Response to five generations of selection for growth performance traits in rainbow trout (*Oncorhynchus mykiss*)

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Running title: Selection for growth in rainbow trout

Key words: heritability, genetic correlation, growth, rainbow trout, selection

ABSTRACT

A pedigreed rainbow trout population (~100 families per generation) was selected for five generations to improve growth performance to the standard ~500-gram US market weight and beyond (>1 kg). Body weights (BW) were recorded each generation at 5, 8, 10, and 13 months post-hatch. Selection was applied to a two-trait index of estimated breeding values for 10-month body weight (i.e., approximate market weight) and thermal growth coefficient between 10 and 13 months, and both traits were given equal weighting in the index. Objectives of this study were to estimate direct selection responses for BW traits and thermal growth coefficient, and correlated response for condition factor traits. Heritability estimates were 0.28 ± 0.08 to 0.43 ± 0.14 for BW between 5 and 13 months post-hatch, 0.12 ± 0.03 for thermal growth coefficient, and 0.40 ± 0.04 and 0.36 ± 0.04 for condition factor at 10 and 13 months post-hatch, respectively. Genetic correlations among the four BW measures were **highest between adjacent BW measures (0.54 ± 0.19 to 0.82 ± 0.05) and decreased as time between measures increased.** Genetic correlation between thermal growth coefficient and 10-month BW was low (0.18 ± 0.13), and genetic correlations between condition factor and BW measured at the same age were moderate (0.39 ± 0.09 to 0.45 ± 0.08). Compared to randomly-mated control lines, selection response in the selected line averaged 10.6% (5-month BW), **9.8%** (8-month BW), **12.7%** (10-month BW), **11.9%** (13-month BW), 2.1% (thermal growth coefficient), 3.4% (10-month condition factor), and 3.3% (13-month condition factor) per generation. The rate of inbreeding averaged 0.86% per generation in the selection line, with a cumulative inbreeding estimate of 4.5% in the last generation. The low heritability of thermal growth coefficient, and low genetic

correlation with 10-month BW, resulted in only modest selection response for thermal growth coefficient. **Larger heritabilities of BW measured at fixed ages, and large genetic correlations between serial BW measures, suggest that direct selection on BW will be more favorable than selection on TGC. This study demonstrates the large and sustained potential for genetic improvement of growth performance in domesticated rainbow trout with only modest accumulation of inbreeding, and provides empirical selection response estimates that have direct inference to commercial breeding programs aiming to select for growth to larger market BW.**

1. Introduction

In contrast to terrestrial livestock species, domestication and systematic breeding of salmonids has occurred within the past 40 years. Growth traits are logical selection criteria because of their economic importance, ease of measurement, large phenotypic variation (coefficient of variation $\approx 30\%$), and moderate-to-large heritabilities (Gjedrem, 1983; Gjerde, 1986; Gjedrem, 1992). Gjedrem (1975; 1983) predicted that substantial genetic gains in growth rates were possible based on available knowledge and emphasized the need to develop breeding programs for fish species. The first family-based selective breeding program in aquaculture was started in Norway by AKVAFORSK in 1975 for Atlantic salmon (Gjedrem, 2010) and rainbow trout (Gjedrem, 1985). To date, results from several selection experiments for improved growth rate in salmonids have been reported, and the data suggest gains of 10 to 15% per generation are possible (Kincaid et al., 1977; Gjerde, 1986; Hershberger et al., 1990; Gjedrem, 2010). These selection experiments represent several commercially-important salmonid species and diverse breeding objectives,

69 population sizes, duration and intensity of selection, production environments, and
70 estimation methods. Data from relatively few rainbow trout breeding programs are
71 available in the literature.

72 In the US, farmed rainbow trout (*Oncorhynchus mykiss*) are typically marketed at a
73 body weight (**BW**) of 400 to 600 g when fish are between 10 and 18 months old (Fornshell,
74 2002; FAO, 2005). There is interest in marketing larger fish (≥ 1 kg), but at sexual
75 maturation (≤ 2 years for male rainbow trout; ≥ 2 years for female rainbow trout) growth
76 and fillet quality traits are adversely affected in salmonids (Aksnes et al., 1986;
77 Aussanasuwannakul et al., 2011). The use of sex control and triploid induction strategies
78 have been effective for averting effects of sexual maturation on growth and fillet quality
79 traits, and thus increasing the marketable size of fish (Piferrer, 2001; Piferrer et al., 2009).
80 Selective breeding is a complimentary strategy that can be used in conjunction with sex
81 control and triploid induction to increase the marketable size of fish. If genetic gains are
82 large enough, selective breeding may reduce the need for sex control and triploid induction
83 strategies by allowing fish to reach a larger market size before the negative effects of sexual
84 maturation manifest.

85 A family-based selective breeding program was initiated at the USDA, ARS,
86 National Center for Cool and Cold Water Aquaculture (NCCCWA; Kearneysville, WV)
87 beginning in 2004 to improve growth performance in diploid rainbow trout to and beyond
88 the standard market BW and before sexual maturation. In addition to the selection line,
89 contemporary, randomly-mated control lines were maintained to estimate direct and
90 correlated selection response. Traits targeted for genetic improvement in the selection line
91 included BW at approximately 10 months post-hatch (i.e., when fish approach standard

market BW) and thermal growth coefficient (**TGC**; Iwama and Tautz, 1981; Jobling, 2003) for the growth period between 10 and 13 months post-hatch. Previous research has shown TGC to be moderately heritable (Silverstein et al., 2009), and TGC measured during an earlier growth period (between 9 and 10 months post-hatch) was a better predictor of BW at a later age (15 months post-hatch) than was a single BW measure at an earlier age of 10 months post-hatch (Lankford and Weber, 2006). Thus, the TGC was included as a novel selection criterion to maintain, and perhaps improve, genetic potential for growth trajectory beyond the standard market BW. The objectives of this study were to: 1) estimate genetic (co)variance parameters for serial BW measures, TGC, and condition factor (**CF**); and 2) estimate direct and correlated response to five generations of selection for improved growth performance in rainbow trout using contemporary control lines and genetic trends.

2. Material and methods

2.1. Resource populations

A synthetic line of 2-year-old winter/spring-spawning rainbow trout was developed at the NCCCWA from 2002 to 2004 by intercrossing seven founder strains. The founder strains were chosen because of their documented domestication and genetic diversity and included 1) College of Southern Idaho, House Creek; 2) Ennis (MT) National Fish Hatchery, Shasta; 3) Kamloops/Puget Sound Steelhead cross; 4) University of Washington, Donaldson; 5) Hagerman (ID) Fish Culture Experiment Station, Arlee; 6) Hagerman (ID) Fish Culture Experiment Station, Kamloops; and 7) Idaho Department of Fish and Game, Hayspur. Unfertilized eggs and milt from the founder strains were received at the NCCCWA from populations testing negative for viral hemorrhagic septicemia virus,

infectious hematopoietic necrosis virus, infectious pancreatic necrosis virus, infectious salmon anemia virus, and *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease. The synthetic line became a closed population in 2004 and has since undergone five generations of selection for improved growth (hereafter referenced as the **Select** line; details of selection are given in 2.6). Five of the founder strains had minimal contribution ($\leq 9\%$ ancestry) to the Select line based on pedigree data, and four of these strains had no contribution after three generations of selection (Table 1). Additionally, remnants of 3 of the founder strains (i.e., Shasta, Kamloops/Puget Sound Steelhead cross, and Donaldson; hereafter referenced as **FS/Control 1**, **FS/Control 2**, and **FS/Control 3**, respectively) were maintained as purebred, randomly-mated control lines; these control lines likewise became closed populations in 2004.

Unlike the Select line, in which the 2004 base population was derived from 143 parents and complete pedigrees were maintained each generation, the FS/Control lines were derived from only 24 to 36 parents with unknown relatedness, and pedigrees were not maintained. Because of the potential for inbreeding depression and genetic drift in the FS/Control lines to affect long-term selection response estimates, and with the development of a microsatellite multiplex system for parental allocation (Johnson et al., 2007), a fully-pedigreed Synthetic Control line was developed in 2010 by randomly mating 2008 year class Select-line fish (details of line development and propagation given below). The Synthetic Control line thus represents two generations of growth selection followed by three generations of random mating, and has nearly identical ancestry (i.e., founder strain composition; Table 1) and levels of inbreeding with that of the Select line (Table 2).

