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On-farm performance of rainbow trout (*Oncorhynchus mykiss*) selectively bred for resistance to bacterial cold water disease: Effect of rearing environment on survival phenotype

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ABSTRACT

Selective fish breeding programs for disease resistance comprise an increasingly important role in aquaculture production and offer an additional management tool for reducing bacterial-caused disease losses. Bacterial cold water disease (BCWD) is one of the most frequent causes of elevated mortality in juvenile salmonids, and we have selectively bred three genetic lines of rainbow trout for varying resistance to BCWD. These lines, designated ARS-Fp-R (resistant), ARS-Fp-C (control) and ARS-Fp-S (susceptible), differ in survival following standardized laboratory challenges with the causative agent of BCWD, *Flavobacterium psychrophilum*. This study evaluated survival of the genetic lines in laboratory challenges and in a production environment. Evaluations of disease resistance demonstrated a reproducible, 30% or greater, survival difference between ARS-Fp-R and ARS-Fp-S lines at body weights ranging from 0.7 to 13 g. Farm trials were performed to evaluate survival over an 80-day growth period starting after the trout began feeding. After a BCWD epizootic, the ARS-Fp-R line displayed significantly greater risk-adjusted survival (95.7%) than the ARS-Fp-S line (91.2%, $P<0.0001$) and the ARS-Fp-C line (92.4%, $P<0.0001$). Phenotype stability in farm-trial fish was also evaluated using laboratory challenges. The ARS-Fp-R line consistently displayed a higher, but not always statistically significant, survival percentage compared to the other lines and the data suggest that the magnitude of the survival phenotype difference is sensitive to environmental influence. In summary, the overall greater survival of the ARS-Fp-R line provides evidence of genetic improvement under production conditions.

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1. Introduction

A frequent cause of freshwater farmed trout loss is bacterial cold water disease (BCWD) that is also synonymous with rainbow trout fry syndrome (Barnes and Brown, 2011; Nematollahi et al., 2003; Starliper, 2011). The etiological agent of BCWD is a Gram-negative, yellow-pigmented bacterium, *Flavobacterium psychrophilum*. In Idaho, a primary location of rainbow trout production in the U.S., most losses due to BCWD occur in fish between 0.2 to 4 g; however, larger fish can also be compromised by BCWD. BCWD can present as an acute outbreak in small fish or as a chronic disease in larger fish, typically causing between 2 and 30% mortality. Production losses from BCWD are due to direct mortality and may also occur due to deformities in fish that survive infection (Madsen et al., 2001; Nematollahi et al., 2003). Current BCWD management frequently involves oral antibiotic therapy, chemical treatment, and/or a reduction in fish density (Barnes and Brown, 2011; Nematollahi et al., 2003; Starliper, 2011).

Genetic improvement in disease resistance accomplished through selective breeding offers an additional management tool for controlling disease in finfish aquaculture (Fjalestad et al., 1993; Gjedrem, 2005; Moen, 2011; Henryon et al., 2005). At the National Center for Cool and Cold Water Aquaculture (NCCCWA), we have investigated whether family-based selective breeding of rainbow trout can increase the innate resistance of naïve fish against BCWD (Hadidi et al., 2008; Leeds et al., 2010; Silverstein et al., 2009). Since 2005, an odd-year spawning line of pedigreed rainbow trout developed from the intercrossing of four domesticated founder strains has been evaluated and selectively bred for increased BCWD survival based on intraperitoneal injection-challenge evaluation (Silverstein et al., 2009). This closed genetic line has been designated ARS-Fp-R. In addition, we have developed a selection control line, designated ARS-Fp-C, and a susceptible line, designated ARS-Fp-S. All lines were derived from the same resource population.

