Response to selection for bacterial cold water disease resistance in rainbow trout


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ABSTRACT: A family-based selection program was initiated at the National Center for Cool and Cold Water Aquaculture in 2005 to improve resistance to bacterial cold water disease (BCWD) in rainbow trout. The objective of this study was to estimate response to 2 generations of selection. A total of 14,841 juvenile fish (BW = 3.1 g; SD = 1.1 g) from 230 full-sib families and 3 randomly mated control lines were challenged intraperitoneally with Flavobacterium psychrophilum, the bacterium that causes BCWD, and mortalities were observed for 21 d. Selection was applied to family EBV derived from a proportional-hazards frailty (animal) model while constraining rate of inbreeding to <1% per generation. After adjusting for nongenetic effects, survival rate of select-line families increased by 24.6 ± 6.8 and 44.7 ± 6.7 (cumulative) percentage points after 1 and 2 generations of selection, respectively (P < 0.01). Genetic trend, estimated from a linear animal model that fit genetic group effects, was 19.0 ± 4.1 percentage points per generation and approached significance (P = 0.07). Heritability estimates from the proportional-hazards frailty model and linear animal model were similar (0.22 and 0.23, respectively), and family EBV from both models were highly correlated (~0.92). Accuracy of selection, estimated as the correlation between mid-parent EBV and progeny survival rate, was 0.20 (P < 0.01) for the proportional-hazards frailty model and 0.18 (P = 0.01) for the linear animal model. Accuracy estimates were not different (P = 0.81) between the models. This study demonstrates that selective breeding can be effective for improving resistance to experimental BCWD challenge in rainbow trout.

Key words: disease resistance, Flavobacterium psychrophilum, heritability, rainbow trout, selection, survival

INTRODUCTION

Infectious disease accounted for 86.6% of the 26.3 million deaths in US trout aquaculture in 2008 (NASS, 2009). Mortality data across the industry are not available for specific diseases, but stakeholders in rainbow trout (Oncorhynchus mykiss) aquaculture have expressed particular concern about losses attributable to Flavobacterium psychrophilum (Nematollahi et al., 2003), the bacterium that causes bacterial cold water disease (BCWD). Furthermore, chronic effects of BCWD, including poor growth and increased incidence of deformities, are responsible for additional economic losses. Licensed antibiotics are available for treatment of BCWD, but antibiotic use increases production costs and antibiotic-resistant pathogens may emerge. A licensed vaccine is not currently available.

Several studies report additive genetic variation for overall survival and resistance to specific disease challenges in aquatic species (reviewed in Gjedrem and Olesen, 2005; Kjøglum et al., 2008), and favorable selection responses have been reported (reviewed in Gjedrem and Thodesen, 2005). However, few studies
Silverstein et al. (2009) reported additive genetic variation for BCWD resistance in Danish and US rainbow trout populations, respectively. Based on these reports, a single-trait, family-based selection program was initiated at the National Center for Cool and Cold Water Aquaculture (NCCCWA) in 2005 to improve BCWD resistance in rainbow trout. The objective of this study was to estimate response to 2 generations of selection for BCWD resistance in Danish and US rainbow trout. Henryon et al. (2005) and Silverstein et al. (2009) described previously the development of a synthetic population of rainbow trout at the NCCCWA based on crosses of 4 domesticated founder strains. To our knowledge, artificial selection in these founder strains was limited to standard production traits (e.g., growth and reproduction) and not disease resistance. During development of the synthetic population at the NCCCWA (i.e., before the current selection experiment), selection was based solely on family growth performance.

Data for the current study were from 3 generations (i.e., 2005, 2007, and 2009 hatch years) of the NCCCWA synthetic line and 3 of the founder strains. The NCCCWA synthetic line has undergone 2 generations of selection for improved BCWD resistance (and herein is referred to as the select line; details of selection given below), whereas remnants of 3 of the founder strains have been maintained as purebred, randomly mated control lines (control 1 to 3). Pedigrees have been maintained as purebred, randomly mated families. Pooling of purebred control-line families at a young age was practiced to limit demands on facility resources. Table 1 summarizes number of families and fertilization and hatching dates by line and year.

Each generation, single-sire × single-dam matings were made during late December, January, and February. Full-sib families were reared separately in vertical incubation trays (2005 and 2007 hatch years) or upwelling incubators (2009 hatch year), and water temperature was manipulated between 7 and 12°C to synchronize hatch times within year. Time from fertilization to hatch is inversely related to water temperature, so early spawns were incubated at colder temperatures and late spawns were incubated at warmer temperatures. Families were subjected to a standard physical shock procedure at the eyed-egg stage to allow differentiation of eggs with and without developing embryos (Piper et al., 1983). Families were moved to 200-L tanks as sac fry (2005 and 2007 hatch years; n « 1,000 per tank) and reared on flow-through spring water (≈12.9°C). All select-line families were maintained in individual tanks to retain family identification. Except for control 2 in 2005, families of each purebred control line were equally pooled within founder strain to create mixed-family lots for each control line (2 replicate lots per line) but otherwise were managed identically to the select-line families. Pooling of purebred control-line families at a young age was practiced to limit demands on facility resources. Table 1 summarizes number of families and fertilization and hatching dates by line and year.

