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ARTICLE

Comparison of the Susceptibility of Four Rainbow Trout Strains to Cold-Water Disease

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Abstract

Susceptibility to cold-water disease was compared among four strains of Rainbow Trout *Oncorhynchus mykiss*: Arlee strain from Ennis National Fish Hatchery, Montana (AL-EN), the Arlee strain from Jocko River Hatchery, Montana (AL-JR), a cold-water disease-resistant strain (WV), and the Harrison–Hofer strain (HH). Bacterial challenges were either by bath or intraperitoneal injection (50 μL of 0.65 optical density). Each strain was exposed at 75 d after hatch to either the CSF 259-93 (Idaho) or 09–104 isolate (Utah) of *Flavobacterium psychrophilum*. Injection controls received a phosphate-buffered saline (PBS) solution and bath controls were exposed to uninoculated sterile broth (tryptone yeast extract salts) mixed 1:1 with hatchery well water. For injected fish, the WV had significantly lower mortality (20.0–36.7%) than HH and AL-EN (76.7–96.7%) but did not significantly differ from AL-JR (46.7–56.7%). Injected fish had significantly higher mortality than bath-exposed fish. For bath-exposed fish, the WV had significantly lower mortality (0%) than the HH (10.0–26.7%), but both Arlee strains had intermediate mortality values (0–13.3%) that did not significantly differ from either the HH or WV strain. There were no significant differences between the two bacterial isolates, indicating similar virulence and similar resistance response of WV to another novel isolate of *F. psychrophilum*.

Cold-water disease, caused by the bacterium *Flavobacterium psychrophilum*, can induce necrotic lesions, partial dark skin coloring (black-tail), exophthalmia, anemia, ascites, and vertebral deformities (Faruk 2002; Nematollahi et al. 2003; Aoki et al. 2005) in salmonids. The bacterium can also induce necrosis of renal tubular epithelium and haematopoietic tissue (Ekman and Norrgren 2003). Cold-water disease has been a significant cause of mortality in a wide variety of cultured fishes worldwide (Cipriano and Holt 2005). In the United Kingdom, annual losses to the disease are estimated to be about 10 million fish (Faruk 2002). In Utah, we estimated 1.3 million hatchery fish have been lost to the disease in the last decade. Ekman (2003) noted that 50–60% of the antibiotics used in Swedish aquaculture are used for treating *F. psychrophilum* or the closely related *F. columnare*.

One of the recent efforts to control the disease has been the advent of a disease-resistant strain of Rainbow Trout *Oncorhynchus mykiss* developed by the team at the National Center for Cool and Cold Water Aquaculture in West Virginia (Silverstein et al. 2009; Leeds et al. 2010). This strain is the result of three generations of selection for resistance to cold-water disease (Leeds et al. 2010). The strain composition of this stock, based the pedigree of the four founder strains, is 16.4% House Creek (College of Southern Idaho), 7.4% Shasta (Ennis National Fish Hatchery), 16.1% Donaldson (University of Washington), and 60.1% Kamloops/Puget Sound Steelhead cross (Trout Lodge).

There are few controlled studies that compare the susceptibility of different fish species or strains to cold-water disease, also known as Rainbow Trout Fry Syndrome. Nagai et al. (2004) compared susceptibility among three stocks of Ayu *Plecoglossus altivelis*; the amphidromous stock had lower mortality than a landlocked or domesticated stock. In a study by Holt et al. (1989) evaluating temperature effects on fish mortality from *F. psychrophilum* injections, presmolts of Chinook Salmon *O. tshawytscha* and Rainbow Trout had similar mortality rates;

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However, the salmon were twice as heavy as the trout, and exposures were not given at a standard age. Nematollahi et al. (2003) suggested that salmonids are the most susceptible, and in nonsalmonids the disease is less severe. However, Nagai et al. (2004) reported that cold-water disease is one of the most serious diseases of Ayu in Japan. Also, Lehmann et al. (1991) observed 40–50% mortality among Common Carp *Cyprinus carpio*, Crucian Carp *Carassius carassius*, and Tench *Tinca tinca* that had *F. psychrophilum* in their spleens and livers. This is similar to the mortality rates reported for Coho Salmon *Oncorhynchus kisutch* (Holt 1987). Rainbow Trout mortality rates around the world have ranged from 20% to 90% (Santos et al. 1992; Madsen et al. 2005; Barnes and Brown 2011).