2.2. General hatchery practices

The NCCCWA Institutional Animal Care and Use Committee (Leetown, WV) reviewed and approved all experimental procedures used in this study. Each generation, single-sire $\times$ single-dam matings were made and full-sib families were reared separately in vertical incubation trays or upwelling jars supplied with spring water. During establishment of the Select and FS/Control lines (i.e., 2002 and 2004 hatch years), matings were made between the months of October and March. Water temperature in the incubation trays was manipulated whenever possible to synchronize hatch dates within generation as previously described (Leeds et al., 2010); however, the extended spawning periods (particularly in 2004) precluded our ability to synchronize the hatch dates of all families. Thereafter (i.e., 2006, 2008, 2010, 2012, and 2014 hatch years), matings were made between late December and early March and water temperature during egg incubation was manipulated between 6 and 13°C so that all families hatched within a 1- to 3-wk period each generation.

Eyed eggs or yolk-sac fry were moved to 200-L tanks and reared on flow-through spring water (ambient temperature range $\approx$ 12 to 13°C). Select-line families were maintained in individual family tanks (1 tank per family) to retain family-of-origin identification. FS/Control-line families were pooled into mixed-family lots by genetic line as eyed eggs or yolk-sac fry to minimize demands on facility resources, and thus family-of-origin information for individual FS/Control-line fish was unknown. Each FS/Control-line mixed-family lot represented an admixture of between 5 and 23 full-sib families. Only one FS/Control-line mixed-family lot was developed per genetic line each generation, but each lot was split into two replicate 200-L tanks. Synthetic Control families were also pooled

into mixed-family lots as eyed eggs or yolk-sac fry to minimize demands on facility resources, but at tagging each fish was fin clipped and subsequently genotyped using a microsatellite multiplex system (Johnson et al., 2007) to reconstruct fish-specific pedigrees. Each generation, the Synthetic Control mixed-family lot represented an initial admixture of 20 to 53 full-sib families; however, approximately 5% of families were not recovered at the time of tagging due either to differential family post-hatch survival, sampling, or ambiguous genotype data. Two mixed-family lots were developed each generation for the Synthetic Control line (~50% of families were assigned to each lot, and each family was assigned to only one lot), and each lot was split into two replicate 200-L tanks. Table 2 summarizes counts of full-sib families, sires, and dams by generation and genetic line used in the study.

2.3. *Sex reversal*

The FS/Control 2 line was derived from an all-female population. Thus, a subset of approximately 50 yolk-sac fry per mixed-family lot were reared separately each generation and masculinized by feeding 2 mg/kg of 17 α -methyltestosterone (Steraloids, Newport, RI) for 60 d post swim-up, and the masculinized females were used as sires of the subsequent generation. The Select, FS/Control 1, FS/Control 3, and Synthetic Control lines were initially comprised of mixed-sex families, but beginning with the 2008 hatch year a subset of approximately 50 yolk-sac fry from every family or mixed-family lot were sampled and treated with 17 α -methyltestosterone in the same manner as for FS/Control 2. All families produced in the 2010, 2012, and 2014 hatch years were derived using sires that were treated as yolk-sac fry with 17 α -methyltestosterone. Non-functional males (i.e., those in which

milt could not be expressed as normal by applying abdominal pressure) were preferentially used as sires because of the increased likelihood that they were genetic females (i.e., neomales). Based on progeny test data and genotyping at the *sdY* locus (Yano et al., 2012), approximately 75% of non-functional males were confirmed to be genetic females, and thus the conversion to all-female lines was complete within two generations (data not shown). Our aim was to convert the Select and all control lines to all-female families to better match industry practice and the needs of stakeholders.

2.4. General husbandry practices

Each 200-L family tank was initially stocked with approximately 600 yolk-sac fry. From 2002 to 2008, fish were hand-fed a commercial fishmeal-based diet (50 to 55% protein, 15% fat; Zeigler Bros Inc., Gardners, PA) by an experienced animal caretaker beginning at swim-up. Beginning in 2010 and thereafter, fish were fed the same diet as in previous years, but were fed using a programmable robotic feeding system (Arvotec, Huutokoski, Finland). Approximately once per month, fish were culled at random (with the exception that fish with visible deformities were preferentially culled) to standardize the number of fish per family tank and maintain biomass densities $< 50 \text{ kg/m}^3$. Fish were immersion-vaccinated (Ermogen; Novartis Aqua Health Ltd., Prince Edward Island, Canada) at 4 to 5 months post-hatch against *Yersinia Ruckeri* serotype 1, the causative agent of enteric redmouth disease.

At 4-5 months post-hatch (BW range = 20-80 g), fish were sampled at random from each Select-line family or mixed-family control lot, anesthetized in approximately 100 mg/L of tricaine methanesulfonate (Tricaine-S, Western Chemical, Ferndale, WA), and

given unique identification by tagging with a passive integrated transponder (Avid Identification Systems Inc., Norco, CA) in the left-side dorsal musculature (hatch years 2002-2010) or intraperitoneally (hatch years 2012 and 2014). Approximately 25 fish per Select-line family, 75 fish per FS/Control line, and 170 Synthetic Control fish were tagged each generation, and generally all fish were tagged within a 1-week period each generation. In addition, sibling fish that were masculinized using 17α -methyltestosterone were tagged at the same time for use as sires of the subsequent generation; approximately 12 fish per Select-line family, 40 fish per FS/Control line, and 100 Synthetic Control fish were tagged each generation for this purpose.

After tagging, fish were commingled in 800-L communal tanks (average = 7.7 tanks per generation for non-masculinized fish; range = 5 – 15 tanks) supplied with processed spring water (ambient temperature range $\approx$ 12.5 to 13.7°C) that was partially recirculated. Masculinized and non-masculinized fish were reared in separate communal tanks, and each tank was stocked with an equal representation of all families and genetic lines. Fish were fed a commercial fishmeal-based diet (42% protein, 16% fat; Zeigler Bros Inc., Gardners, PA) using programmable automatic feeders (Arvotec). The initial daily feeding rate in young fish was approximately 2.5% of BW, and the daily feeding rate was gradually decreased to approximately 1.0% of BW as fish grew. This feeding schedule is generally similar to that described in Hinshaw (1999). Fish hatched in 2006 were also fed once per day to apparent satiation by hand, and the base feeding schedule was increased by 10 to 20% in 2012 and 2014 to maximize growth. Once every 2 to 3 months the non-masculinized fish were anesthetized, individually weighed and measured for fork length, and divided into additional 800-, 3,800-, or 13,400-L communal tanks to maintain biomass

densities $\leq 130 \text{ kg/m}^3$. At the end of the 13-month growth trial, fish were stocked in an average of 16.7 communal rearing tanks per generation (range = 3 – 23 tanks).

2.5. Data collection and editing

Individual BW and fork length were measured on non-masculinized fish at tagging (**5-month BW**; mean post-hatch age = 165 d; SD = 11.4 d) and at 230 d (**8-month BW**; SD = 7.0 d), 295 d (**10-month BW**; SD = 18.8 d), and 386 d (**13-month BW**; SD = 12.9 d) post-hatch each generation beginning in 2002 using a Biomark tagging station (Biomark, Boise, ID). The BW data were checked for outliers by first identifying records less or greater than 3 SD compared to the respective family mean BW, and then evaluating each outlier datum against the corresponding fork length measurement and serial BW data for the respective fish. Approximately 0.25% of the BW records were considered erroneous and thus were removed from the dataset; however, BW records from fish with confirmed static or decreasing BW across time remained in the dataset. The TGC was calculated for all fish with valid 10- and 13-month BW records as $[(\sqrt[3]{W_f} - \sqrt[3]{W_i}) / (T \times t)] \times 1,000$, where W_f and W_i are 13-month BW and 10-month BW, respectively, T is water temperature ($^{\circ}\text{C}$), and t is time in days (Iwama and Tautz, 1981; Jobling, 2003). A constant 13°C was assumed for all TGC calculations because 1) all communal tanks were supplied with water from the same source; 2) daily water temperatures were not recorded for each communal tank; and 3) ambient water temperature did not vary substantially during this growth period. Condition factor of each fish was calculated at 10- and 13-months of age (**10-mo CF** and **13-mo CF**, respectively) as $\text{CF} = (10^5 \times \text{BW}) / \text{FL}^3$, where BW is in grams and FL is fork length in millimeters.

2.6. Selection and mating design

Breeding values for Select-line fish were estimated for 10-month BW and TGC with a multiple-trait animal model using MTDFREML (Boldman et al., 1995). The animal model included fixed effects of hatch year and age (linear covariate), random animal additive genetic effects, and uncorrelated random effects of full-sib family and communal rearing tank (nested within generation). The random full-sib family effect was included to account for non-additive genetic effects and effects of family rearing tank until the time of tagging. Each generation, breeding values were estimated using all available ancestor data and all known pedigree relationships.