At present, factors influencing BCWD resistance under field conditions remain poorly understood and optimal trial design for evaluating genetic resistance has not been investigated. Studies reported herein were initiated as part of a multi-year laboratory and field evaluation process to: 1) obtain off-site validation of the resistant and susceptible

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phenotypes using standardized laboratory challenges, 2) test the hypothesis that the ARS-Fp-R line will exhibit higher survival than either the ARS-Fp-C or ARS-Fp-S lines, and 3) measure the stability of the disease-resistance phenotype at select time points after farm exposure by removing fish from field evaluation and subjecting them to standardized laboratory challenges.

2. Materials and methods

2.1. Rainbow trout genetic lines, rearing conditions and water quality parameters

Development of the single, 2-year-old spawning, closed resource population consisting of 71 full-sib families from which the ARS-Fp-R, -C, and -S lines were derived has been previously described (Silverstein et al., 2009). Differentiation of the three lines via selective breeding is shown schematically in Fig. 1. Briefly, the ARS-Fp-R line has been selected each generation for improved survival following laboratory challenge with *F. psychrophilum* as previously described (Leeds et al., 2010). We have utilized survival at 21 days post-challenge as a surrogate measure of disease resistance. The ARS-Fp-S line has undergone 1 generation of selection for poor survival following laboratory challenge with *F. psychrophilum*, and has since been randomly mated as a reference susceptible line. The ARS-Fp-C line represents random mating of the ARS-Fp-R line after only one generation of selection and was developed to directly quantify survival improvement due to effects of continued selection. An average of 76, 19, and 25 full-sib families has been produced and evaluated for BCWD resistance each generation for the ARS-Fp-R, -S, and -C lines, respectively. Details of general animal husbandry practices and rearing conditions are given in Silverstein et al. (2009) and Leeds et al. (2010).

A random sub-sample of each generation (n = 60/lot) were tested once or twice per year and determined to be free of viral hemorrhagic septicemia virus, infectious hematopoetic necrosis virus (IHNV), infectious pancreatic necrosis virus, *Oncorhynchus* masou virus, infectious salmon anemia virus, and spring viremia of carp virus. One-year-old fish or older were also tested and confirmed to be free of *Aeromonas salmonicida*, *Yersinia ruckeri*, *Renibacterium salmoninarum*, *Myxobolus cerebralis* and *Ceratomyxa shasta*. While not required for certification, broodstock and one-year-old fish at our facility were swabbed from kidney tissue (n = 60 fish/lot) and cultured on Tryptone yeast glucose agar slants (AFS-FHS, 2010), incubated at 15 °C for 14 days, and

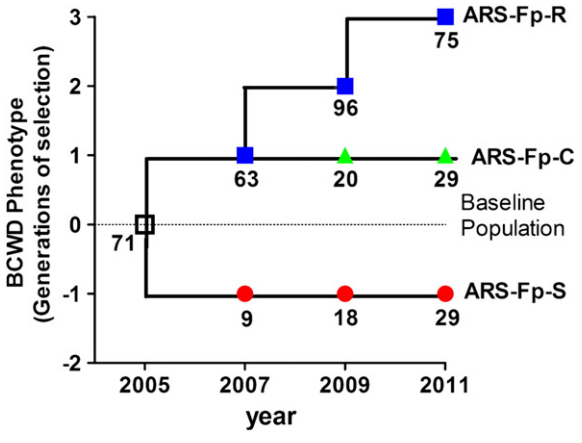

Fig. 1. Schematic of disease resistance breeding and the relative direction of selection applied to three rainbow trout lines. Symbols indicate year class in which BCWD challenges were performed while the steps indicate the generations of selection applied either for increased BCWD resistance (+1) or for increased BCWD susceptibility (−1). Number of full-sib families is given for each line and generation. The ARS-Fp-R was selected for three generations while the ARS-Fp-C and ARS-Fp-S lines were selected for only one generation, and since, randomly bred as reference lines with similar genetic background to the ARS-Fp-R line.

were negative for *F. psychrophilum*. Testing was performed either by Kennebeck River Biosciences (Richmond, ME) or by the U.S. Fish and Wildlife Service (Lamar, PA).