In an effort to control cold-water disease, the Utah Division of Wildlife's (UDWR) fish hatchery system has imported the Rainbow Trout strain developed by Leeds et al. (2010), hereafter referred to as the 'West Virginia' strain (WV). The UDWR has also recently imported other strains of unknown susceptibility to cold-water disease, including the Arlee strain from Ennis Hatchery, Montana (AL-EN), the Arlee strain from Jocko River Hatchery, Montana (AL-JR), and the Harrison–Hofer strain (HH) obtained from the Colorado Division of Wildlife Resources. The HH strain is a cross between fish from a wild population in Harrison Lake, Montan, and the domesticated Hofer strain originating from Germany (Wagner et al. 2012). The source strains and the cross have demonstrated greater resistance to whirling-disease than other Rainbow Trout strains (Hedrick et al. 2003; Wagner et al. 2006; Wagner et al. 2012). However, this strain and the Arlee strains of Rainbow Trout have not been tested for susceptibility to cold-water disease. The objective of our study was to compare the susceptibility of these strains with the resistant WV strain. We evaluated the resistance using two different isolates of *Flavobacterium psychrophilum* and used both injection and bath exposures.

METHODS

To make our comparisons consistent with other studies, we used the same challenge methodology as Leeds et al. (2010). For the cold-water disease challenges, fish were acclimated to aquaria 1 week prior to exposure. Aquaria (110 L) were each stocked with 20 fish and three replicate tanks were used per treatment. Treatments were bacteria isolate (CSF 259-93, 09-104, or a phosphate-buffered saline injected control) and fish strain (WV, AL-JR, AL-EN, HH). Fish were exposed at 75 d after hatching to *Flavobacterium psychrophilum* either by intraperitoneal injection or bath.

For injections, 50 μL of a 0.65 optical density (OD; 525 nm) mixture in sterile phosphate-buffered saline (PBS) was injected into 10 fish per tank using a tuberculin syringe. The bacteria used for the mixture were first grown in Petri plates with tryptone yeast extract salt (TYES; Holt et al. 1993) media. After 3–4 d of culture, the growth was then transferred with sterile bacteria loops in sterile test tubes and mixed with sterile water to achieve the target OD. For the bath exposures, the bath was composed of 2 L of TYES broth (0.3 OD) and 2 L of well water and aerated during the exposure. The OD was based on maximum ODs achievable in our broth cultures in the incubation time alloted. For bath exposures, all 30 fish for a treatment were fin-clipped (adipose fin) and exposed together for 30 min. After bath exposure, the fish were rinsed with freshwater, and 10 fish were returned to each treatment aquarium. Thus, 10 bath-exposed fish and 10 injected fish shared each aquarium.

Two isolates of *Flavobacterium psychrophilum* were tested for each Rainbow Trout strain, the CSF 259-93 isolate from Idaho, and the 09-104 isolate isolated from sick Rainbow Trout in Utah. The CSF 259-93 isolate has been used in multiple studies (Crump et al. 2001; LaFrentz et al. 2003; Crump and Kay 2008; Castillo et al. 2012). Control fish were injected with sterile PBS or exposed to a 30-min bath with 2 L sterile TYES broth and 2 L well water.