An index of estimated breeding values for 10-month BW and TGC was used as the selection criterion in the latter 4 generations (i.e., selection of parents of 2008, 2010, 2012, and 2014 year classes), but only the 10-month BW estimated breeding value was used in the first generation (i.e., selection of parents of 2006 year class). To derive the 2-trait selection index, estimated breeding values for both traits were first standardized by expressing them as standard deviation units above and below the mean for the current generation. The 2-trait selection index value was then calculated as the mean of the standardized EBVs for the two traits. Whereas fish-specific EBV index values were available for females in all generations, neomales were not performance tested and thus only had family-specific EBV index values available for use. Selection and mating decisions were made each generation to maximize genetic gain while constraining the rate of inbreeding to $\leq 1\%$ per generation using an evolutionary algorithm (EVA software; Berg et al., 2006). Neomales were generally selected each generation from full-sib families in

the top ~15% for the selection index, and females were selected from families in the top ~35%. Across all five generations of selection, selected neomales had an EBV index that averaged 0.83 SD units greater, and selected females 0.86 SD units greater, than the population mean for the respective generation.

Parents for each of the FS/Control lines were mated at random within line with respect to growth performance, and pedigrees were unknown. Parents for the Synthetic Control line were sampled at random with respect to growth performance from all available families within the line to maintain maximum genetic diversity, and mated to minimize inbreeding in the progeny families. Some paternal half-sib families were produced each generation in the Select and FS/Control lines, and in 2014 (generation 5) for the Synthetic Control line (Table 2). Maternal half-sib families were only produced in the Select line in the 2004 base generation (Generation 0).

2.7. Selection response and genetic parameter estimation

Selection response was estimated using 2 approaches. First, BW, TGC, and CF data were described with linear mixed-effects models using PROC MIXED (SAS Inst. Inc., Cary, NC) to adjust for non-genetic effects and obtain least squares means for Select and control lines each generation. The model for 5-month BW included fixed effects of line, hatch year, and line $\times$ hatch year interaction, post-hatch age in days (linear covariate), and a random effect of family nested within line. Communal rearing tank nested within hatch year was added to the model as a **fixed** effect to describe the other BW, TGC, and CF traits. For TGC, post-hatch age was the age of the fish at the beginning of the growth performance period. The Satterthwaite option was used to approximate denominator degrees of freedom

for tests of fixed effects. Linear contrasts of the line $\times$ hatch year least squares means were constructed to estimate differences between the Select line versus the mean of the three FS/Control lines, or the Select line versus the Synthetic Control line, for each trait in successive generations to estimate cumulative phenotypic selection response. Linear contrasts between the Select and the mean of the three FS/Control lines for each i^{th} generation of selection were constructed to adjust for differences already existing between the lines in the 2004 base generation (0) as:

$$\text{response}_{(i)} = [\text{Select line}_{(i)} - (\text{FS/Control } 1_{(i)} + \text{FS/Control } 2_{(i)} + \text{FS/Control } 3_{(i)})/3] \\ - [\text{Select line}_{(0)} - (\text{FS/Control } 1_{(0)} + \text{FS/Control } 2_{(0)} + \text{FS/Control } 3_{(0)})/3]$$

Alternatively, additive genetic trend was estimated using breeding values derived from a 7-trait animal model using WOMBAT (Meyer, 2007). **The mixed model is:**

$$\begin{bmatrix} y_1 \\ \vdots \\ y_7 \end{bmatrix} = \begin{bmatrix} X_1 & 0 & 0 \\ \vdots & \ddots & \vdots \\ 0 & 0 & X_7 \end{bmatrix} \begin{bmatrix} \beta_1 \\ \vdots \\ \beta_7 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 & 0 \\ \vdots & \ddots & \vdots \\ 0 & 0 & Z_7 \end{bmatrix} \begin{bmatrix} a_1 \\ \vdots \\ a_7 \end{bmatrix} + \begin{bmatrix} F_1 & 0 & 0 \\ \vdots & \ddots & \vdots \\ 0 & 0 & F_7 \end{bmatrix} \begin{bmatrix} f_1 \\ \vdots \\ f_7 \end{bmatrix} + \begin{bmatrix} e_1 \\ \vdots \\ e_7 \end{bmatrix}$$

in which y_i is the vector of observations for the i^{th} trait; β_i is the vector of fixed effects; a_i , f_i , and e_i are vectors of random animal additive genetic, full-sib family, and residual effects, respectively; and X_i , Z_i , **and** F_i are known incidence matrices relating observations to β , a_i , **and** f_i , respectively. Fixed effects included hatch year, **communal rearing tank**, and age (linear covariate). **Communal rearing tank effects were fitted for all traits except 5-month BW because this effect was completely confounded with family at the time of tagging.** First and second moments of the model **are:**

$$\mathbf{E} \begin{bmatrix} y_i \\ a_i \\ f_i \\ e_i \end{bmatrix} = \begin{bmatrix} X_i \beta_i \\ 0 \\ 0 \\ 0 \end{bmatrix}; \quad \text{var} \begin{bmatrix} a_i \\ f_i \\ e_i \end{bmatrix} = \begin{bmatrix} G & 0 & 0 \\ 0 & I\sigma_{f_i}^2 & 0 \\ 0 & 0 & R \end{bmatrix};$$

319 in which $G = \begin{bmatrix} A\sigma_{a_1}^2 & & sym \\ \vdots & \ddots & \\ A\sigma_{a_1a_7} & \dots & A\sigma_{a_7}^2 \end{bmatrix}$, $R = \begin{bmatrix} I\sigma_{e_1}^2 & & sym \\ \vdots & \ddots & \\ I\sigma_{e_1e_7} & \dots & I\sigma_{e_7}^2 \end{bmatrix}$, A is the numerator

320 relationship matrix, and I are identity matrices of the appropriate order. Change in the log
 321 likelihood less than 1×10^{-4} , change in the parameter vector less than 1×10^{-8} , and a norm
 322 of gradient vector less than 1×10^{-3} were used as the convergence criteria. A total of
 323 71,340 BW records, 15,791 TCG records, and 25,555 CF records from 21,630 fish
 324 (including pre-2004 founder fish) were used in the analyses, and 22,163 fish were used in
 325 the calculation of the inverse of the numerator relationship matrix.

326 Mean breeding values of Select-line families were calculated each generation. For
 327 convenience, all breeding value means were adjusted such that the mean Select-line
 328 breeding value in the base generation was 0. Genetic trends were estimated using PROC
 329 REG (SAS Inst. Inc., Cary, NC) by regressing Select-line mean breeding values on
 330 generation number, and the null hypothesis $b_{BV_{select} \bullet generation} \neq 0$, where b is the regression
 331 coefficient, was tested using a t test.

332

333 **3. Results**

334 *3.1. Genetic parameter estimates*

335 Heritability estimates (Table 3) were moderate for BW traits (0.27 ± 0.03 to $0.43 \pm$
 336 0.14), low for TGC (0.12 ± 0.03), and moderate for CF (0.36 ± 0.04 and 0.40 ± 0.04). Non-
 337 additive full-sib family effects accounted for a greater proportion of phenotypic variation
 338 for 5-month BW ($28 \pm 6.7\%$) and 8-month BW ($28 \pm 4.8\%$) than 10-month BW ($17.4 \pm$
 339 2.3%), 13-month BW ($14.7 \pm 1.3\%$), TGC ($10.4 \pm 1.1\%$), 10-month CF ($15.7 \pm 1.6\%$), or

13-month CF ($14.3 \pm 1.4\%$), likely because of confounding between family and pre-tagging rearing tank.

Genetic correlations (Table 3) were large (0.54 ± 0.19 to 0.82 ± 0.05) **between adjacent BW measures** and decreased as the time between measures increased. The genetic correlation between TGC and BW traits was greatest at 13-months (0.50 ± 0.08) and consistently decreased as age at the BW measurement decreased. The 10- and 13-month CF measures were moderately correlated with 10- and 13-month BW (0.38 ± 0.09 to 0.45 ± 0.08) and not correlated with TGC (-0.05 ± 0.12 and 0.16 ± 0.12 , respectively).

3.2. Selection response

3.2.1. Phenotypic selection response: The interaction between genetic line and generation was significant ($P \leq 0.025$) for all traits except TGC ($P = 0.41$), thus indicating divergence of BW and CF traits between the lines over generations as a result of selection (Table 4).

Genetic gains per generation given as original trait units (Figure 1) were larger for all traits except TGC when using the Synthetic Control line as the reference control population.

