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Domestic-wild hybridization to improve aquaculture performance in Chinook salmon

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ABSTRACT

Salmon farming is one of Canada's fastest growing industries and contributes to Canada's economy as well as creating jobs in rural areas; however, the industry is challenged by the need to balance production economics against environmental impacts. While Atlantic salmon (*Salmo salar*) are the most commonly farmed species on the west coast of Canada, Chinook salmon (*Oncorhynchus tshawytscha*) are a valuable alternative, as they fill a niche market and generate reduced environmental concerns because they are a native species. However, Chinook salmon have not been systematically domesticated, and their performance remains highly variable. Here we report on the results of a research program designed to develop a performance-enhanced hybrid Chinook salmon stock. Growth and survival were estimated for seven domestic-wild hybrid Chinook salmon crosses at various freshwater stages and during 15 months of saltwater rearing at a British Columbia Chinook salmon farm and compared with domestic-domestic crosses (control). The project included 8640 individually (PIT) tagged offspring from the domestic stock and seven domestic-wild hybrid stocks originating from the Lower Fraser Valley, Lower Mainland Vancouver, and Vancouver Island, British Columbia, Canada. Within each stock, milt from 10 sires was used to fertilize eggs pooled from 15 highly inbred domestic females to produce 80 half-sib families. Our breeding design allows the partitioning of stock and sire effects, and minimises maternal genetic and maternal environment effects. Replicates of all families were reared under common environmental conditions in both fresh- and salt water and monitored for body size and survival. There was significant variation in survival, body size, and saltwater biomass among the Chinook salmon hybrid stocks. The performance of some of the hybrid crosses exceeded that of the fully domesticated stock, although the pattern of performance varied with rearing stage. Overall, two hybrid stocks consistently outperformed the domestic stock in terms of survival, growth, and biomass estimates. We systematically assess production performance across a wide range of wild-domestic hybrid crosses in a Pacific salmon species, and our results highlight opportunities to improve the production performance of Chinook salmon culture.

1. Introduction

To meet the increasing global demand for dietary animal protein (Gjedrem et al., 2012), commercial farming of numerous fish species has increased dramatically over the past 3–4 decades (Diana, 2009; Subasinghe et al., 2009), with captive rearing production now

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surpassing global wild fishery production (FAO, 2016). Farming of salmonid species continues to be a major growth industry worldwide (Liu and Sumaila, 2008), and in salt water is currently dominated by the culture of Atlantic salmon (*Salmo salar*). Nevertheless, the farming of indigenous Pacific salmon species (*Oncorhynchus* spp.) for North American west coast aquaculture is increasingly important economically due to niche market price advantages, and possible higher disease and parasite resistance as a result of adaptation to local pathogens (e.g., Evans and Neff, 2009). Moreover, because Pacific salmon are reared at lower stocking densities and are native to the Pacific Northwest, there is significant interest in developing improved lines of Pacific salmonids for aquaculture purposes due to relatively reduced environmental concerns. Chinook salmon (*Oncorhynchus tshawytscha*) is a common farm-raised Pacific salmon species; however, unlike Atlantic salmon, Chinook salmon aquaculture performance has not been systematically assessed, although in-house broodstock management is generally practiced. In North America, the development of high-performance domesticated stocks is constrained by the limited number of small-scale commercial facilities rearing Chinook salmon, limiting the availability of genetically diverse stocks for targeted breeding programs.

