Field evaluation of rainbow trout (*Oncorhynchus mykiss*) selectively bred for resistance to bacterial cold water disease

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Abstract

Bacterial cold water disease (BCWD) is a frequent cause of elevated mortality in rainbow trout (*Oncorhynchus mykiss*) and the development of effective control strategies is a priority within the U.S. Since 2005, the NCCCWA has implemented a selective breeding program designed to increase survival following BCWD exposure [1–4]. We hypothesized that this strategy may protect early life stages prior to and through typical vaccination size (hatching to >2g), and once established, the trait would be low-cost to maintain and distribute to rainbow trout producers. Factors influencing BCWD resistance under field conditions remain poorly understood and optimal field-trial design has not been investigated. Studies were initiated in 2010 and 2011, as part of a multi-year field evaluation process, to address: 1) egg transport and hatching under different commercial production conditions; 2) evaluation of different trial designs utilizing first-use and re-use water; 3) determine survival at locations that routinely experience natural BCWD outbreaks; and 4) investigate the distribution and impact of *Flavobacterium psychrophilum* strain variants on resistance.

In order to quantify breeding progress and investigate the effects of selection, we have created three genetic lines of outbred rainbow trout: ARS-Fp-R (resistant), ARS-Fp-C (control) and ARS-Fp-S (susceptible) (Fig. 1). The ARS-Fp-R line was originally developed as a synthetic cross among four domesticated founder strains (House Creek, College of Southern Idaho; Shasta, Ennis National Fish Hatchery, MT; Kamloops/Puget Sound Steelhead cross, Troutlodge, Inc., WA; and Donaldson, University of Washington) and became a closed population beginning with the 2005 year class (YC). Beginning with the YC2005 base population and in each subsequent generation (i.e., YC2007, YC2009, and YC2011), approximately 76 full-sib families per generation have been produced and evaluated for BCWD resistance using experimental challenges with *F. psychrophilum*. Details of the experimental challenges include: 1) fish were challenged at a mean age of 83 days [approximately 1,048 degree days (water temperature, °C × post-hatch age, days)] at a mean body weight of 3.1 grams; 2) the mean challenge dose was approximately 1.4×10^7 colony forming units per fish delivered via intraperitoneal injection; 3) BCWD challenges were conducted using a single, genome-sequenced bacterial clone from a virulent strain of *F. psychrophilum* (CSF259-93); 4) approximately 40 fish per full-sib family were challenged in 2.4-liter tanks (1 family per tank) supplied with 1.9 liters per minute of flow-through spring water (approximately 12.9°C), with approximately 50% of families challenged in replicate tanks; and 5) mortalities were observed once per day for a total of 21 days. In 2005, we also identified highly susceptible families and continued to randomly breed these starting in 2007

as a reference susceptible line, designated ARS-Fp-S. In order to directly quantify survival improvement due to effects of continued selection, we initiated a third line, designated ARS-Fp-C, which represents the base-line survival improvement achieved after one generation of selection. The ARS-Fp-C line has similar ancestry compared to the ARS-Fp-R and ARS-Fp-S lines, has been randomly bred since 2007, and has an intermediate survival phenotype between the ARS-Fp-R and ARS-Fp-S lines.

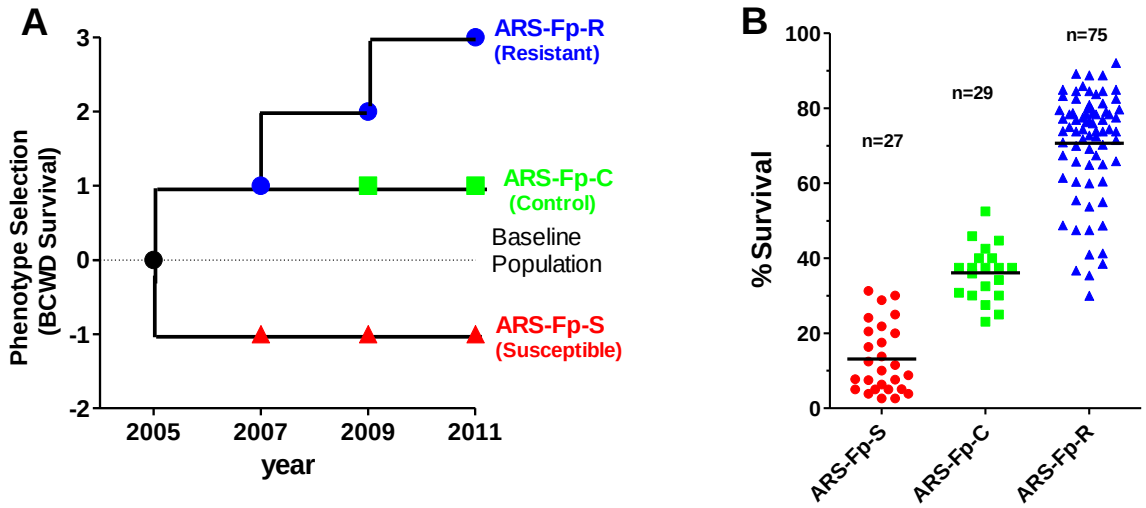

Figure 1. (A) Schematic showing the direction and generations of selection (y-axis) applied to rainbow trout lines in comparison to the original baseline population. (B) Phenotype of genetic lines measured in 2011. Percent family survival after 21 days challenge, n=number of families evaluated per line, each point representing a separate family (ARS-Fp-R and ARS-Fp-S lines), while for the ARS-Fp-C line, each point represents a challenge tank containing pooled families.

Field evaluation was carried out at multiple sites in Utah in cooperation with the Utah Division of Wildlife Resources, and at one site in Idaho in cooperation with Clear Springs Foods, Inc. Approximately 175,000 eyed eggs from the ARS-Fp-R line have been distributed to farms in Idaho and Utah in 2010 and 2011 as part of designed field trial evaluations. Primary objectives of these field trials were to evaluate BCWD-specific mortality in the ARS-Fp-R line in production settings where fish are naturally exposed to the pathogen, and compare mortality in the ARS-Fp-R line to an industry reference line (Gunnison River/Harrison Lake Triploids; GHTP) and two NCCCWA reference lines ARS-Fp-C and ARS-Fp-S. In completed field trials to date (n = 4) in which fish have had confirmed exposure to *F. psychrophilum* and BCWD was diagnosed, survival of the ARS-Fp-R line has been $\geq$ 95.5% (mean = 96.4%) through a minimum of 80 days post initial feeding. By comparison, mean survival of the GHTP line averaged 83.8% over three field trials, did not exceed 86.8% for any single field trial, and was statistically different from the ARS-Fp-R line for each field trial. Survival of the ARS-Fp-C line was 92.4% in a single field trial and was statistically different from the ARS-Fp-R line. Survival of the ARS-Fp-S line was 91.2% and statistically different from the ARS-Fp-R line in one field trial, but did not differ (97.3% survival) from the ARS-Fp-R line in another field trial. Consistent with these results, the percentage of ARS-Fp-R fish yielding a positive culture for *F. psychrophilum* has generally been smaller than that for the various reference lines, and sample analyses are ongoing to quantify pathogen load. In all field trials, observations of feeding behavior and growth rate

have been favorable for the ARS-Fp-R line, but long-term growth trials (i.e. to a standard market weight) have yet to be conducted for this line under production settings. To date there has been no reported outbreak of the ARS-Fp-R line to IHNV or other major pathogens. These preliminary findings support the release of germplasm and the continued evaluation of the ARS-Fp-R genetic line in large-scale production trials.

References

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