With reference to the FS/Control and Synthetic Control lines, the phenotypic selection response estimates in Figure 1 represent gains of 8.5 and 12.6% (5-month BW), **7.9** and **11.8%** (8-month BW), **12.3** and **13.1%** (10-month BW), **12.5** and **11.4%** (13-month BW), **3.3** and **0.9%** (TGC), **3.0** and 3.8% (10-month CF), and **2.1** and **4.5%** (13-month CF) per generation, respectively, compared to the respective base population means (i.e., hatch years 2004 and 2008 in the Select line).

3.2.2. *Genetic trends*: All genetic trends, estimated as the regression of mean Select-line family breeding values on generation, were positive and significantly differed from 0 ($P \leq 0.0001$; Figure 2). Genetic trend estimates were generally more conservative than estimates of phenotypic selection response given in Figure 1; genetic trend **was** only larger than the phenotypic selection response estimate for TGC versus the Synthetic Control line. The rate of inbreeding averaged 0.86% (range = 0.3 to 1.6%) per generation over five generations in the Select line, and 0.80% (range = 0.2 to 1.3%) per generation over three generations in the Synthetic Control line (Table 2).

4. Discussion

4.1. *Direct and correlated selection response*

Compared to terrestrial livestock species (e.g., Zollinger and Nielsen, 1984; Chen et al., 2003), selection response estimates for growth in fish are generally large and often exceed 10% per generation (reviewed in Gjerde, 1986; Gjedrem and Thodesen, 2005). The favorable and consistent selection response of ~11% per generation for BW traits in the Select line was anticipated due to the relatively high heritabilities and genetic correlations among the four BW traits, and also due to the large coefficient of variation of traits (~25%; data not shown) which are comparable to other estimates in salmonids (reviewed in Gjedrem and Olesen, 2005). The average rate of inbreeding of 0.86% per generation in the Select line resulted in cumulative inbreeding of 4.5% after five generations of selection. Kause et al. (2005) selected two rainbow trout populations for two generations each using an index of BW at sea and in fresh water and reported an average genetic trend of approximately 7% per generation in growth to various market weights (1 - 3 kg). Similar to

the current study, their resource populations were developed by intercrossing multiple ($n =$ 4) founder strains, and inbreeding was constrained to $<1\%$ per generation. Neira et al. (2006) selected for increased harvest weight (≥ 2.5 kg) in even- and odd-year Coho salmon populations for four generations each and estimated mean selection responses of 10.5 and 9.9% per generation, respectively. In that study, the rates of inbreeding averaged over four generations of selection were 2.5 (even year) and 1.1% (odd year). In a historical review of the AKVAFORSK Atlantic salmon breeding program that started in 1975, Gjedrem (2010) cites a selection response of approximately 14% per generation for BW at harvest. Hershberger et al. (1990) selected two lines of coho salmon for four generations using an index of growth and smoltification traits and reported a 6.4 to 10.1% increase per generation in BW at 7 to 8 months of age (~ 400 g). Cumulative inbreeding levels in that study were 5.9 and 8.3%, which equate to inbreeding rates of 1.5 and 2.1% per generation, respectively, assuming no inbreeding in the base population. O’Flynn et al. (1999) selected Atlantic salmon for two generations using an index of growth, smoltification, maturation, and disease resistance traits and increased harvest BW by 880 g ($\sim 10\%$ per generation). Chevassus et al. (2004) describe the use of the “PROSPER” method of mass selection for increased body length in brown trout at 1 year of age. After four generations of intense truncation selection (1.2 to 9.7%), they reported a correlated response in BW of 21.5% per generation. Rate of inbreeding in that experiment was not reported, but the “PROSPER” method aimed to keep an effective population size of approximately 100. By comparison, effective population size of the Select line in the current study, estimated as $1/(2 \times \Delta F)$ where ΔF is the average rate of inbreeding over time (Table 2), was 66. Kincaid et al. (1977) practiced three generations of selection for BW in young rainbow trout (< 7 g) and

reported a cumulative selection response of 30.1% and cumulative inbreeding in the selection line of 6.25% (approximate rate of inbreeding = 2.1% per generation).

Despite a similar weighting to that of 10-month BW in the selection index, TGC in the current study was lowly heritable and only modestly correlated with 10-month BW and thus resulted in a selection response of only **0.9** and **3.3%** per generation when compared to the Synthetic Control and FS/Control lines, respectively. The design of this selection experiment and the four serial BW measures do not allow us to directly compare the efficacy of using TGC versus BW at a fixed age as selection criteria to improve later growth. Cleveland et al. (2016) provide data that suggest improved growth performance of the Select line compared to the fastest-growing commercial line at 1 kg and beyond (i.e., ≥ 13 months post-hatch). It is **likely** that inclusion of TGC between 10 and 13 months post-hatch in the selection index contributed to the improved late-growth performance in the Select line. However, **the data suggest** that differences between the two lines would have been even greater had 13-month BW been included as a selection criterion instead of TGC **based the following predictions. Using genetic parameter estimates from the current population (i.e., heritabilities and genetic correlations in Table 3 and an additive genetic variance of 13.77 kg² for 13-month BW) and assuming truncation selection at 1 SD above the population mean (i.e., selection intensity = 1.5), correlated response (Falconer and Mackay, 1996) in 13-month BW would be more than two-fold greater using 10-month BW as the selection criterion (79.1 g/generation) compared to using TGC (30.5 g/generation), despite 13-month BW being used as the final body weight in the TGC calculation. Similarly, correlated response in 13-month BW is predicted to be larger when selecting on 10-month BW than TGC between 8 and 10 months post-**

hatch, although it is interesting to note that we found larger genetic correlation of TGC measured at this earlier age, compared to TGC measured between 10 and 13 months post-hatch, with 13-month BW (data not shown).

Contrary to the data of Lankford and Weber (2006), in the current study TGC during an early growth period (e.g., between 5 and 8 months post-hatch) is not a better predictor of 13-month BW compared to 8-month BW (data not shown). Whereas the predictive ability of TGC was similar in both studies (i.e., phenotypic correlation ≈ 0.58), phenotypic correlation between 10- and 15-month BW in Lankford and Weber (2006) was smaller ($r = 0.37$) than between 8- and 13-month BW in the current study ($r = 0.71$). Exact reasons for this discrepancy are unknown, but there were differences between the studies in age and growth rate during the approximately 160-day growth period that may be important (i.e., fish in the current study were approximately 70 days younger and increased BW by approximately 5.3-fold compared to 3.2-fold). Thus, the merit of using TGC as a selection criterion for improving later growth may depend on the magnitude of phenotypic and genetic correlation observed among serial BW measures in the population.

In agreement with the current study, O'Flynn et al. (1999) reported a positive correlated response in CF of approximately 1.3% per generation in a Canadian Atlantic salmon population. Likewise, Thodesen et al. (1999) reported a larger final CF in fish from the AKVAFORSK Atlantic salmon selective breeding program compared to progeny from wild-caught salmon, but their use of wild-caught salmon rather than a contemporary, randomly-mated control line limits conclusions that can be made regarding the indirect effect of selection on CF. Contrary to the current study, Chevassus et al. (2004) did not detect a correlated response in CF in a brown trout population after four generations of

selection for growth; when their data were adjusted for BW, control-line fish actually had larger CF compared to the selected line. After adjusting the 13-month CF data from the current study for BW according to Chevassus et al. (2004), or for fork length, CF remained larger in the Select line compared to the FS/Control lines in 2012 ($P \leq 0.008$; data not shown) and compared to the Synthetic Control line in 2014 ($P \leq 0.0003$; data not shown). It is important to note that BW was the selection criterion in the current study, whereas body length was the criterion used in the other studies (O'Flynn et al., 1999; Chevassus et al., 2004).

Sae-Lim et al. (2012) analyzed farmer survey data using an analytic hierarchy process to derive objective consensus preference values for traits in a rainbow trout breeding program and found that farmers generally preferred a modest increase in CF. Trout with excessive CF are not desirable due to their unnatural appearance, but the threshold for 'excessive' CF likely varies widely among different segments of the industry. At BW greater than 1 kg, Select-line fish in the current study appear to have larger CF (≥ 1.9) compared to rainbow trout >1 kg from other populations (e.g., Elvingson and Johansson, 1993; Kause et al., 2002; Weber et al., 2014; Cleveland et al., 2016), but comparisons among the studies must be made with caution as the age of the fish, and thus growth rate, varies considerably. Cleveland et al. (2016) report a more modest CF of fish from the Select line that was similar to a commercial population when reared under production conditions to 1.3 kg, so it seems unlikely that the correlated increase in CF after 5 generations of selection will result in production fish with excessively-large CF.