For the WV strain, an additional treatment was added for which fish were injected with 50 μL of a 0.4-OD solution of the CSF 259-93 isolate. This dose has been used in several previous bacteria challenges related to *F. psychrophilum* vaccine evaluations at the UDWR's Fisheries Experiment Station. There was insufficient tank space for an additional 09-104 treatment at 0.4 OD. Additional treatments at 0.4 OD for the other strains was not possible due to tank space limitations. For the WV trial, the injected fish of the 0.4 OD treatment were cohabitated with bath-exposed fish as in the other treatments.

Tank system, fish, and water quality.—The tanks were part of a recycle system that had a biofilter and sump from which water was pumped to a headbox and distribution manifold (see Wagner et al. 2006 for further system details). Each fish strain was tested on separate dates due to differences in hatching dates, but the same methods were applied in each case. Mean lengths and weights of the fish at the end of the trials were AL-EN = 94.2 mm, 8.4 g; AL-JR = 86.7 mm 7.8 g; HH = 83.1 mm, 6.8 g; and WV = 95.9 mm, 9.5 g.

Fish were fed daily ad libitum with a commercial pellet feed (Skretting trout diet). Each day, about a fourth (56 L) of the total volume of water was removed, siphoning waste feed and feces at the same time. This water was replaced with fresh well water. Water quality was monitored periodically with a Hach test kit to ensure temperature, ammonia, and nitrite levels were not affecting survival. Temperatures were dependent on air temperature in the room, but only varied between 13.5°C and 16.1°C during the trials. The well water pH was 7.6, the total hardness was 222 mg/L, and the total alkalinity was 222 mg/L. Mortalities were removed and recorded daily until the challenge ended after 21 d. This duration was the same as in the study by Leeds et al. (2010), in which most mortality occurred within 14–16 d. Kidney and spleen samples from dead fish were streaked on TYES plates and monitored for growth of yellow pigmented bacteria.

Broth culture preparation.—The broth cultures used for the bath exposures were initiated by adding frozen bacteria stock to

a flask with 50–100 mL sterile TYES broth. After 2 d, the contents were transferred to a larger flask with 1–2 L of iron-limited TYES broth and incubated at room temperature (18–20°C) for 3–4 more days. Iron-limited media were used to increase virulence (LaFrentz et al. 2009; Long et al. 2013). This media was made by adding 834 μL of 0.03M 2,2 bipyridyl (DPD) per liter of TYES broth. The DPD was first made in a stock solution (0.3 M: 0.47 g in 10 mL of 70% ethanol). This stock solution was diluted 1:10 in sterile water, then filter-sterilized (0.2 μm) to make the 0.03-M working solution. In addition to making injection solutions, the bacteria from the plates were used to augment the number of bacteria in the broth cultures. A portion of the broth cultures were also centrifuged in 50 mL tubes. The pellets of these tubes were resuspended in a small amount of the supernatant and added to other broth cultures of the same isolate to concentrate bacteria into 2 L for the bath. Serial 10-fold dilutions of both injection and bath solutions were made on TYES plates to estimate the number of bacteria in each solution.

Statistics.—We used SPSS version 13.0 for all analyses and significance was set at $\alpha = 0.05$. A fully saturated general linear model was used to compare the percent mortality among the four trout strains, the three challenges of bacteria isolates (CSF 259-93, 09-104, and PBS control), and between exposure method (bath or injection). Tanks were considered replicates. Given the significant interactions, separate tests were conducted for each exposure method. The percent mortality was transformed prior to analysis using the arcsine-square root procedure (Kirk 1982). Scheffé statistic was used for mean separation.

Cox regression analysis, using the backward stepwise likelihood ratio option of SPSS, was used to compare time to death (d) among the trout strains and between bacterial isolates. For survivors (censored values), 21 d was inserted as the days-to-death value; mean days to death is the average of all individual days to death and includes survivors in the calculation. The analysis used only the data for injected fish in the 0.65-OD treatments because the mortality rate was so low in the bath-treatment data set that the models using that data would not converge. A Kaplan-Meier test featuring the log-rank (Mantel–Cox) option was used to compare (1) differences, pairwise, among fish strains, and (2) the 0.40 and 0.65 OD data for the WV strain (pooling the data for the bacterial isolates for both analyses because the isolates were not significantly different).