Fish were fed a commercial fishmeal-based diet (Ziegler Bros Inc., Gardners, PA) beginning at swim-up. Once per month, fish were culled at random (with the exception that fish with visible deformities were pre-
Each year, an aliquot was cultured on tryptone yeast extract with salts agar plates for 5 d at 15°C. Bacterial cells were harvested, resuspended in PBS, and diluted to an optical density of 0.325 measured at 525 nm. Serial dilutions of the bacterial suspension were recultured on tryptone yeast extract with salts agar plates on the same day as the challenge to determine actual cfu per inoculation.

Fish were anesthetized in 90 mg/L of tricaine methanesulfonate (Tricaine-S, Western Chemical, Ferndale, WA) and injected intraperitoneally with 25 (2005 and 2007 hatch years) or 50 µL (2009 hatch year) of the bacterial suspension. Fish were heavier at challenge in 2009 compared with 2005 and 2007 (Table 2), and we chose to inject a larger volume of the bacterial suspension, rather than increasing its optical density, to obtain a dosage (i.e., cfu per gram of BW) comparable with 2005 and 2007. Each year, all fish in the experiment were challenged on the same day. In 2007 and 2009, contemporary fish (n = 229 and 159, respectively) were given sham injections of PBS to serve as negative controls. The posthatch age of fish at the time of challenge was approximately 84 d [alternatively, 1,050 degree days (°C x d)]. Average BW of a family or mixed-family lot was estimated from a sample of 75 (2005 hatch year) or 50 |µL (2009 hatch year) on the date fish were moved to the challenge facility. In 2009, the total weight of fish in each challenge tank was recorded at the time of challenge, and average BW were calculated for each challenge tank.

Mortalities were recorded once daily for a 21-d period. Moribund fish were killed and recorded as mortalities. During the challenge, fish were fed to apparent satiation 3 times per week in 2005 and 2007, and once

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**Table 2. Summary statistics by line and year for bacterial cold water disease challenges**

<table>
<thead>
<tr>
<th>Hatch year/line</th>
<th>Challenge dose, cfu</th>
<th>No. challenged</th>
<th>Full-sib families</th>
<th>No. of challenge tanks</th>
<th>Mean age at BW measure, d</th>
<th>Mean (SD) BW, g</th>
<th>Mean age at challenge, d</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005 (generation 0)</td>
<td>8.8 x 10⁶</td>
<td>4,496¹</td>
<td>71¹</td>
<td>114</td>
<td>68</td>
<td>2.33 (0.35)</td>
<td>82</td>
</tr>
<tr>
<td>Select</td>
<td>Control 1</td>
<td>Control 2</td>
<td>Control 3</td>
<td>Subtotal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2007 (generation 1)</td>
<td>6.5 x 10⁶</td>
<td>3,921</td>
<td>63</td>
<td>99</td>
<td>73</td>
<td>2.37 (0.33)</td>
<td>86</td>
</tr>
<tr>
<td>Select</td>
<td>Control 1</td>
<td>Control 2</td>
<td>Control 3</td>
<td>Subtotal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2009 (generation 2)</td>
<td>1.2 x 10⁷</td>
<td>5,472</td>
<td>96</td>
<td>137</td>
<td>84</td>
<td>4.17 (0.96)</td>
<td>84</td>
</tr>
<tr>
<td>Select</td>
<td>Control 1</td>
<td>Control 2</td>
<td>Control 3</td>
<td>Subtotal</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹All families reared separately in 2005 were selection-eligible; these numbers thus include the counts of fish (n = 430) and full-sib families (n = 7) given for control 2 in 2005.

²Number of pure-strain families pooled into mixed-family lots. Fish used in the challenge experiment were sampled from the mixed-family lot and thus had unknown family of origin.

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**Disease-Challenge Protocol**

The BCWD challenge protocol has been described previously in detail (Hadidi et al., 2008; Silverstein et al., 2009), and a brief outline is given here. Forty fish from each select-line family and control line were moved to 2.4-L challenge tanks (1 family/tank) in an isolated challenge facility at approximately 75 d posthatch. Two replicate challenge tanks were used for each of 43, 36, and 41 select-line families in 2005, 2007, and 2009, respectively, and a minimum of 3 replicate challenge tanks were used for each control line each year. Except for control 2 in 2005, fish from the purebred control lines were sampled from mixed-family lots, and thus had unknown family of origin. After moving to the challenge tanks, fish were given a 1-wk acclimation period in 2005 and 2009 and a 2-wk acclimation period in 2007. Fish were fed once daily to apparent satiation during the acclimation period. Challenge tanks were supplied with 1.9 L/min of flow-through spring water (i.e., the same source that supplied the 200-L family tanks), and water temperature was ≈12.9°C.