The practice of selective breeding in aquaculture facilities has been essential for maximizing growth and survival, but has also led to increased differentiation from wild strains (Einum and Fleming, 1997; McGinnity et al., 2003) and loss of genetic diversity, with potential deleterious effects (Heath et al., 2003). Differences between naturally produced and selected populations can have a genetic basis (Fleming and Einum, 1997), although genetic-by-environment (G × E) interactions and phenotypic plasticity can also contribute (Winkelman and Peterson, 1994). The potential for outbreeding (hybridizing genetically divergent groups) to affect performance in growth efficiency, survival, and flesh quality is high; theory predicts outcomes that vary from elevated performance (heterosis; Bryden et al., 2004; Whitlock et al., 2000) to loss of performance due to hybrid breakdown (Lehnert et al., 2014; Lynch, 1991). Heterosis has been demonstrated in both wild and cultured salmonids for traits such as growth (Bryden et al., 2004; Wangila and Dick, 1996), survival (Ayles and Baker, 1983), behaviour (Einum and Fleming, 1997; Tymchuk et al., 2006), and disease resistance (Becker et al., 2014). Hybrid breakdown (or outbreeding depression) has been shown in only a few studies (e.g., Tymchuk et al., 2007) while one study reported no measureable outbreeding effects in Chinook salmon F1 and F2 outbred families (Lehnert et al., 2014). Predictions of outbreeding performance outcomes may be confounded by the potential for genetic and environmental interactions (i.e., G × E effects), which must be carefully analyzed in a replicated statistical framework. However, while the analysis of complex interactions that contribute to hybrid stock performance is critical for identifying and selecting optimal stocks (or even optimal breeding individuals within stocks), this type of work is generally beyond the resources of individual salmon farms. Indeed, obtaining wild gametes, having access to dedicated aquaculture facilities, and the ability to undertake the rearing, sampling and analyses necessary for the suitable development of hybrid production stock requires a highly integrative approach combining government, commercial, and academic partners.

The present study is part of a large, multi-investigator project funded by the Natural Science and Engineering Research Council of Canada (Strategic Partnership Grant for Projects) designed to explore the potential for farmed Chinook salmon performance enhancement through outbreeding, via hybridization (Scientia, 2017). Our design involved hybrid crosses of males from seven Chinook salmon populations with varying degrees of hatchery supplementation (hereafter termed “wild”) and one domestic population with eggs from highly inbred (self-fertilized hermaphrodite offspring) domestic females to produce a total of 8 stocks, with 10 half-sib families within each stock (80 families total). Our goals were to assess the potential impact of outbreeding on three performance metrics critical for aquaculture production: i) survival during four life stages (at incubation, in fresh water, transition to salt water and in salt water), ii) size in fresh water, and iii) size in salt water under standard culture conditions. Using cumulative survival estimates throughout development for each stock, we assessed overall survival for each stock, and by combining survival during the grow-out saltwater phase at 1.5 years post-fertilization with their average masses at this sampling time, we calculated total biomass to determine whether differential stock performance exists. We expected wild-sired hybrid offspring to exhibit increased performance relative to the domestic-by-domestic crosses due to the possibility of increased heterozygosity, especially given our use of highly inbred dams. However, the pure domestic stock has been selected for survival over multiple generations in the farmed, organic-rearing captive environment (Heath et al., 2003), thus selecting for traits suited to captivity. Our proximate intent is to determine whether the highly inbred female line produced by our aquaculture partner can successfully contribute to improved production (i.e., fish for harvesting) via hybridization with wild-sourced stocks. Should hybrid crosses exhibit increased growth performance and survival above that of the control domesticated cross, the production of these fish for harvest (i.e., the crossing of the inbred-line with a specific stock) would then become a proprietary process. Our ultimate goal is to generate production-relevant data for Chinook salmon production stocks that will serve to improve salmon farming efficiency which will help make Chinook salmon a viable alternative for the salmon farming industry in Canada and potentially, globally.

2. Materials and methods

2.1. Source populations and breeding design

Breeding and rearing was conducted at Yellow Island Aquaculture Ltd. (YIAL), a Chinook salmon production facility that follows organic standards, with both freshwater hatchery and saltwater netcage facilities. The YIAL facility has been in production since 1985 and is located on Quadra Island, British Columbia, Canada [Lat - N 50° 7′ 59.124“, Long - W 125° 19’ 51.834”]. The YIAL production and brood stock are derived from the (hatchery supplemented) Robertson Creek and Big Qualicum River (Vancouver Island, British Columbia, Canada) Chinook salmon stocks.