The FS/Control lines used in this study, and the method of estimating phenotypic selection response using these three lines as selection controls, were similar to that used in our breeding program for bacterial cold water disease resistance (Leeds et al., 2010). Because FS/Control lines were performance tested and propagated using non-pedigreed fish and with smaller effective population sizes compared to the Select line, sampling effects and random drift within a given FS/Control line may be large. However, by maintaining three independent FS/Control lines and using their average as the selection control, effects of sampling and random drift are expected to be minimized. Estimated rates of inbreeding in the three FS/Control lines based on effective population sizes (Falconer and Mackay, 1996) averaged 2.2%, resulting in a mean predicted inbreeding level of 8.4% in the last generation. Interestingly, estimates of selection response derived using the fully-pedigreed Synthetic Control line, compared to those estimated using the average of the three FS/Control lines, were larger for all traits except TGC. This was largely unexpected for two reasons. First, management of the FS/Control lines make them more subject to the effect of inbreeding depression, which is known to adversely affect growth performance (Su et al., 1996; Pante et al., 2001; Neira et al., 2006). Second, two generations of selection had occurred before development of the Synthetic Control line. Thus, the theoretical reduction in genetic variance (i.e., Bulmer effect) as a result of selection is expected to decrease selection response in subsequent generations.

As discussed in Leeds et al. (2010) and references therein, the standard errors of genetic trend estimates do not explicitly account for drift variance. However, because of limited inbreeding in the Select line, and the large test statistics for regressions of mean

breeding values on generation number, accounting for drift variance in the regressions had little impact on tests of significance ($P \leq 0.0004$ for all traits; data not shown).

Genetic trend estimates were generally more conservative compared to phenotypic selection response estimates. The limited use of nested matings in the breeding program likely increased confounding between additive genetic effects and the effects of full-sib family that were included in the animal model. Therefore, the ability of the animal model to accurately partition these sources of variation was limited, and likely led to conservative estimates of additive (co)variance components and estimation of genetic trend (Janhunen et al., 2013). In a subsequent 7-trait animal model in which random full-sib family effects were excluded, estimates of heritabilities and genetic trends increased, particularly for 10- and 13-mo BW and TGC (heritabilities = 0.53 ± 0.02 , 0.51 ± 0.02 , and 0.34 ± 0.02 , respectively). Resulting genetic trends for these three traits were 33.6 ± 1.3 grams, 80.3 ± 3.4 grams, and 0.054 ± 0.004 TGC units, respectively, and thus more similar to, or even larger than (TGC), phenotypic selection response estimates.

4.2. Heritabilities and genetic correlations

The moderate heritability of BW in salmonids has been well-documented in the literature (for review, see: Gjedrem and Olesen, 2005), and the current estimates of 0.27 to 0.43 for BW between 5 and 13 months of age are consistent with the average of literature estimates. Silverstein et al. (2009) reported larger heritabilities for 9- and 12-month BW (0.59 ± 0.08 and 0.61 ± 0.09 , respectively) from our odd-year synthetic population, which was largely developed using the same founder strains reported herein, but their estimates were derived using data from a single generation. The larger heritability of BW at the time

of tagging (5-mo BW in the current study) compared to heritabilities at later ages may be partially attributable to difficulty in partitioning additive, maternal, and common environmental variance when fish are initially reared separately by family. However, Sae-Lim et al. (2013a) reported a similar trend in BW heritabilities despite communal rearing of all families beginning at the eyed egg stage and accounting for maternal effects that have been shown to affect early growth performance (Haffray et al., 2012).

Relatively few heritability estimates are available in the literature for TGC. Furthermore, heritability estimates have been shown to differ across production environments (Sae-Lim et al., 2013b) and interval during which growth performance is assessed (Kause et al., 2006; Le Boucher et al., 2011; Sae-Lim et al., 2013a). The current TGC heritability estimate of 0.12 ± 0.03 is considerably smaller than that reported by Silverstein et al. (2009) despite similarities in production environment and growth interval. Heritability estimates for CF have consistently been moderate to high in magnitude (McKay et al., 1986; Gjerde and Schaeffer, 1989; Elvingson and Johansson, 1993; Rye and Refstie, 1995; Fishback et al., 2002; Kause et al., 2002; Neira et al., 2004; Sae-Lim et al., 2013a), and thus the current heritability estimates of 0.36 ± 0.04 and 0.40 ± 0.04 for CF in this population were typical.

The large genetic correlations among adjacent measures of BW, and the decreasing magnitude of these correlations as time between measurements increased, is very consistent with previous literature reports (Elvingson and Johansson, 1993; Su et al., 2002; Kause et al., 2005; Sae-Lim et al., 2013a). Genetic correlation between TGC and 13-month BW in the current study (0.50 ± 0.08) is somewhat smaller than corresponding estimates of 0.69, 0.80, and 0.71 to 0.81 given in Silverstein et al. (2009), Sae-Lim et al. (2013a), and Sae-

Lim et al. (2013b), respectively, but the increase of genetic correlation between TGC and BW measured at later ages is consistent with that observed in those studies. Estimates of genetic correlation between BW and CF measured at the same age (0.39 ± 0.09 and 0.45 ± 0.08) are intermediate to estimates from salmonids in the literature (McKay et al., 1986; Elvingson and Johansson, 1993; Rye and Refstie, 1995; Fishback et al., 2002; Kause et al., 2002; Neira et al., 2004; Haffray et al., 2012; Sae-Lim et al., 2013a), which have ranged considerably across studies (0.02 to 0.93). The large genetic correlation between CF measured at different ages (0.91 ± 0.03), and small genetic correlation between CF and TGC (-0.05 ± 0.12 to 0.16 ± 0.12), are consistent with the available literature (Fishback et al., 2002; Sae-Lim et al., 2013a).

5. Conclusions

Improving growth performance of rainbow trout populations beyond traditional market BW through selective breeding is effective. The genetic gain in 13-month BW (≥ 1 kg) of 11.9% per generation in the current study is similar to genetic gains made in other salmonid species marketed at larger BW. Through five generations, there was no apparent decrease in selection response over time, and selection resulted in only modest inbreeding accumulation (0.86% per generation). Selection on an index of BW and TGC resulted in more rotund fish (i.e., greater CF) compared to randomly-mated controls. The low heritability (0.12 ± 0.03) and limited selection response of TGC (2.1% per generation) suggests that selection at a fixed age, or when the population mean reaches a target BW, may be more effective for improving late growth performance, particularly when genetic correlations among serial body weight measures are large.

Acknowledgments

The authors acknowledge J. Everson, J. Harper, M. Hostuttler, K. Jenkins, J. Kretzer, J. Leasor, J. McGowan, K. Melody, T. Moreland, D. Payne, and E. Squires for technical assistance. This work was supported by the USDA, Agricultural Research Service CRIS Project 1930-31000-010 “Utilizing Genetics and Physiology for Enhancing Cool and Cold Water Aquaculture Production” **and in part by an appointment of DGP to the ARS Research Participation Program administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the U.S. Department of Energy (DOE) and the USDA. ORISE is managed by ORAU under DOE contract number DE-AC05-06OR23100.** The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the USDA or the ARS of any product or service to the exclusion of others that may be suitable. USDA is an equal opportunity provider and employer.

References

- Aksnes, A., Gjerde, B., Roald, S.O., 1986. Biological, chemical and organoleptic changes during maturation of farmed Atlantic salmon, *Salmo salar*. *Aquaculture*. 53, 7-20.
- Aussanasuwannakul, A., Kenney, P.B., Weber, G.M., Yao, J., Slider, S.D., Manor, M.L., Salem, M., 2011. Effect of sexual maturation on growth, fillet composition, and texture of female rainbow trout (*Oncorhynchus mykiss*) on a high nutritional plane. *Aquaculture*. 317, 79-88.
- Berg, P., Nielsen, J., Sørensen, M.K., 2006. EVA: Realized and predicted optimal genetic contributions, *Proc. 8th World Cong. Genet. Appl. Livest. Prod.*, Belo Horizonte, Brazil, pp. N° 27-09.

