

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
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NOTICE OF RELEASE OF RAINBOW TROUT LINE ARS-Fp-R (YC2012) SELECTED FOR IMPROVED
RESISTANCE TO BACTERIAL COLD WATER DISEASE

The USDA, ARS, National Center for Cool and Cold Water Aquaculture (NCCCWA), Kearneysville, WV announces the release of the ARS-Fp-R (YC2012) line of rainbow trout (*Oncorhynchus mykiss*) that has been selected for improved survival following laboratory challenge with *Flavobacterium psychrophilum*, the bacterium that causes bacterial cold water disease (BCWD).

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*. Salient details of the experimental challenges include: 1) fish were challenged at a mean posthatch age of 83 days [approximately 1,048 degree days (water temperature, °C × posthatch 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. Samples of results obtained from experimental challenges at the NCCCWA have been independently validated by an off-site collaborator.

Genetic selection within the ARS-Fp-R line has been applied solely to family breeding values for survival estimated from a proportional-hazards frailty model and using all accumulated survival data and known pedigree relationships back to the founder strains. Selection and mating decisions were made each generation using optimal genetic contribution theory to maximize genetic response while constraining the rate of inbreeding to $\leq 1\%$ per generation. Leeds et al. (J. Anim. Sci., 2010, 88:1936-1946) estimated response to two generations of selection within the ARS-Fp-R line and reported a genetic trend of 19.0 ± 4.1 percentage points in survival per generation. Compared to the mean of three randomly-mated control lines, phenotypic selection response following two generations of selection was estimated as 44.7 ± 6.7 percentage points in survival. Hadidi et al. (J. Immunol., 2008, 180:4156-4165) conducted serial *F. psychrophilum* challenges on a limited number of families at body weights of approximately 2.4, 10 and 800 grams and reported that the resistant and susceptible phenotypes are stable traits as size increased > 300-fold. Silverstein et al. (J. Anim. Sci., 2009, 87:860-867) reported that survival following *F. psychrophilum* challenge in the ARS-Fp-R line was not genetically correlated with growth to various market sizes, thus the growth rate of the ARS-Fp-R line is not expected to have been impacted by selection for improved BCWD resistance.

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 [randomly-mated control (ARS-Fp-C) and BCWD susceptible (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. Spleen somatic index (i.e., spleen weight \div body weight) was smaller in pathogen-exposed fish from the ARS-Fp-R line compared to the ARS-Fp-S and GHTP lines, giving further indication of a smaller prevalence of *F. psychrophilum* infection in the ARS-Fp-R line. 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. Eleven families sampled from the ARS-Fp-R line were experimentally challenged with infectious hematopoietic necrosis virus (IHNV) at approximately 0.75 grams. Mean survival rate of the ARS-Fp-R line was only 7.6%, indicating susceptibility to IHNV, and did not differ from the ARS-Fp-S line. However, in field trials to date there has been no reported outbreak of the ARS-Fp-R line to IHNV or other major pathogens.

Despite substantial genetic diversity among *F. psychrophilum* isolates as measured by multi-locus sequence typing, approximately half of the U.S. isolates characterized to date belong to the same sequence type (ST10) as the CSF259-93 strain. In experimental challenges using different *F. psychrophilum* variants, survival in the ARS-Fp-R line has been consistently greater than that of the ARS-Fp-S line for the limited number of variants tested. Although these results suggest that resistance to this bacterium is broad based, the magnitude of difference in survival between the lines is clearly affected by the *F. psychrophilum* variant used. Research is underway to identify *F. psychrophilum* virulence factors and host immunological mechanisms of BCWD resistance to better characterize the biology underlying this apparent host \times pathogen interaction.

Development and selective breeding of the ARS-Fp-R line has occurred at the NCCCWA in Kearneysville, WV. Water is supplied to this facility from a combination of springs and wells. Mean annual water temperature is approximately 12.9°C, with monthly averages ranging from approximately 12.3 to 13.3°C. All fish are reared indoors under ambient, artificial lighting. When fed a commercial fishmeal-based diet at the NCCCWA and at a fixed rate based on body weight, mean body weights in the ARS-Fp-R line have averaged approximately 368, 861, and 1,953 grams at 9, 13, and 19 months posthatch, respectively. Females from the ARS-Fp-R line spawn annually beginning as two-year-olds between the months of January and March, and mean fecundity is approximately 4,000 eggs per two-year-old female. In the most recent generation (i.e., YC2011), mean pedigree-based estimates of ancestry to the founder strains across all families were 17% (House Creek), 8% (Shasta), 59% (Kamloops/ Puget Sound Steelhead cross), and 16% (Donaldson), and the mean pedigree-based estimate of inbreeding was 4.4%. The ARS-Fp-R population has been subjected to a twice-per-year fish health inspection protocol, and all samples to date have tested negative for the viral, bacterial, and protozoal pathogens screened. Pending negative results for the most recent inspection (October 2011), the ARS-Fp-R population will be certified disease-free per the Canada Fisheries & Oceans Fish Health Protection Regulations.

A limited quantity of eyed eggs from the ARS-Fp-R line will be available in February or March of 2012 to producers with a documented broodstock program that are interested in propagating and/or introgressing the line. Eyed eggs will be derived from the matings of three-year-old females (i.e., YC2009) to one-year-old males (i.e., YC2011), and can be made available as separate full-sib families with complete pedigree information. Although most families as part of this release are expected to be all-female, no guarantee of gender ratio will be made. Producers interested in obtaining eyed eggs should contact Drs. Timothy Leeds (Research Geneticist), Gregory Wiens (Molecular Biologist), or Caird Rexroad, III (Center Director & Research Leader) at the USDA, ARS, NCCCWA, 11861 Leetown Road, Kearneysville, WV, 25430. It is requested that appropriate recognition be made if this germplasm contributes to the producer's broodstock program. Furthermore, producers receiving germplasm are encouraged to engage in collaborative research efforts with NCCCWA scientists aimed at documenting extensive production characteristics of the ARS-Fp-R line and advancing knowledge of immunological mechanisms of disease resistance, disease epidemiology, durability of disease resistance, and synergism among multiple disease control strategies.

A handwritten signature in cursive script, appearing to read "Jeffrey Siler".