For the purposes of generating outbred stocks, milt from males taken from seven wild populations was collected from across Vancouver Island and lower mainland British Columbia (Fig. 1) with permission and support from Department of Fisheries and Oceans (DFO) Canada. We chose the Chinook stocks based on the need for “ocean-type” (coastal) populations to avoid possible hybrid incompatibilities (Clarke et al., 1994). Additionally, we targeted populations experiencing similar environmental conditions and evolutionary history as the YIAL fish (Waples et al., 2004), that is, locations in southwestern British Columbia. The target populations were: Big Qualicum River, Capilano River, Chilliwack River, Nitinat River, Puntledge River, Robertson Creek, and Quinsam River (Fig. 1; Supplementary Table S1). We collected milt from 10 males at the salmon enhancement facilities associated with each wild population (individuals were haphazardly selected from unclipped – i.e., naturally produced males) as well as from 10 production stock males at YIAL (see Supplementary Table S2 for list of male lengths and weights). As the breeding times for the various source populations varied, we cryopreserved milt from all males from all 8 populations following a commercial cryopreservation protocol (Canada Cryogenetics Services; www.cryogenetics.com). Briefly, sperm was collected from individual males and each sample of sperm was density tested using the SDM6 Photometer to allow us to pack the same number of sperm cells per Square Pack® (designed to hold enough sperm cells to fertilize 3000 Chinook salmon eggs). After the sperm was density tested it was diluted with cryoprotectant and the resulting solution was measured into individual Square Packs®, heat sealed and frozen in liquid nitrogen. The cryopreserved sperm was then stored in

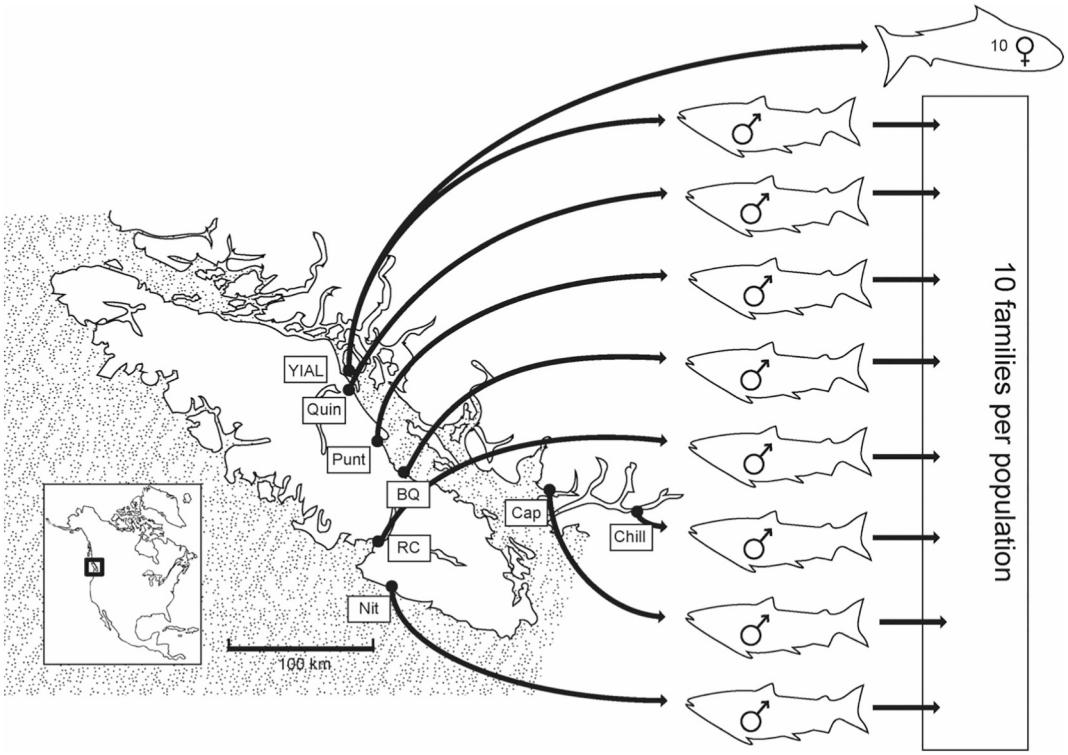

Fig. 1. Map and schematic diagram showing source locations for Chinook salmon stocks used in the breeding design. Note that the eggs came from dams (N = 17) that were offspring from a self-crossed female XX Chinook salmon - the eggs were mixed for the creation of the 80 half-sib families. "**BQ**" = Big Qualicum River, "**Cap**" = Capilano River, "**Chill**" = Chilliwack River, "**Nit**" = Nitinat River, "**Punt**" = Puntledge River, "**RC**" = Roberston Creek, and "**Quin**" = Quinsam River. For latitudinal and longitudinal coordinates of river mouth locations, please see Supplementary Table S1.

liquid nitrogen until it was required for fertilization of the eggs, from 5 days to 32 days. At the time of fertilization, individual Square Packs® were thawed in a warm water bath and the milt mixed with the eggs as Aqua Boost Activator® was mixed in with the eggs to promote fertilization.