RESULTS

The general linear model indicated that effects of Rainbow Trout strain, bacteria isolate, exposure method were all highly significant ($P < 0.001$), as well as all two-way and three-way interactions ($P \leq 0.007$). Injected fish had significantly ($P < 0.001$) higher mortality than bath-exposed fish, which had low to no mortality. When analyzed separately for each exposure method, the mortality rates varied significantly among the strains of Rainbow Trout that were injected ($P < 0.001$, df = 3, $F = 21.6$) or exposed in baths ($P = 0.003$, df = 3, $F = 6.2$). For injected fish, the WV had significantly lower mortality (20.0–36.7%) than HH and AL-EN (76.7–96.7%; $P < 0.001$) but did not significantly differ from AL-JR (46.7–56.7%; $P = 0.27$; Table 1). The percent mortality of injected fish did not differ between the two bacteria isolates ($P = 0.77$), but both differed significantly from controls ($P < 0.001$). Of all the control fish, only one died. This one died the day after injection, indicating the death was likely due to needle damage intraperitoneally, rather than disease or water quality issues. Water quality was maintained throughout all the exposure trials, so ammonia (<0.9 mg/L total ammonia nitrogen), nitrite (≤0.17 mg/L), and temperatures (13.5–16.1°C) were not a source of mortality.

The comparison of days until death among strains indicated that there were significant differences among the strains (Cox regression: $P < 0.001$, $\chi^2 = 67.4$, df = 3) but not between bacterial isolates ($P = 0.99$, $\chi^2 = 0$, df = 1; Table 1; Figure 1). Pooling data for both isolates, the mean days until death was significantly higher ($P < 0.001$, $\chi^2 \geq 7.1$) for the WV strain

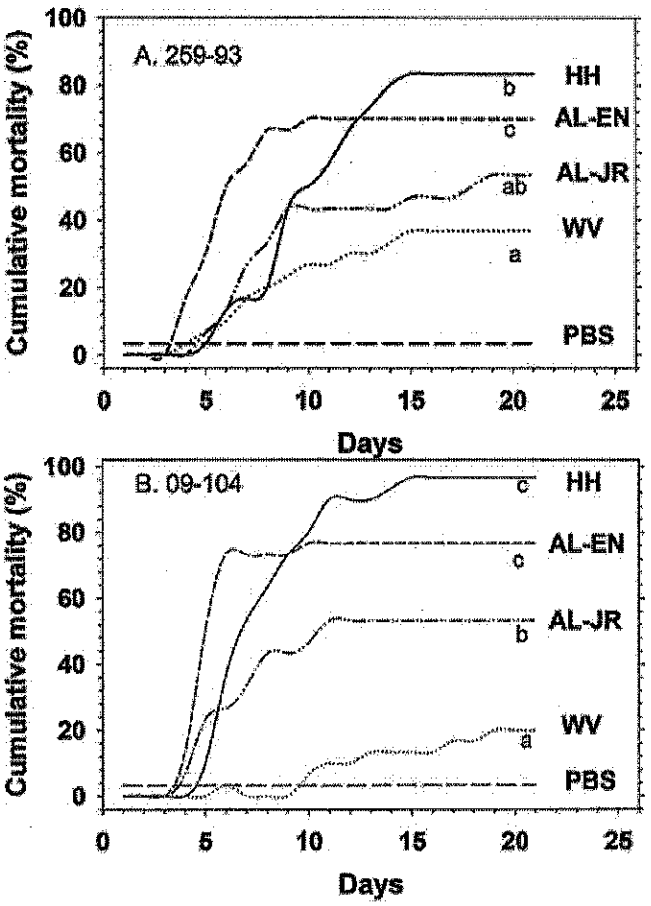

FIGURE 1. Comparison of the cumulative percent mortality among four Rainbow Trout strains (see Table 1) injected with one of two isolates of *Flavobacterium psychrophilum* at 0.65 OD: (A) CSF 259-93 from Idaho, and (B) 09-104 from Utah. Significant differences among strains in the mean days to death are noted for each isolate by different lower case letters (a–c).