Previously, a bank of frozen aliquots was prepared from the culture of a single, genome-sequenced bacterial clone from a virulent strain of *F. psychrophilum* (CSF259-93; Crump et al., 2001; MacLean et al., 2001). Each year, an aliquot was cultured on tryptone yeast extract with salts agar plates for 5 d at 15°C. Bacterial cells were harvested, resuspended in PBS, and diluted to an optical density of 0.325 measured at 525 nm. Serial dilutions of the bacterial suspension were recultured on tryptone yeast extract with salts agar plates on the same day as the challenge to determine actual cfu per inoculation.

Fish were anesthetized in 90 mg/L of tricaine methanesulfonate (Tricaine-S, Western Chemical, Ferndale, WA) and injected intraperitoneally with 25 (2005 and 2007 hatch years) or 50 µL (2009 hatch year) of the bacterial suspension. Fish were heavier at challenge in 2009 compared with 2005 and 2007 (Table 2), and we chose to inject a larger volume of the bacterial suspension, rather than increasing its optical density, to obtain a dosage (i.e., cfu per gram of BW) comparable with 2005 and 2007. Each year, all fish in the experiment were challenged on the same day. In 2007 and 2009, contemporary fish (n = 229 and 159, respectively) were given sham injections of PBS to serve as negative controls. The posthatch age of fish at the time of challenge was approximately 84 d [alternatively, 1,050 degree days (°C x d)]. Average BW of a family or mixed-family lot was estimated from a sample of 75 (2005 hatch year) or 300 fish (2007 hatch year) on the date fish were moved to the challenge facility. In 2009, the total weight of fish in each challenge tank was recorded at the time of challenge, and average BW were calculated for each challenge tank.

Mortalities were recorded once daily for a 21-d period. Moribund fish were killed and recorded as mortalities. During the challenge, fish were fed to apparent satiation 3 times per week in 2005 and 2007, and once
per day in 2009. Fish surviving at the end of challenge were killed with an overdose of Tricaine-S. Table 2 gives summary data by year and line for the BCWD challenge.

**Breeding Value Estimation**

Each generation, family breeding values were estimated from all available survival data and all known pedigree relationships. Estimates were derived using 2 approaches: 1) survival status (dead or alive) and time to death or censoring data were described with a proportional-hazards frailty model using Survival Kit v.3.12 (Ducrocq and Sölkner, 1998), and 2) binary survival data were described with a linear animal model using MTDFREML (Boldman et al., 1995).

The proportional-hazards frailty model was

\[ h(t, X, Z_1, Z_2) = h_0(t) \exp(X\beta + Z_1a + Z_2r), \quad [1] \]

where \( h_0(t) \) is the baseline hazard function at time \( t \), \( \beta \) is a vector of fixed effects, \( a \) is a vector of random animal genetic effects with a multivariate normal distribution \( a \sim MVN(0, \text{A} \text{a}_{\text{animal}} \text{a}) \), where \( \text{A} \) is the numerator relationship matrix, \( r \) is a vector of random effects of challenge tank nested within family, and \( X, Z_1, \) and \( Z_2 \) are incidence matrices. Fixed effects were average BW of fish in a family or challenge tank (linear covariate) and hatch year (when appropriate). The baseline hazard function was unspecified and estimated according to the grouped-data approach of Prentice and Gloeckler (1978) because failure time was measured with limited precision (i.e., mortalities were recorded only once daily, thus resulting in many ties). Only one random effect in the model can be estimated from the mode of the marginal posterior density with Survival Kit, so sequential program runs were performed iteratively using updated variance estimates (i.e., alternating estimation of animal genetic and challenge tank effects while assuming the second random effect is known). Analysis was considered complete when estimates for both variance components were identical to the second decimal place after "cold" restarts were used as parents of the 96 families produced in 2007. Twelve families were selected, and a total of 35 sires and 63 dams were used as parents of the 63 families produced in 2007. The relative contribution of the 12 selected families to the progeny generation ranged from 1 to 11%. For the second generation of selection, 16 families were selected (out of 63 families in 2007) and a total of 47 sires and 90 dams were used as parents of the 96 families produced in 2009. The relative contribution of the 16 selected families to the progeny generation ranged from 1 to 11%. Matings in the purebred control lines were made at random with respect to BCWD resistance among fish with unknown family origin sampled from the mixed-family lots. Parents of control 2 in 2007 were sampled at random from the 7 control 2 families in 2005. Counts of full-sib and paternal and maternal half-sib families by generation and line are given in Table 1.

**Selection Protocol**

Selection and mating decisions were made each generation to maximize genetic response while constraining the rate of inbreeding to \( \leq 1\% \) per generation using EVA software (Berg et al., 2006). Selection was applied to family breeding values estimated from the proportional-hazards frailty model [1] and using all accumulated data and known pedigree relationships. All full-sib families maintained separately in the 2005 base generation (\( n = 71 \); this includes control 2 families) were selection-eligible (Table 1). Twelve families were selected, and a total of 35 sires and 63 dams were used as parents of the 63 families produced in 2007. The relative contribution of the 12 selected families to the progeny generation ranged from 2 to 17%. For the second generation of selection, 16 families were selected (out of 63 families in 2007) and a total of 47 sires and 90 dams were used as parents of the 96 families produced in 2009. The relative contribution of the 16 selected families to the progeny generation ranged from 1 to 11%. Matings in the purebred control lines were made at random with respect to BCWD resistance among fish with unknown family origin sampled from the mixed-family lots. Parents of control 2 in 2007 were sampled at random from the 7 control 2 families in 2005. Counts of full-sib and paternal and maternal half-sib families by generation and line are given in Table 1.