594 Boldman, K.G., Kriese, L.A., Van Vleck, L.D., Van Tassell, C.P., Kachman, S.D., 1995. A manual for
 595 the use of MTDFREML. A set of programs to obtain estimates of variances and
 596 covariances [Draft]. USDA-ARS, Clay Center, NE.
 597 Chen, P., Baas, T.J., Mabry, J.W., Koehler, K.J., Dekkers, J.C.M., 2003. Genetic parameters and
 598 trends for litter traits in U.S. Yorkshire, Duroc, Hampshire, and Landrace pigs. *J. Anim*
 599 *Sci.* 81, 46-53.
 600 Chevassus, B., Quillet, E., Krieg, F., Hollebecq, M.G., Mambrini, M., Fauré, A., Labbé, L., Hiseux,
 601 J.P., Vandeputte, M., 2004. Enhanced individual selection for selecting fast growing
 602 fish: The "PROSPER" method, with application on brown trout (*Salmo trutta fario*).
 603 *Genet. Sel. Evol.* 36, 643-661.
 604 Cleveland, B.M., Leeds, T.D., Rexroad III, C.E., Summerfelt, S.T., Davidson, J.W., May, T., Crouse,
 605 C., Wolters, W.R., Plemmons, B., Kenney, P.B., 2016. Genotype by environment
 606 interaction on rainbow trout growth and carcass traits. *N. Am. J. Aquacult.* Submitted
 607 for publication.
 608 Elvingson, P., Johansson, K., 1993. Genetic and environmental components of variation in body
 609 traits of rainbow trout (*Oncorhynchus mykiss*) in relation to age. *Aquaculture.* 118,
 610 191-204.
 611 Falconer, D.S., Mackay, T.F.C., 1996. Introduction to Quantitative Genetics, 4th ed. Longman
 612 Group Ltd., Essex, England.
 613 FAO, 2005. Cultured Aquatic Species Information Programme. *Oncorhynchus mykiss*. FAO
 614 Fisheries and Aquaculture Department.
 615 Fishback, A.G., Danzmann, R.G., Ferguson, M.M., Gibson, J.P., 2002. Estimates of genetic
 616 parameters and genotype by environment interactions for growth traits of rainbow
 617 trout (*Oncorhynchus mykiss*) as inferred using molecular pedigrees. *Aquaculture.* 206,
 618 137-150.
 619 Fornshell, G., 2002. Rainbow Trout - Challenges and Solutions. *Rev. Fish. Sci.* 10, 545-557.
 620 Gjedrem, T., 1975. Possibilities for genetic gain in salmonids. *Aquaculture.* 6, 23-29.
 621 Gjedrem, T., 1983. Genetic variation in quantitative traits and selective breeding in fish and
 622 shellfish. *Aquaculture.* 33, 51-72.
 623 Gjedrem, T., 1985. Improvement of productivity through breeding schemes. *GeoJournal.* 10,
 624 233-241.
 625 Gjedrem, T., 1992. Breeding plans for rainbow trout. *Aquaculture.* 100, 73-83.
 626 Gjedrem, T., 2010. The first family-based breeding program in aquaculture. *Rev. Aquacult.* 2,
 627 2-15.
 628 Gjedrem, T., Thodesen, J., 2005. Selection. in: Gjedrem, T. (Ed.), Selection and Breeding
 629 Programs in Aquaculture. Springer, Dordrecht, The Netherlands, pp. Pages 89-112.
 630 Gjedrem, T., Olesen, I., 2005. Basic statistical parameters. in: Gjedrem, T. (Ed.), Selection and
 631 Breeding Programs in Aquaculture. Springer, Dordrecht, The Netherlands, pp. Pages
 632 45-72.
 633 Gjerde, B., 1986. Growth and reproduction in fish and shellfish. *Aquaculture.* 57, 37-55.
 634 Gjerde, B., Schaeffer, L.R., 1989. Body traits in rainbow trout. II. Estimates of heritabilities and
 635 of phenotypic and genetic correlations. *Aquaculture.* 80, 25-44.
 636 Haffray, P., Vandeputte, M., Petit, V., Pincent, C., Chatain, B., Chapuis, H., Mériaux, J.C.,
 637 Coudurier, B., Quillet, E., Dupont-Nivet, M., 2012. Minimizing maternal effect in
 638 salmonid families mixed since eyed stages and a posteriori DNA-pedigree. *Livestock*
 639 *Science.* 150, 170-178.

640 Hershberger, W.K., Myers, J.M., Iwamoto, R.N., McAuley, W.C., Saxton, A.M., 1990. Genetic
 641 changes in the growth of coho salmon (*Oncorhynchus kisutch*) in marine net-pens,
 642 produced by ten years of selection. *Aquaculture*. 85, 187-197.
 643 Hinshaw, J.M., 1999. Trout production: Feeds and feeding methods. Southern Regional
 644 Aquaculture Center Publication No. 223.
 645 Iwama, G.K., Tautz, A.F., 1981. A simple growth model for salmonids in hatcheries. *Can. J. Fish.*
 646 *Aquat. Sci.* 38, 649-656.
 647 Janhunen, M., Kause, A., Vehviläinen, H., Nousiainen, A., Koskinen, H., 2013. Accounting for
 648 early rearing density effects on growth in the genetic evaluation of rainbow trout,
 649 *Oncorhynchus mykiss*. *Journal of Animal Science*. 91, 5144-5152.
 650 Jobling, M., 2003. The thermal growth coefficient (TGC) model of fish growth: A cautionary
 651 note. *Aquacult. Res.* 34, 581-584.
 652 Johnson, N.A., Rexroad Iii, C.E., Hallerman, E.M., Vallejo, R.L., Palti, Y., 2007. Development and
 653 evaluation of a new microsatellite multiplex system for parental allocation and
 654 management of rainbow trout (*Oncorhynchus mykiss*) broodstocks. *Aquaculture*. 266,
 655 53-62.
 656 Kause, A., Ritola, O., Paananen, T., Mäntysaari, E., Eskelinen, U., 2002. Coupling body weight
 657 and its composition: a quantitative genetic analysis in rainbow trout. *Aquaculture*.
 658 211, 65-79.
 659 Kause, A., Ritola, O., Paananen, T., Wahlroos, H., Mäntysaari, E.A., 2005. Genetic trends in
 660 growth, sexual maturity and skeletal deformations, and rate of inbreeding in a
 661 breeding programme for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 247, 177-
 662 187.
 663 Kause, A., Tobin, D., Houlihan, D.F., Martin, S.A.M., Mäntysaari, E.A., Ritola, O., Ruohonen, K.,
 664 2006. Feed efficiency of rainbow trout can be improved through selection: Different
 665 genetic potential on alternative diets. *J. Anim Sci.* 84, 807-817.
 666 Kincaid, H.L., Bridges, W.R., von Limbach, B., 1977. Three generations of selection for growth
 667 rate in fall-spawning rainbow trout. *Transactions of the American Fisheries Society*.
 668 106, 621-628.
 669 Lankford, S.E., Weber, G.M., 2006. Associations between plasma growth hormone, insulin-like
 670 growth factor-I, and cortisol with stress responsiveness and growth performance in a
 671 selective breeding program for rainbow trout. *N. Am. J. Aquacult.* 68, 151-159.
 672 Le Boucher, R., Quillet, E., Vandeputte, M., Lecalvez, J.M., Goardon, L., Chatain, B., Médale, F.,
 673 Dupont-Nivet, M., 2011. Plant-based diet in rainbow trout (*Oncorhynchus mykiss*
 674 Walbaum): Are there genotype-diet interactions for main production traits when fish
 675 are fed marine vs. plant-based diets from the first meal? *Aquaculture*. 321, 41-48.
 676 Leeds, T.D., Silverstein, J.T., Weber, G.M., Vallejo, R.L., Palti, Y., Rexroad, C.E., III, Evenhuis, J.,
 677 Hadidi, S., Welch, T.J., Wiens, G.D., 2010. Response to selection for bacterial cold water
 678 disease resistance in rainbow trout. *J. Anim Sci.* 88, 1936-1946.
 679 McKay, L.R., Ihssen, P.E., Friars, G.W., 1986. Genetic parameters of growth in rainbow trout,
 680 *Salmo gairdneri*, prior to maturation. *Canadian Journal of Genetics and Cytology*. 28,
 681 306-312.
 682 Meyer, K., 2007. WOMBAT—A tool for mixed model analyses in quantitative genetics by
 683 restricted maximum likelihood (REML). *J. Zhejiang Univ. - Sci. B*. 8, 815-821.
 684 Neira, R., Lhorente, J.P., Araneda, C., Díaz, N., Bustos, E., Alert, A., 2004. Studies on carcass
 685 quality traits in two populations of Coho salmon (*Oncorhynchus kisutch*): phenotypic
 686 and genetic parameters. *Aquaculture*. 241, 117-131.