TABLE 1. Comparison of the mean percent mortality among four strains of Rainbow Trout exposed at 75 d posthatch to two different isolates of *Flavobacterium psychrophilum* by injection or bath exposure. The optical density (OD; 525 nm wavelength) and plate count dilution estimates are also given. Injected fish received 50 µL (1/20th the amount shown). Significant differences among strains in days to death are noted separately for each bacteria isolate by different letters: x to z for 09-104 or a to c for 259-93 and the asterisk (*) indicates a significant difference from the 0.65 OD for isolate 259-93. Abbreviations: Arlee strain from Ennis National Fish Hatchery, Montana (AL-EN), the Arlee strain from Jocko River Hatchery, Montana (AL-JR), a cold-water disease-resistant strain (WV), and the Harrison–Hofer strain (HH).

Exposure method	Rainbow Trout strain	Bacteria isolate	OD	Number of bacteria/mL	Mean ± SE mortality (%)	Mean ± SE days to death
Injected	AL-EN	09-104	0.65	1.44×10^9	76.7 ± 15.3	9.0 ± 1.2 z
		CSF 259-93	0.65	6.30×10^7	86.7 ± 5.8	7.8 ± 1.0 c
		PBS control			0.0 ± 0.0	
	AL-JR	09–104	0.65	No growth	46.7 ± 25.2	14.3 ± 1.3 y
		CSF 259-93	0.65	No growth	56.7 ± 15.3	14.6 ± 1.2 ab
		PBS control			0.0 ± 0.0	
	HH	09–104	0.65	2.2×10^6	96.7 ± 5.8	8.4 ± 0.6 z
		CSF 259-93	0.65	3.6×10^6	83.3 ± 11.5	11.8 ± 0.9 b
		PBS control			3.3 ± 5.7	
	WV	09–104	0.65	3.9×10^6	20.0 ± 10.0	19.3 ± 0.7 x
		CSF 259-93	0.65	1.4×10^7	36.7 ± 20.8	16.3 ± 1.1 a
		CSF 259-93	0.4	1.1×10^7	0.0 ± 0.0	20.6 ± 0.4 *
		PBS control			0.0 ± 0.0	
Bath	AL-EN	09–104	0.3	1.6×10^7	3.3 ± 5.8	
		CSF 259-93	0.3	1.9×10^7	6.7 ± 5.8	
		PBS control			0.0 ± 0.0	
	AL-JR	09–104	0.3	No growth	0.0 ± 0.0	
		CSF 259-93	0.3	No growth	13.3 ± 15.3	
		PBS control			0.0 ± 0.0	
	HH	09–104	0.3	4.3×10^9	26.7 ± 5.8	
		CSF 259-93	0.3	8.0×10^9	10.0 ± 10.0	
		PBS control			0.0 ± 0.0	
	WV	09–104	0.3	4.6×10^6	0.0 ± 0.0	
		CSF 259-93	0.3	6.0×10^6	0.0 ± 0.0	
		CSF 259-93	0.3	4.0×10^6	0.0 ± 0.0	
		PBS control			0.0 ± 0.0	

than the other strains. Each strain was significantly different from each other ($P < 0.034$, $\chi^2 \geq 4.5$), ranking from highest to lowest: WV (17.9 d) > JR (14.5 d) > HH (10.1 d) > AL-EN (8.4 d). The mean days to death for WV fish exposed to 0.4 OD (20.6 d) was significantly higher than for WV fish exposed to 0.65 OD (17.9 d; $P = 0.006$, $\chi^2 = 7.4$).