**Response to Selection**

Selection response was estimated using 2 approaches. First, survival percentages of select-line families and control-line mixed-family lots (\( n = 261 \) records) were described with a linear model using PROC MIXED (SAS Inst. Inc., Cary, NC) to adjust for nongenetic effects and obtain least squares means for select and control lines each generation. The model included fixed effects of line (4 levels), year (3 levels), and line \( \times \) year also tested without fitting genetic group effects. Variance of the likelihood in the simplex less than \( 1 \times 10^{-9} \) was used as the convergence criterion. Global minimum was assumed when \( -2 \times \log \text{likelihood} \) was identical to the third decimal place after "cold" restarts of the simplex with prior estimates of variance components. At convergence, estimates of covariate and genetic group effects, contrasts, and sampling variances were obtained.

Because family information for mixed-family lots in the control lines was unknown, unrelated dummy parents were included in the pedigree to estimate genetic merit for each control line each year. A total of 15,268 fish (not including genetic groups) were obtained. The proportional-hazards frailty model was

\[ h(t, X, Z_1, Z_2) = h_0(t) \exp(X\beta + Z_1a + Z_2r), \quad [1] \]

where \( h_0(t) \) is the baseline hazard function at time \( t \), \( \beta \) is a vector of fixed effects, \( a \) is a vector of random animal genetic effects with a multivariate normal distribution \( a \sim MVN(0, \text{A} \text{a}_{\text{animal}} \text{a}) \), where \( \text{A} \) is the numerator relationship matrix, \( r \) is a vector of random effects of challenge tank nested within family, and \( X, Z_1, \) and \( Z_2 \) are incidence matrices. Fixed effects were average BW of fish in a family or challenge tank (linear covariate) and hatch year (when appropriate). The baseline hazard function was unspecified and estimated according to the grouped-data approach of Prentice and Gloeckler (1978) because failure time was measured with limited precision (i.e., mortalities were recorded only once daily, thus resulting in many ties). Only one random effect in the model can be estimated from the mode of the marginal posterior density with Survival Kit, so sequential program runs were performed iteratively using updated variance estimates (i.e., alternating estimation of animal genetic and challenge tank effects while assuming the second random effect is known). Analysis was considered complete when estimates for both variance components were identical to the second decimal place in successive runs. Heritability of survival was estimated as

\[ \frac{\sigma_{\text{animal}}^2}{1 + \left(\frac{1}{4} \times \sigma_{\text{animal}}^2\right)} \]

modified from Yazdi et al. (2002).

The linear animal model was

\[ y = X\beta + Z_1a + Z_2r + e, \quad [2] \]

where \( y \) is a vector of binary observations (0 = died; 1 = survived) and the remaining model components were as described for the proportional-hazards frailty model [1]. The mixed-model equations for the linear animal model [2] were augmented to account for genetic group effects (i.e., founder strain effects; \( n = 4 \)) according to Westell et al. (1988). The linear animal model [2] was used as parents of the 96 families produced in 2007. Twelve families were selected, and a total of 35 sires and 63 dams were used as parents of the 63 families produced in 2007. The relative contribution of the 12 selected families to the progeny generation ranged from 1 to 11%. Matings in the purebred control lines were made at random with respect to BCWD resistance among fish with unknown family origin sampled from the mixed-family lots. Parents of control 2 in 2007 were sampled at random from the 7 control 2 families in 2005. Counts of full-sib and paternal and maternal half-sib families by generation and line are given in Table 1.
Table 3. Unadjusted means of family survival rate and days to death by line and year