- Neira, R., Díaz, N.F., Gall, G.A.E., Gallardo, J.A., Lhorente, J.P., Manterola, R., 2006. Genetic improvement in Coho salmon (*Oncorhynchus kisutch*). I: Selection response and inbreeding depression on harvest weight. *Aquaculture*. 257, 9-17.
- O'Flynn, F.M., Bailey, J.K., Friars, G.W., 1999. Responses to two generations of index selection in Atlantic salmon (*Salmo salar*). *Aquaculture*. 173, 143-147.
- Pante, M.J.R., Gjerde, B., McMillan, I., 2001. Effect of inbreeding on body weight at harvest in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*. 192, 201-211.
- Piferrer, F., 2001. Endocrine sex control strategies for the feminization of teleost fish. *Aquaculture*. 197, 229-281.
- Piferrer, F., Beaumont, A., Falguière, J.-C., Flajšhans, M., Haffray, P., Colombo, L., 2009. Polyploid fish and shellfish: Production, biology and applications to aquaculture for performance improvement and genetic containment. *Aquaculture*. 293, 125-156.
- Rye, M., Refstie, T., 1995. Phenotypic and genetic parameters of body size traits in Atlantic salmon *Salmo Salar* L. *Aquaculture Research*. 26, 875-885.
- Sae-Lim, P., Komen, H., Kause, A., van Arendonk, J.A.M., Barfoot, A.J., Martin, K.E., Parsons, J.E., 2012. Defining desired genetic gains for rainbow trout breeding objective using analytic hierarchy process. *Journal of Animal Science*. 90, 1766-1776.
- Sae-Lim, P., Komen, H., Kause, A., Martin, K.E., Crooijmans, R., van Arendonk, J.A.M., Parsons, J.E., 2013a. Enhancing selective breeding for growth, slaughter traits and overall survival in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 372-375, 89-96.
- Sae-Lim, P., Kause, A., Mulder, H.A., Martin, K.E., Barfoot, A.J., Parsons, J.E., Davidson, J., Rexroad, C.E., van Arendonk, J.A.M., Komen, H., 2013b. Genotype-by-environment interaction of growth traits in rainbow trout (*O*): A continental scale study. *Journal of Animal Science*. 91, 5572-5581.
- Silverstein, J.T., Vallejo, R.L., Palti, Y., Leeds, T.D., Rexroad III, C.E., Welch, T.J., Wiens, G.D., Ducrocq, V., 2009. Rainbow trout resistance to bacterial cold-water disease is moderately heritable and is not adversely correlated with growth. *Journal of Animal Science*. 87, 860-867.
- Su, G.-S., Liljedahl, L.-E., Gall, G.A.E., 1996. Effects of inbreeding on growth and reproductive traits in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 142, 139-148.
- Su, G.-S., Liljedahl, L.-E., Gall, G.A.E., 2002. Genetic correlations between body weight at different ages and with reproductive traits in rainbow trout. *Aquaculture*. 213, 85-94.
- Thodesen, J., Grisdale-Helland, B., Helland, S.J., Gjerde, B., 1999. Feed intake, growth and feed utilization of offspring from wild and selected Atlantic salmon (*Salmo salar*). *Aquaculture*. 180, 237-246.
- Weber, G.M., Hostuttler, M.A., Cleveland, B.M., Leeds, T.D., 2014. Growth performance comparison of intercross-triploid, induced triploid, and diploid rainbow trout. *Aquaculture*. 433, 85-93.
- Yano, A., Guyomard, R., Nicol, B., Jouanno, E., Quillet, E., Klopp, C., Cabau, C., Bouchez, O., Fostier, A., Guiguen, Y., 2012. An Immune-Related Gene Evolved into the Master Sex-Determining Gene in Rainbow Trout, *Oncorhynchus mykiss*. *Current Biology*. 22, 1423-1428.
- Zollinger, W.A., Nielsen, M.K., 1984. An evaluation of bias in estimated breeding values for weaning weight in Angus beef cattle field records. I. Estimates of within herd genetic trend. *J. Anim Sci*. 58, 545-549.

Figure 1. Phenotypic selection response by generation of selection for body weight, thermal growth coefficient (TGC), and condition factor (CF) traits in the Select line. Values in parentheses on the x-axis represent the number of generations of selection with respect to development of the randomly-mated Synthetic Control line. Selection response was estimated from linear contrasts of the genetic line $\times$ generation least-squares means given in Table 4. Selection response estimates are given using the mean of three Founder-Strain control lines (solid black lines), or Synthetic Control line (dashed black lines), as reference control lines. Gray lines represent regression of cumulative selection response on generation of selection (i.e., genetic gain per generation) with reference to the Founder-strain control lines (solid gray lines) or Synthetic Control line (dashed gray lines).

Figure 2. Plots of Select-line mean family breeding values (black lines) by generation for body weight, thermal growth coefficient (TGC), and condition factor (CF) traits. Breeding values were estimated using a 7-trait animal model. Genetic trends (dashed gray lines) represent regression of mean family breeding values on generation of selection. All genetic trends differed from 0 ($P \leq 0.0001$).

Table 1. Pedigree-based estimates of founder-strain composition in Select and Synthetic Control lines by generation

hatch year (generation)	genetic line	House Creek	Shasta ^a	Kamloops/Puget Sound Steelhead ^b	Donaldson ^c	Arlee	Kamloops	Hayspur
2004 (0)	Select	0.9%	0.9%	47.5%	36.7%	7.7%	4.5%	1.8%
2006 (1)	Select	0.0%	2.0%	39.9%	50.9%	5.8%	1.0%	0.5%
2008 (2)	Select	0.0%	3.3%	45.4%	50.4%	0.7%	0.0%	0.2%
2010 (3)	Select	0.0%	6.5%	44.0%	49.5%	0.0%	0.0%	0.0%
	Synthetic Control	0.0%	4.4%	47.3%	43.9%	3.1%	0.0%	1.3%
2012 (4)	Select	0.0%	9.0%	42.0%	49.0%	0.0%	0.0%	0.0%
	Synthetic Control	0.0%	4.7%	47.3%	44.3%	2.8%	0.0%	0.9%
2014 (5)	Select	0.0%	7.8%	42.6%	49.6%	0.0%	0.0%	0.0%
	Synthetic Control	0.0%	4.1%	47.9%	44.0%	3.1%	0.0%	0.8%

^a Also maintained as a separate, purebred, randomly-mated control line (FS/Control 1)

^b Also maintained as a separate, purebred, randomly-mated control line (FS/Control 2)

^c Also maintained as a separate, purebred, randomly-mated control line (FS/Control 3)

Table 2. Counts of full- and half-sib families by hatch year and genetic line

Hatch year/genetic line	Full-sib families	Paternal/maternal half-sib families ^a	Mean family inbreeding, %
pre-2004 (generation -1)	107	22/29 (44)	0
2004 (generation 0)			
Select	111	22/33(27)	0.2
FS/Control 1	12	0/0 (12)	-
FS/Control 2	14	0/0 (14)	-
FS/Control 3	23	5/1 (7)	-
2006 (generation 1)			
Select	99	31/0 (25)	1.5
FS/Control 1	16	3/0 (8)	-
FS/Control 2	14	3/0 (8)	-
FS/Control 3	22	3/0 (14)	-
2008 (generation 2)			
Select	104	18/0 (62)	1.8
FS/Control 1	5	1/0 (3)	-
FS/Control 2	12	4/0 (2)	-
FS/Control 3	7	2/0 (3)	-
2010 (generation 3)			
Select	98	30/0 (38)	2.4
FS/Control 1	11	0/0 (11)	-
FS/Control 2	15	1/0 (13)	-
FS/Control 3	15	0/0 (15)	-
Synthetic Control	20	0/0 (20)	2.0
2012 (generation 4)			
Select	99	21/0 (57)	4.0
FS/Control 1	18	0/0 (18)	-
FS/Control 2	19	0/0 (19)	-
FS/Control 3	19	1/0 (17)	-
Synthetic Control	20	0/0 (20)	3.3
2014 (generation 5)			
Select	102	26/0 (48)	4.5
Synthetic Control	53	14/0 (25)	4.2

^a Numbers in parentheses are counts of full-sib families not nested within a half-sib family.

