*. Only one fish out of a total of 360 tested positive. The cage containing the positive fish had been deployed near highly-positive Rainbow Trout. These results suggest the rate of horizontal transmission to fish both on, and in the river below a positive unit may be insignificant, even when conditions are optimal for the bacteria to persist.

Vertical Transmission

Eggs from unique male-female pairs of Cutthroat Trout were collected during the spawn at PRU in May 2019. Adult fish were tested for *R. salmoninarum* after being spawned to determine the potential infection status of the progeny created from these spawns. Thirty-two tanks of progeny are being reared at the FRH: one containing progeny from a male negative × female negative pair, six from a male positive × female negative pair, four from a male negative × female positive pair, and 21 from a male positive × female positive pair. Progeny from each tank will be tested for *R. salmoninarum* and associated antibodies, indicating that the progeny had mounted an immune response to the bacteria at some point prior to the testing, at six months and one year of age.



Progeny collected from positive adults

The results will be used to understand vertical transmission rates, male and female contributions to vertical transmission, and the bacterial load at which transmission occurs from parent to offspring. Overall, the results from the transmission studies will be used to inform future management options. Results from these transmission studies will be available in 2021.

Associated Literature

- Fetherman, E. R., G. J. Schisler, and B. W. Avila. 2018. Sport Fish Research Studies. Federal Aid Project F-394-R18. Federal Aid in Fish and Wildlife Restoration, Job Progress Report. Colorado Parks and Wildlife, Aquatic Wildlife Research Section. Fort Collins, Colorado.
- Fetherman, E. R., B. Neuschwanger, T. Davis, C. L. Wells, and A. Kraft. *In review*. Use of Erymin 200 injections for reducing vertical transmission of *Renibacterium salmoninarum* in a Rainbow Trout brood stock. Diseases of Aquatic Organisms.