For bath-exposed fish, the WV had significantly lower mortality (0%) than the HH (10.0–26.7%), but both Arlee strains had intermediate mortality values (0–13.3%) that did not significantly differ ($P > 0.10$) from either the HH or WV strain (Table 1). There were no significant differences between the two bacteria isolates, though mortality in both treatments was significantly higher than the PBS control ($P \leq 0.02$). Cumulative mortality after bath exposure for each strain and each isolate is shown in Figure 2.

Bath-exposed fish, either alive or dead, did not have observable lesions, whereas several of the injected fish had lesions develop around the injection site. Gram-negative, yellow-pigmented bacteria with typical *Flavobacterium psychrophilum* morphology (long thin rods) were observed in the kidney and spleen samples of dead fish (Table 2).

DISCUSSION

The results of this study indicated that there were significant differences among the strains in their susceptibility to cold-water disease. Not surprisingly, the strain selected for cold-water disease resistance had the lowest mortality. The HH had higher susceptibility to cold-water disease, despite selection for resistance to whirling disease. Selection for one disease does not necessarily confer resistance to other diseases, and may even hinder resistance (Fevolden et al. 1992; Allendorf and Spruell 1999; Hedrick et al. 2001). Nagai et al. (2004) found that a strain of Ayu resistant to cold-water disease was just as susceptible to vibriosis (caused by *Vibrio anguillarum*) as two other strains of Ayu that were not resistant to cold-water disease. However,

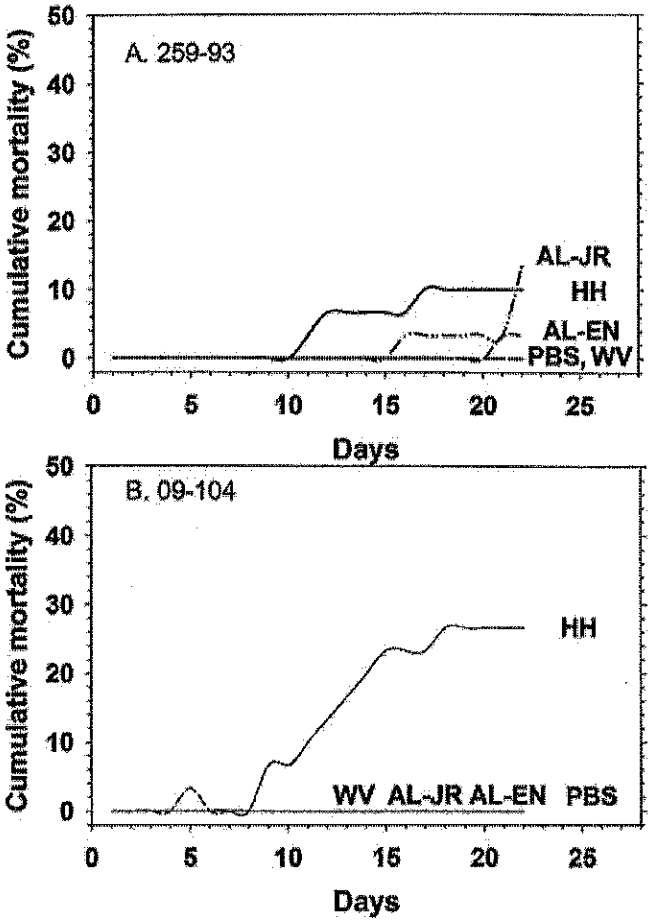

FIGURE 2. Comparison of the cumulative percent mortality among four Rainbow Trout strains (see Table 1) exposed by bath immersion to one of two isolates of *Flavobacterium psychrophilum*: (A) CSF 259-93 from Idaho, and (B) 09-104 from Utah.

Henryon et al. (2005) noted some weak positive correlations for Rainbow Trout resistance if fish were previously exposed to either *Yersinia ruckeri* (cause of enteric redmouth disease), *F. psychrophilum* (Rainbow Trout Fry Syndrome), or viral haemorrhagic septicemia (VHS).