Table 3. Estimates of heritabilities (on diagonal), genetic correlations (below diagonal), and environmental correlations (above diagonal)

	5-month BW, g	8-month BW, g	10-month BW, g	13-month BW, g	TGC	10-month CF	13-month CF
5-month BW, g	0.43 ± 0.14	0.30 ± 0.10	0.20 ± 0.08	0.15 ± 0.05	0.00 ± 0.04	0.08 ± 0.06	0.05 ± 0.06
8-month BW, g	0.54 ± 0.19	0.28 ± 0.08	0.65 ± 0.04	0.46 ± 0.03	-0.01 ± 0.03	0.22 ± 0.04	0.10 ± 0.04
10-month BW, g	0.18 ± 0.17	0.69 ± 0.12	0.30 ± 0.05	0.74 ± 0.02	0.10 ± 0.02	0.35 ± 0.03	0.16 ± 0.03
13-month BW, g	0.20 ± 0.11	0.54 ± 0.11	0.82 ± 0.05	0.27 ± 0.03	0.63 ± 0.01	0.25 ± 0.03	0.26 ± 0.03
TGC	-0.02 ± 0.15	0.02 ± 0.16	0.18 ± 0.13	0.50 ± 0.08	0.12 ± 0.03	-0.07 ± 0.02	0.22 ± 0.02
10-month CF	0.08 ± 0.10	0.27 ± 0.11	0.39 ± 0.09	0.39 ± 0.08	-0.05 ± 0.12	0.40 ± 0.04	0.59 ± 0.02
13-month CF	0.06 ± 0.11	0.23 ± 0.11	0.38 ± 0.09	0.45 ± 0.08	0.16 ± 0.12	0.91 ± 0.03	0.36 ± 0.04

BW, body weight; TGC, thermal growth coefficient measured for growth period between 10 and 13 months post-hatch; CF, condition factor.

Table 4. Trait least-squares means ($\pm$ SE) by genetic line and generation and model statistics used to estimate phenotypic selection response

Hatch year / genetic line	No. records ^a	5-month BW, g	8-month BW, g	10-month BW, g	13-month BW, g	TGC	10-month CF	13-month CF
2004 (generation 0)								
Select	1,822 / 3,122	47.4 $\pm$ 1.0	144.2 $\pm$ 2.4	320.7 $\pm$ 7.6	635.4 $\pm$ 19.9	1.47 $\pm$ 0.03	1.37 $\pm$ 0.01	1.53 $\pm$ 0.02
FS/Control 1	78 / 79	32.8 $\pm$ 7.3	97.7 $\pm$ 16.4	228.4 $\pm$ 40.4	434.3 $\pm$ 84.1	1.38 $\pm$ 0.11	1.30 $\pm$ 0.06	1.48 $\pm$ 0.08
FS/Control 2	69 / 122	52.8 $\pm$ 4.7	155.3 $\pm$ 10.8	354.7 $\pm$ 26.8	621.3 $\pm$ 58.3	1.37 $\pm$ 0.08	1.43 $\pm$ 0.04	1.56 $\pm$ 0.06
FS/Control 3	183 / 192	48.2 $\pm$ 4.3	153.7 $\pm$ 9.6	341.3 $\pm$ 24.2	654.6 $\pm$ 50.9	1.47 $\pm$ 0.07	1.38 $\pm$ 0.04	1.53 $\pm$ 0.05
2006 (generation 1)								
Select	1,857 / 2,033	85.7 $\pm$ 1.1	219.0 $\pm$ 2.4	508.4 $\pm$ 9.1	960.2 $\pm$ 16.2	1.63 $\pm$ 0.03	1.56 $\pm$ 0.01	1.63 $\pm$ 0.02
FS/Control 1	25 / 30	71.1 $\pm$ 10.4	148.3 $\pm$ 23.2	338.9 $\pm$ 56.1	667.8 $\pm$ 115.6	1.54 $\pm$ 0.16	1.55 $\pm$ 0.08	1.70 $\pm$ 0.11
FS/Control 2	40 / 45	91.5 $\pm$ 7.4	203.1 $\pm$ 16.6	444.9 $\pm$ 40.6	822.4 $\pm$ 83.2	1.53 $\pm$ 0.12	1.47 $\pm$ 0.06	1.52 $\pm$ 0.08
FS/Control 3	24 / 30	72.8 $\pm$ 10.4	177.5 $\pm$ 23.2	393.4 $\pm$ 56.3	790.7 $\pm$ 115.8	1.64 $\pm$ 0.16	1.52 $\pm$ 0.08	1.63 $\pm$ 0.11
2008 (generation 2)								
Select	2,886 / 6,182	60.3 $\pm$ 1.2	175.7 $\pm$ 2.4	387.6 $\pm$ 6.5	905.5 $\pm$ 13.2	1.91 $\pm$ 0.02	1.47 $\pm$ 0.01	1.57 $\pm$ 0.01
FS/Control 1	52 / 110	55.5 $\pm$ 7.3	129.3 $\pm$ 16.5	270.6 $\pm$ 39.5	654.0 $\pm$ 81.9	1.82 $\pm$ 0.11	1.50 $\pm$ 0.06	1.73 $\pm$ 0.08
FS/Control 2	55 / 112	60.3 $\pm$ 7.3	160.5 $\pm$ 16.4	325.5 $\pm$ 39.3	694.3 $\pm$ 81.4	1.64 $\pm$ 0.11	1.44 $\pm$ 0.06	1.56 $\pm$ 0.08
FS/Control 3	54 / 102	51.2 $\pm$ 7.3	145.5 $\pm$ 16.5	309.9 $\pm$ 39.4	729.8 $\pm$ 81.6	1.84 $\pm$ 0.11	1.48 $\pm$ 0.06	1.60 $\pm$ 0.08
2010 (generation 3)								
Select	1,601 / 1,654	70.7 $\pm$ 1.2	151.8 $\pm$ 2.8	338.4 $\pm$ 7.7	845.6 $\pm$ 17.3	2.07 $\pm$ 0.02	1.52 $\pm$ 0.01	1.74 $\pm$ 0.02
FS/Control 1	45 / 50	63.8 $\pm$ 7.4	118.4 $\pm$ 16.6	248.7 $\pm$ 40.1	656.1 $\pm$ 82.8	2.02 $\pm$ 0.11	1.32 $\pm$ 0.06	1.52 $\pm$ 0.08
FS/Control 2	49 / 50	58.2 $\pm$ 7.4	119.5 $\pm$ 16.6	235.8 $\pm$ 40.0	574.7 $\pm$ 82.6	1.78 $\pm$ 0.11	1.34 $\pm$ 0.06	1.51 $\pm$ 0.08
FS/Control 3	50 / 50	57.7 $\pm$ 7.4	132.0 $\pm$ 16.5	274.4 $\pm$ 39.7	691.3 $\pm$ 81.7	1.96 $\pm$ 0.11	1.44 $\pm$ 0.06	1.64 $\pm$ 0.08
Synthetic Control	187 / 193	59.8 $\pm$ 2.8	135.3 $\pm$ 6.4	292.6 $\pm$ 15.8	750.8 $\pm$ 33.2	2.06 $\pm$ 0.05	1.42 $\pm$ 0.02	1.60 $\pm$ 0.03
2012 (generation 4)								
Select	997 / 1,466	86.7 $\pm$ 1.8	185.7 $\pm$ 2.5	498.4 $\pm$ 5.9	1,264.4 $\pm$ 15.2	2.54 $\pm$ 0.02	1.66 $\pm$ 0.01	1.90 $\pm$ 0.01
FS/Control 1	30 / 47	62.2 $\pm$ 7.5	126.7 $\pm$ 16.6	306.4 $\pm$ 39.8	845.3 $\pm$ 82.6	2.34 $\pm$ 0.11	1.43 $\pm$ 0.06	1.75 $\pm$ 0.08
FS/Control 2	35 / 50	60.9 $\pm$ 7.5	120.4 $\pm$ 16.5	293.8 $\pm$ 39.6	753.3 $\pm$ 81.9	2.09 $\pm$ 0.11	1.48 $\pm$ 0.06	1.75 $\pm$ 0.08
FS/Control 3	28 / 44	61.1 $\pm$ 7.6	132.4 $\pm$ 16.7	323.8 $\pm$ 40.0	853.1 $\pm$ 83.0	2.25 $\pm$ 0.11	1.55 $\pm$ 0.06	1.78 $\pm$ 0.08
Synthetic Control	86 / 126	67.7 $\pm$ 3.1	149.8 $\pm$ 6.4	397.5 $\pm$ 15.4	1,065.5 $\pm$ 33.4	2.51 $\pm$ 0.05	1.49 $\pm$ 0.02	1.73 $\pm$ 0.03
2014 (generation 5)								
Select	1,458 / 1,551	81.8 $\pm$ 1.1	263.8 $\pm$ 2.4	609.1 $\pm$ 9.1	1,452.7 $\pm$ 14.3	2.28 $\pm$ 0.02	1.65 $\pm$ 0.01	1.98 $\pm$ 0.01

Figure 1.

line type	estimate	reference control line
—●—	cumulative response	Founder-Strain control
—	gain per generation	Founder-Strain control
- -▲- -	cumulative response	Synthetic Control
- -	gain per generation	Synthetic Control

Figure 2.

— • — mean family EBV per generation

— — — genetic trend