<table>
<thead>
<tr>
<th>Hatch year/line</th>
<th>Mean (SD)</th>
<th>Range of survival, %</th>
<th>Mean (SD)</th>
<th>Range of survival, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>survival, %</td>
<td></td>
<td>days to death</td>
<td></td>
</tr>
<tr>
<td>2005 (generation 0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Select</td>
<td>20.4 (17.6)</td>
<td>1.3 to 72.5</td>
<td>8.4 (1.4)</td>
<td>3.3 to 15.5</td>
</tr>
<tr>
<td>Control 1</td>
<td>15.9 (5.1)</td>
<td>10.3 to 20.0</td>
<td>7.5 (0.5)</td>
<td>4.0 to 9.8</td>
</tr>
<tr>
<td>Control 2</td>
<td>33.6 (15.1)</td>
<td>10.0 to 48.7</td>
<td>8.8 (1.7)</td>
<td>3.5 to 13.1</td>
</tr>
<tr>
<td>Control 3</td>
<td>5.8 (3.8)</td>
<td>2.5 to 10.0</td>
<td>5.5 (0.6)</td>
<td>2.0 to 8.0</td>
</tr>
<tr>
<td>2007 (generation 1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Select</td>
<td>75.2 (12.0)</td>
<td>35.0 to 94.7</td>
<td>10.8 (1.6)</td>
<td>5.0 to 16.0</td>
</tr>
<tr>
<td>Control 1</td>
<td>51.7 (3.1)</td>
<td>48.8 to 55.0</td>
<td>11.7 (1.2)</td>
<td>7.0 to 15.0</td>
</tr>
<tr>
<td>Control 2</td>
<td>42.3 (14.3)</td>
<td>27.0 to 55.3</td>
<td>10.1 (0.8)</td>
<td>5.0 to 15.0</td>
</tr>
<tr>
<td>Control 3</td>
<td>27.7 (14.3)</td>
<td>15.8 to 43.5</td>
<td>9.6 (1.3)</td>
<td>4.0 to 14.0</td>
</tr>
<tr>
<td>2009 (generation 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Select</td>
<td>82.5 (13.2)</td>
<td>33.8 to 100.0</td>
<td>10.0 (2.5)</td>
<td>5.0 to 15.0</td>
</tr>
<tr>
<td>Control 1</td>
<td>14.9 (7.2)</td>
<td>7.5 to 22.0</td>
<td>8.8 (0.6)</td>
<td>4.0 to 12.0</td>
</tr>
<tr>
<td>Control 2</td>
<td>50.8 (6.3)</td>
<td>43.6 to 55.0</td>
<td>9.9 (1.4)</td>
<td>5.0 to 14.0</td>
</tr>
<tr>
<td>Control 3</td>
<td>11.7 (3.8)</td>
<td>7.5 to 15.0</td>
<td>6.7 (0.3)</td>
<td>3.0 to 9.0</td>
</tr>
</tbody>
</table>

1Means, SD, and ranges were calculated using challenge tank as the observational unit.

interaction, and BW as a linear covariate. Survival percentage of each challenge tank was included as a record for control 1 and control 3 in 2005 and control 1 to 3 in 2007 and 2009. Records of the 7 control 2 families in 2005 were duplicated, and 1 record each was assigned to control 2 and the select line. Linear contrasts of line × year least squares means were constructed to estimate differences between the select line and the average of the 3 control lines each generation.

Alternatively, additive genetic trend was estimated using family EBV derived from the linear animal model [2] and full data set. Mean EBV of select-line families and control lines were calculated each generation. Breeding values of control 2 families in 2005 were estimable and thus were weighted according to the relative contribution of the family to the progeny generation. A single control-line mean EBV was calculated each generation as the arithmetic average of the 3 control lines. For convenience, all EBV means were adjusted such that mean select-line EBV in 2005 was 0. Select- and control-line mean EBV were regressed on generation number, and the null hypotheses $b_{BVselect\,-\,generation} \neq 0$ and $b_{BVcontrol\,-\,generation} \neq 0$, where $b$ are the resulting regression coefficients for select and control lines, were each tested using a $t$-test with 1 df.

Accuracy of Selection

Breeding values estimated using collateral relative and ancestor data (i.e., no progeny data) for select-line families hatched in 2005 and 2007 were used to calculate mid-parent EBV for families hatched in 2007 and 2009, respectively. Mid-parent EBV and phenotypic survival of progeny families were standardized within generation and expressed in units of SD. Pearson correlations between standardized mid-parent EBV and progeny survival (n = 159 full-sib families) were calculated for the proportional-hazards frailty model [1] and linear animal model [2]. The $t$ and $z$ statistics for these correlations were calculated to test null hypotheses $r > 0$ and $r_1 = r_2$.

RESULTS

A total of 14,841 fish were challenged with BCWD during this study, and mean 21-d survival rate was 61.1%. Mortality in the negative controls was minimal (2.6 and 2.5% in 2007 and 2009, respectively); thus, all mortalities in experimental groups were assumed to result from the BCWD challenge. Mean survival rates and days to death by line and generation are given in Table 3, and observed Kaplan-Meier survival functions (Kaplan and Meier, 1958) by line and generation are given in Figure 1.

A likelihood ratio test comparing the linear animal model [2] with and without genetic group effects suggests that effects of founder strains were important ($\chi^2 = 30.7$ with 4 df; $P < 0.001$). Thus, only variance component and breeding value estimates from the model including group effects will be described. Solutions for genetic group effects, after adjusting base-generation EBV to 0, were $-18.6$, $10.0$, and $-17.7$ percentage points for control 1, 2, and 3, respectively, and $-3.3$ percentage points for the founder strain contributing germplasm to the select line but not maintained as a purebred control line. Pedigree-based estimates of founder-strain contribution of germplasm to the select line are given in Table 4 by year to illustrate the effect of selection on germplasm composition.

Heritability estimates from the proportional-hazards frailty model [1] and linear animal model [2] were similar ($0.22$ and $0.23 \pm 0.03$, respectively). When rescaled to the underlying continuous scale using the transformation given by Robertson in Dempster and Lerner.
(1950), the estimate of heritability from the linear animal model increased to 0.37. The Pearson correlation between family breeding values estimated from the 2 models was −0.92, indicating similar rankings of families.