The bath exposure data indicated that healthy fish can withstand a challenge by a substantial dose of bacteria. Several bath exposure studies have had varying success in producing infection, but wounds or injuries that break the skin and mucus barrier contributed to higher rates of infection by *F. psychrophilum* (Madsen and Dalsgaard 1999) and *F. columnare* (Bader et al. 2006). The higher mortality of injected fish corroborates this earlier work on the effects of injury. A high percentage of injected fish died in most strains, indicating that if injected bacteria numbers are high enough, disease will follow, regardless of trout strain. The lack of mortality in WV injected with a 0.4 OD solution of *F. psychrophilum*, compared with 20–37% in 0.65 OD, indicated that a critical concentration of bacteria is needed to lead to disease. This effect has been observed in numerous

TABLE 2. Summary of data (percent positive for each tissue) on reisolation of yellow-pigmented bacteria (YPB, Gram-negative rods) from mortalities after injection of four rainbow trout strains (see Table 1) with one of two different isolates of *Flavobacterium psychrophilum*. ND = no data.

Strain	Isolate	YPB In kidney (%)	YPB in spleen (%)
WV	259–93	16.7	33.3
	09–104	25.0	50.0
GH	259–93	100.0	100.0
	09–104	81.8	81.8
AL-EN	259–93	100.0	100.0
	09–104	ND	ND
AL-JR	259–93	64.3	64.3
	09–104	0.0	20.0

challenges studies with *F. psychrophilum* in which higher doses of bacteria led to higher mortality (Obach and Laurencin 1991; Madsen and Dalsgaard 1999; Rahman et al. 2000, 2001, 2002; Plant et al. 2011). This effect suggests that lower doses can be controlled by the immune system, but higher bacteria concentrations overcome these defenses.

The implication of this dose effect for aquaculture is that cold-water disease can be managed by keeping bacteria growth and numbers below threshold levels. This is a working hypothesis, so further research is needed in an aquaculture setting to determine what these thresholds are. Once determined, regular monitoring and adaptive management (e.g., adjustment of densities, water temperature, fish numbers, water flow rates, rapid removal of mortalities) can keep bacteria loads low and fish healthy. Chemical treatments such as hydrogen peroxide could be considered as well (Speare and Arsenault 1997; Rach et al. 2000; Giménez-Papiol et al. 2009), if doses and durations are kept below levels that have been shown to increase the incidence of cold-water disease (Henriksen et al. 2013). Probiotic bacteria may also be a tool to keep *F. psychrophilum* levels low via competition (Burbank et al. 2011, 2012). The data also suggest that if the bacteria concentrations are sufficient, even resistant strains of Rainbow Trout will succumb to cold-water disease.

There was little difference between the two isolates of bacteria used for the challenges. Soule et al. (2005) observed that a different Utah isolate of *F. psychrophilum* (03-009) was in the same lineage (II) as the CSF 259-93 isolate. In this study the virulence was comparable between the two bacteria isolates, whether the fish were exposed by bath or injection. This suggests that for future research, one isolate could be used. The CSF 259-93 isolate would be preferable because it has been used more widely as a reference strain (e.g., Crump et al. 2001; LaFrentz et al. 2003). The results for the WV exposed to the 09-104 isolate also indicated that the resistance developed in the WV strain using the CSF 259-93 isolate will be applicable to Utah and probably other sites beyond Idaho.

The relationship between OD and the plate-count numbers is worth discussing as well. It appears that the predictive ability

of OD for bacteria abundance is not as accurate as one would like. For example, at a 0.65 OD, the plate counts varied from 3.9×10^6 to 1.4×10^9, which is a range of 3 logs. Perhaps variance increases with OD, but the variation in numbers around the bath ODs, which were about half that for the injection, was similarly wide. The scatter in plots by Michel et al. (1999; $r^2 = 0.61$ between OD and *F. psychrophilum* numbers) also suggested that variance was similar across a range of bacteria abundance. Another factor to consider is that OD measures both live and dead bacteria, whereas plate counts would count only the live colony forming units. So, it appears that although a general relationship between OD and bacteria numbers exists (e.g., Holt 1987; Michel et al. 1999), meaningful differences related to OD values do not correspond well to bacteria numbers. Unfortunately, for doing research, one cannot wait several days for bacteria counts on plates to do challenge studies.