Flow-through spring water was used to maintain early life stage growth and pre-spawning broodstock. The average water quality parameters (±1 s.d.) of the NCCCWA spring-source, as measured from January 2008 through August 2012, were the following: water temperature 12.8 ± 0.4 °C, pH 7.2 ± 0.12, ammonia 0.03 ± 0.06 mg L^{-1}, CO_2 30.3 ± 4.3 mg L^{-1}, and water hardness 285 ± 25 mg L^{-1} $CaCO_3$. Fish were fed a commercial fishmeal-based diet (Zeigler Bros, Inc., Gardners, PA).

At the Clear Springs Foods (CSF), Inc. farm site (Buhl, ID), where fish were grown for the farm trial, as well as the CSF Research Facility, where animals were transferred for experimental challenge, fish were fed a standard rainbow trout fishmeal-based diet (45% protein: 20% fat; CSF, Inc.). Both the farm site and CSF Research Facility are supplied by spring water from the Snake River Plain Aquifer. At the farm site, water quality parameters were the following: temperature of 14.5 °C, pH 8.2, ammonia <0.01 mg L^{-1}, nitrate–nitrite nitrogen 3.22 mg L^{-1}, and water hardness 255 mg L^{-1} $CaCO_3$. At the CSF Research Facility the water quality parameters were the following: temperature of 14 °C, pH 7.9, ammonia <0.01 mg L^{-1}, nitrate–nitrite 2.48 mg L^{-1}, and water hardness 216 mg L^{-1} $CaCO_3$.

2.2. Bacterial and viral strains, propagation, enumeration and standardized laboratory challenge

All BCWD challenges used *F. psychrophilum* strain CSF259-93 originally isolated by Dr. S. LaPatra from a clinical case of BCWD in rainbow trout. In 2003, a frozen broth culture was sent to the NCCCWA and a single colony was isolated on Tryptone yeast extract salts (TYES) plates (Cain and LaFrentz, 2007), amplified in TYES broth, and a bank of approximately 200 1-mL frozen stocks, containing 10% glycerol, were prepared from this culture and stored at −80 °C to provide a standardized challenge source. Virulence of the NCCCWA subculture was confirmed to be identical to the original stock and the complete genome sequence of this isolate has been determined (G. Wiens, unpublished data). For challenge, a single vial was thawed, dilutions cultured on TYES plates at 15 °C for five days, and the harvested bacteria re-suspended in either Dulbecco's PBS (Sigma) or sterile saline. Cell concentration was measured by optical density of the suspension at 525 nm and challenge dose adjusted for body weight to maintain a dose above 300,000 CFU per gram average fish body weight, although this value varied between challenge experiments (Table S1). Bacterial cell number was verified at the NCCCWA by direct plate counting. Fish were challenged by i.p. injection as previously described (Hadidi et al., 2008; Overturf et al., 2010). This challenge route results in reproducible mortality when challenging large numbers of fish.

Viral challenge was carried out using IHNV strain 220-90 (LaPatra et al., 1994). Briefly, IHNV was propagated in *Epithelioma papulosum cyprini* (EPC) cell line and virus re-isolated as previously described (LaPatra et al., 1994). Fish were waterborne challenged with a concentration of 10^4 PFU mL^{-1} tank water (Overturf et al., 2010).

2.3. Phenotype validation studies

Off-site phenotype validation was carried out at the CSF Research Facility using 21 full-sib rainbow trout families from the NCCCWA 2009 year class. Egg hatching was temperature-synchronized as described previously (Leeds et al., 2010) and all families hatched within a 4-day period. At 39 days post-hatching, individual families were transported overnight to the CSF Research Facility using supplemental oxygen. Upon receipt, fish were acclimated for 8 days in ambient water (14.5 °C) prior to BCWD and IHNV challenge. PBS injected groups, or mock waterborne challenge groups, were included