Average family BW and year had important effects on survival ($P < 0.05$). For every 1-g increase in BW, survival increased $2.9 \pm 1.2$ percentage points (linear animal model [2]), or risk of death decreased approximately 26% (proportional-hazards frailty model [1]). Solutions for year effects were 1, 0.20, and 0.24 (expressed as risk ratios) from the proportional-hazards frailty model [1] and −4.3, 15.5, and 5.4 percentage points from the linear animal model [2] for 2005, 2007, and 2009, respectively. Variance attributable to challenge tank replicate accounted for less than 1% of phenotypic variance.

Phenotypic least squares means by line and year are given in Table 5. Response to selection, estimated from the contrast of differences between the select line and the average of the 3 control lines, was $24.6 \pm 6.8$ ($P < 0.01$) and $44.7 \pm 6.7$ ($P < 0.01$) percentage points after 1 and 2 generations, respectively. Genetic trend, estimated from the regression of EBV on generation, was $19.0 \pm 4.1$ percentage points ($P = 0.07$) for the select line and $-3.3 \pm 2.2$ ($P = 0.38$) percentage points for the average of 3 control lines (Figure 2). Genetic trends of select and control lines were significantly different ($t = 4.81$ with 2 df; $P = 0.04$).

Accuracy of selection, given as the correlation between mid-parent EBV and progeny survival, was $0.20 \pm 0.08$ ($P < 0.01$; absolute value of correlation is given) for the proportional-hazards frailty model [1] and $0.18 \pm 0.08$ ($P = 0.01$) for the linear animal model [2]. Accuracy estimates did not differ between the models ($P = 0.81$).

**DISCUSSION**

Henryon et al. (2005) were the first to report additive genetic variation for BCWD resistance in a Danish population of rainbow trout, and the authors concluded that selective breeding for improved resistance would be successful. We subsequently detected additive genetic variation for BCWD resistance in our population of rainbow trout and came to a similar conclusion (Silverstein et al., 2009). The current selection experiment provides empirical data to support these independent conclusions. Phenotypic survival in our select line increased by 24.6 and 44.7 percentage points after 1 and 2 generations of selection, respectively. Genetic trend, estimated using a linear animal model, was +19 percentage points per generation for the select line and approached significance. Although our estimate of genetic trend is quite large, the short duration of the selection experiment necessitates a test of significance with only 1 df and thus limits the statistical power to detect a genetic trend.

To our knowledge, this is the first report to quantify response to selection for BCWD resistance in rainbow trout. Moreover, there are relatively few literature reports that document response to selection for improved disease resistance in salmonids. Embody and Hyford...
Leeds et al.

Table 4. Pedigree-based estimates of mean strain composition (%) of select line by year

<table>
<thead>
<tr>
<th>Founder strain</th>
<th>2005 (0)</th>
<th>2007 (1)</th>
<th>2009 (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>8.5</td>
<td>9.5</td>
<td>7.6</td>
</tr>
<tr>
<td>Control 2</td>
<td>54.9</td>
<td>59.9</td>
<td>60.2</td>
</tr>
<tr>
<td>Control 3</td>
<td>22.9</td>
<td>14.3</td>
<td>15.2</td>
</tr>
<tr>
<td>Strain 4</td>
<td>13.7</td>
<td>16.3</td>
<td>16.9</td>
</tr>
</tbody>
</table>

1The select line was developed as a synthetic cross of control 1 to 3 and an additional founder strain (strain 4) not maintained as a purebred control line.

(1925) selectively bred surviving brook trout from a population with endemic furunculosis and increased survival rate by 67 percentage points after 3 generations (reviewed in Gjedrem and Thodesen, 2005). Dorson et al. (1995) tested rainbow trout from 14 sire families for resistance to viral hemorrhagic septicemia virus. Fish from a single-sire family exhibiting superior resistance were selected and mated, and survival of their progeny was 60.7 percentage points better than control fish. Storset et al. (2007) reported a 37 to 47 percentage point difference between Atlantic salmon divergently selected for 1 generation for resistance to infectious pancreatic necrosis virus. Okamoto et al. (1993) described a commercial strain of rainbow trout naturally selected for infectious pancreatic necrosis virus resistance. Although selection response was not quantified in their study, the authors reported that resistance was stable when the strain was maintained in a virus-free environment (i.e., in the absence of selective pressure).

Accurate estimation of selection response using an ordinary least-squares approach requires the use of a randomly mated control line to properly account for environmental effects (Blair and Pollak, 1984). In the absence of our contemporary controls, selection response would likely have been over- and underestimated in the first and second generations of selection, respectively.

It would seem likely that differences in challenge dose (i.e., total cfu or cfu per gram of BW) across years would largely describe year effects. However, the correlation between survival and estimated colony-forming units per gram of BW was only moderate (—0.51), and year effects were not absorbed when BW was replaced with colony-forming units per gram of BW (log10) and control-line data were analyzed separately (data not shown). Thus, control lines may be adjusting for unknown year effects that are independent of apparent challenge dose. Our estimates of challenge dose should be interpreted with caution because 1) colony-forming units are calculated from dilutions (e.g., 1:100,000) and are subject to substantial error; and 2) BW were recorded at different times relative to the start of challenge across years.