Plate counts have their pitfalls as well, with variables such as moisture content or air diffusion that may affect colony counts. Another example is our finding that the serial dilutions for the trial with AL-JR had no bacterial growth, yet mortality indicated that bacteria were present. We suspect this may have been a result of pouring plates when the media was still too hot, leading to potentially toxic polystyrene breakdown products such as benzaldehyde and benzene (Ciucanu et al. 2002) being released, but we cannot conclude that with certainty. Also, differences among serial dilutions of a single sample in this study, which should have been 10-fold, indicated that values were often approximately but inconsistently 10-fold. Clumping of bacteria could also affect colony development, which would underestimate actual numbers that would be replicating inside challenged fish. In our experience, auto-agglutination of *F. psychrophilum* is a common problem when growing broth cultures. The variance in bacterial number estimates that results from plate counts is of concern in this study because the resulting numbers suggest that perhaps the fish strains were exposed to different doses that affected mortality differences. Ideally all strains would have been exposed at the same time to the same batch of bacteria, but given the different spawning times inherent to the strains, this was not possible. However, every effort was made to minimize differences among trials: Fish were exposed to the same OD, the same injection volume, the same age of the broth culture and at the same fish age in the same tank systems. We do not have the data to calculate the variance around a plate count estimate, but given the variance observed for a given OD, the experimental error suggests that fish strains were similarly challenged across trials. The similarity in results between the two isolates also provides support for the reproducibility of the methods for bacterial challenges.

As an alternative to plate counts, bacteria counts on a filter or a slide after dilution could be done, after staining with fluorochromes to indicate viability (APHA et al. 1989; Boulos et al. 1999). Flow cytometry is another alternative for obtaining bacteria population, cell size, DNA, and biomass-distribution data (Button and Robertson 1993), though the cost of the instrument prohibits its widespread use for aquatic animal health. Comparison of these various methods by Paulse et al. (2007) has indicated that direct microbial counts are about 43% of that obtained by flow cytometry; similarly, heterotrophic plate counts are about 4% of the flow cytometry estimates. Wohlsen et al. (2006) found that the pour-plate method and the commercial product Petrifilm produced more consistent and better estimates of coliform bacteria numbers than membrane filtration, the MPN (most probable number) method, or two commercial coliform counting kits.

In summary, the data showed significant differences among strains of Rainbow Trout tested, the WV strain showing the greatest resistance to cold-water disease. These trials represent the first efforts at comparing susceptibility to cold-water disease among Rainbow Trout strains. Because the bacterium can be vertically transmitted (Brown et al. 1997; Cipriano 2005), eggs from infected brood, coupled with inadequate disinfection methods (Cipriano 2005; Wagner et al. 2008), lead to transfer of the bacterial pathogen to rearing hatcheries. By using resistant strains and making efforts to develop pathogen-free broodstock, the disease can be better controlled when the bacterium is not present to cause disease. The data also provide a foundation for future studies comparing resistance of other Rainbow Trout strains or among fish species under standard conditions established by Leeds et al. (2010). The data also indicate that the virulence of *F. psychrophilum* isolates from Utah is similar to the Idaho isolate. Bath trials indicated that Rainbow Trout are able to withstand exposure to high numbers of bacteria without developing the disease, or at least experience much lower mortality levels than fish injected with the bacteria. The exposures indicated that susceptibility to disease is dependent on bacteria numbers, which if controlled, could control or limit mortality and morbidity related to cold-water disease.

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