Alternatively, mixed-model methodology using an animal model allows separation of genetic and environmental effects, even in the absence of control lines.

Table 5. Least squares means (SE) by line and year for survival rate (%) and contrasts of line differences between years

<table>
<thead>
<tr>
<th>Year</th>
<th>Select</th>
<th>Control 1</th>
<th>Control 2</th>
<th>Control 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>31.1 ± 1.9</td>
<td>9.7 ± 8.4</td>
<td>34.9 ± 5.4</td>
<td>18.5 ± 8.3</td>
</tr>
<tr>
<td>2007</td>
<td>76.9 ± 2.0</td>
<td>44.6 ± 8.2</td>
<td>52.7 ± 8.1</td>
<td>29.3 ± 8.2</td>
</tr>
<tr>
<td>2009</td>
<td>79.7 ± 2.1</td>
<td>13.0 ± 8.2</td>
<td>50.1 ± 8.1</td>
<td>11.8 ± 8.1</td>
</tr>
<tr>
<td>Contrast&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Estimate, percentage points</td>
<td>P-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2007 minus 2005</td>
<td>24.6 ± 6.8</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2009 minus 2007</td>
<td>20.0 ± 7.1</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2009 minus 2005</td>
<td>44.7 ± 6.7</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Least squares were estimated from a model that fit line, year, and line × year interaction as fixed effects and BW as a linear covariate.

<sup>2</sup>Contrast of differences of select-line survival rate and average control-line survival rate between years shown. For example, the contrast “2007 minus 2005” = (76.9 — (44.6 + 52.7 + 29.3)/3 — (31.1 — (9.7 + 34.9 + 18.3)/3).
(Sorensen and Kennedy, 1986). Control-line data were included in our mixed-model analyses, but these data were expected to have little effect on select-line EBV because there were no pedigree relationships between select and control lines beyond genetic groups. Subsequent analysis confirmed this; correlation of EBV from the analyses with and without control-line data was 1.00. As discussed in al-Shorepy and Notter (1997), EBV give the best estimate of the genetic merit of an animal, and thus genetic trend for the population under selection, provided estimated genetic parameters are correct and the model is correctly specified.

The genetic trend estimate was similar to phenotypic response (19.0 and 22.3 percentage points/generation, respectively), which suggests that the genetic model was generally appropriate. The difference between these estimates may be partially attributable to effects of sampling on the estimates of control-line phenotypic performance each generation. Because fish with unknown ancestry were used for BCWD challenges and breeding, sampling effects may have been substantial for a given control line. However, the global effect of sampling was expected to be minimal because 3 independent control lines were maintained. Furthermore, the estimate of phenotypic response may be biased upward due to accumulation of inbreeding in the control lines. Inbreeding of control-line fish hatched in 2009 was calculated using estimates of effective population size (Falconer and Mackay, 1996) and ranged from 6.8 to 8.6%. Although inbreeding depression for general survival has been documented (reviewed in Fjalestad, 2005), the effect of inbreeding on BCWD resistance is unknown. Because inbreeding levels of individual control-line fish are not known, our data are not well-suited to estimate this effect.

Genetic gains in the select line were achieved without a substantial increase in inbreeding. Meuwissen and Woolliams (1994) estimated an effective population size between 31 and 250 animals per generation is needed to prevent a decline in fitness, and they discuss assumptions implicit in these estimates. Effective population size of our last generation (2009 hatch), given as $\frac{1}{2\Delta F}$, where $\Delta F$ is inbreeding rate calculated as $-\left[(F_t - 1)^{1/4} - 1\right]$, with $F_t$ representing inbreeding at generation $t$, was approximately 88 (Falconer and Mackay, 1996). Rate of inbreeding in our select population (0.57%) was similar to those reported for 3 nucleus breeding populations of rainbow trout in Norway (Pante et al., 2001).

The SE given for genetic trend does not explicitly account for drift variance. Exact calculation of drift variance is given by Sorensen and Kennedy (1986), but is difficult for reasons described in al-Shorepy and Notter (1997). Drift variance ($\sigma_d^2$) of a selected line can be approximated as $2F \sigma_A^2$, where $\sigma_A^2$ is additive genetic variance (Falconer and Mackay, 1996). Variance of the estimator ($\sigma_R^2$), including drift, is then estimated as $\sigma_R^2 = \sigma_A^2 + \sigma_e^2$, where $\sigma_e^2$ is error variance of the regression estimate. Resulting SE of genetic trend was then $19.0 \pm 5.5$ and still approached significance ($P = 0.09$). Because inbreeding in our select line was minimal (1.7%), accounting for drift had little effect on our interpretation of genetic trend.

The EBV derived from the proportional-hazards frailty model were used as selection criteria, but we chose to report genetic trend using estimates derived from the linear animal model for 3 reasons. First, the linear animal model is appealing because of its relative simplicity in terms of computation and interpretation. Second, MTDFREML allowed fitting of genetic group effects, which were important in this study because founder strains differed for resistance. Finally, family breeding values estimated from both models were highly correlated, and there was no difference in accuracy of EBV between the models. Ødegård et al. (2007) used a linear sire-dam model to predict progeny survival after infectious salmon anemia challenge and reported an accuracy similar to our linear animal model (0.22 and 0.18, respectively). Comparable estimates from the 2 models in this study suggest that the linear animal model correctly modeled the trait upon which selection was practiced and thus was appropriate for estimating genetic trend.

Survival models, such as our proportional-hazards frailty model, make full use of survival status, censoring, and time-to-event data in the analysis, whereas cross-sectional linear models only use survival status (Kachman, 1999). Furthermore, linear models can perform poorly when fit to dichotomous data (Gianola, 1980). Thus, survival models have proven superior in theory and empirical research for analysis of survival data and have gained widespread use in animal breeding contexts (Ducrocq and Casella, 1996). However, similarity between the survival and linear models in our study was not entirely unexpected because 1) frequency of either outcome was not extreme (i.e., mean survival during the study was 61%); 2) censoring only occurred at the end of the 21-d challenge; and 3) mortality had effectively ceased by the end of the challenge (i.e., during the final 3-d period of our challenges, only 2.2% of remaining fish died or became moribund). As a result, the binomial distribution was approximately normal, and survival status essentially contained all of the information available from the data. Other scientists have reported comparable results from survival and linear models when estimating genetic parameters from similar disease-challenge models in fish species (Ødegård et al., 2006; Kettunen et al., 2007).

Although results from the linear animal model fitting genetic group effects suggest that the founder strains differed in their resistance to BCWD, indirect selection for the most-resistant founder strain, and against the least-resistant strains, did not appear to describe the observed selection response. Founder-strain composition of the select line was relatively static across
gests that survival increased only 1 percentage point without error, weighting the genetic group solutions by the constraints imposed on inbreeding accumulation. To 0.95 between survival of Atlantic salmon in experi-

ceration appeal because of the large reduction of pathogen load. Data from the current experiment do not allow us to conclude if differences in resistance or tolerance mechanisms, or both, are responsible for differences in postchallenge survival rates of rainbow trout families. Our synonymous use of improved survival and improved resistance in the current and previous (Silverstein et al., 2009) reports is strictly a matter of convenience. We have, however, found smaller splenic bacterial loads in families with decreased mortality after BCWD challenge (Hadidi et al., 2008), thus implicating a resistance mechanism. These data were from a small subset of our population and thus should be considered preliminary.

In future studies we aim to evaluate the effect of selection for survival on BCWD resistance and tolerance. If it is found that both mechanisms of protection are responsible for improved survival, there will likely be debate as to which mechanism is the best target for selection. Selection for resistant genotypes has intuitive appeal because of the large reduction of pathogen load in a population. However, resistant genotypes can increase selective pressure for pathogen variants capable of evading the resistance mechanism of the host (Rausher, 2001; Woolhouse et al., 2002). Effects of increased pathogen load on other economically important traits (e.g., growth rate, feed efficiency, product quality, and resistance to other bacterial and viral diseases) is needed for development of multiple-trait selection indic-

The large estimates of phenotypic response and genetic trend from this selection experiment are encouraging for rainbow trout breeding programs, but 3 important caveats remain. First, it is important to validate our selection protocol in a field setting where fish are exposed to the pathogen via natural routes of infec-

Laboratory-based challenge models are laborious, and resistance cannot be measured directly on breeding candidates. Thus, identification of genetic markers and easily measured, nonlethal proxy traits will allow breeding programs to utilize within-family genetic variation for BCWD resistance. We previously reported a suggestive association between microsatellite markers in the major histocompatibility complex and BCWD resistance (Johnson et al., 2008), and are currently developing genomic tools to allow identification of QTL for BCWD resistance (Rexroad et al., 2008). Furthermore, we found that normalized spleen weight was correlated with BCWD resistance in a subset of our population (Hadidi et al., 2008). Because spleen weight and resistance did not share common environmental effects in that study (i.e., individual fish were not measured for both traits), the observed correlation was likely genetic in nature. Currently, nonlethal methods to measure spleen weight are not available.

It is well established that plant and animal species have 2 mechanisms for protection against harmful effects of pathogenic infection (Read et al., 2008; Schneider and Ayres, 2008; Råberg et al., 2009). Resistance is a mechanism by which the host directly attacks the pathogen, resulting in a reduced pathogen load. Tolerance, on the other hand, is a mechanism by which the host circumvents harmful effects of a given pathogen load. Data from the current experiment do not allow us to conclude if differences in resistance or tolerance mechanisms, or both, are responsible for differences in postchallenge survival rates of rainbow trout families. Our synonymous use of improved survival and improved resistance in the current and previous (Silverstein et al., 2009) reports is strictly a matter of convenience. We have, however, found smaller splenic bacterial loads in families with decreased mortality after BCWD challenge (Hadidi et al., 2008), thus implicating a resistance mechanism. These data were from a small subset of our population and thus should be considered preliminary.

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production traits will need to be minimal to merit selection for tolerant genotypes.

LITERATURE CITED


Leeds et al. 1946