To minimize potential maternal effects (Wellband et al., 2017), we used mixed eggs from highly inbred domestic females. The female fish were the progeny of self-fertilization of a single hermaphrodite XX Chinook salmon. The hermaphrodite parent was produced by exposing all-female (monosex) developing embryos (eyed-egg stage; ~250 ATUs) to 17-alphamethyltestosterone (17-aMT). Specifically, the 17-aMT treatment was used on up to a maximum of 4000 embryos per batch (at 50% hatch) for an initial treatment of 20 mg in a 50 L aerated water bath for 2 h (400 μg/L). The treatment was repeated 7–10 days later when the embryos were at 100% hatch. This protocol yielded a very small proportion of hermaphrodite offspring (4 out of ~400 mature fish examined). We successfully self-fertilized two of the four mature hermaphrodite Chinook salmon, and reared their offspring to sexual maturity (Komsa, 2012). These hermaphrodite offspring had an inbreeding coefficient of at least 0.50 (likely higher due to previous inbreeding in the farmed stock that was used for the production of the hermaphrodite). Approximately 3000 eggs were collected from each of 17 mature female offspring from a single hermaphrodite parent, and mixed. By mixing the eggs of these highly related female Chinook salmon, female genetic and maternal effect variation among crosses was minimized and standardized.

Fertilization occurred on November 1, 2013. To perform the crosses, 0.25 mL of thawed cryopreserved milt (see above) was used per male to fertilize the mixed eggs. The mixed eggs were divided into 80 groups of ~600 eggs, with each group of mixed eggs being fertilized with sperm from one of 10 males from each of seven wild and one domestic (YIAL) stocks, generating a total of 10 'families' within each cross (80 families total for the study; Fig. 1). The domestic production cross (YIAL x YIAL) served as the internal control/standard for comparative assessment of performance across stocks. Once fertilized, eggs were reared in divided vertical-stack incubation trays (16 wells per tray) in replicate (80 families × 2 replicates = 160 wells), haphazardly distributed across trays within the incubation stacks. The incubation stacks were supplied with untreated ground water (temperature range: 7–9 °C).

2.2. Rearing (fresh- and saltwater)

Hatching occurred from January 12–15, 2014 (10 wpf (weeks post-fertilization)), and unfertilized eggs and mortalities were counted and removed from incubation trays every second day until the end of incubation, to the "swim-up" stage (~1000 ATUs). Following completion of swim-up, exogenous-feeding alevins from replicate incubation tray wells were combined and haphazardly redistributed to replicate 200 L tanks for rearing (March 14–17, 2014 (19 wpf); Fig. 2a). A maximum of 120 alevins per family (range: 27–120; 75%–25% quartiles: 120–111 alevins) were transferred into each tank to minimize potential density effects. Crosses sired by four Chilliwack River, four Capilano River and two Puntledge River males produced too few offspring for replicate tanks, resulting in a total of 150 identical rearing tanks (Fig. 2a). All tanks were supplied with flow-through ground water at 1.0 L/ min. Exogenously feeding offspring were fed ad libitum three to four times daily. Tank dissolved oxygen, which was maintained at above 80% saturation, and water temperature, approximately 8 °C (temperature range: 7–10 °C), were regularly monitored. Tanks received light from 7 a.m. to 5 p.m. daily, and mortalities were counted and removed when each tank was cleaned every 5 days.

From June 12–16, 2014 (32 wpf), fish from replicate family tanks were mixed and a subset of the mixed fish from each family received Passive Integrated Transponder (PIT) identification tags and were placed in communal and replicated 2500 L recovery troughs, keeping stocks separate, but combining families. We tagged 108 fish per family

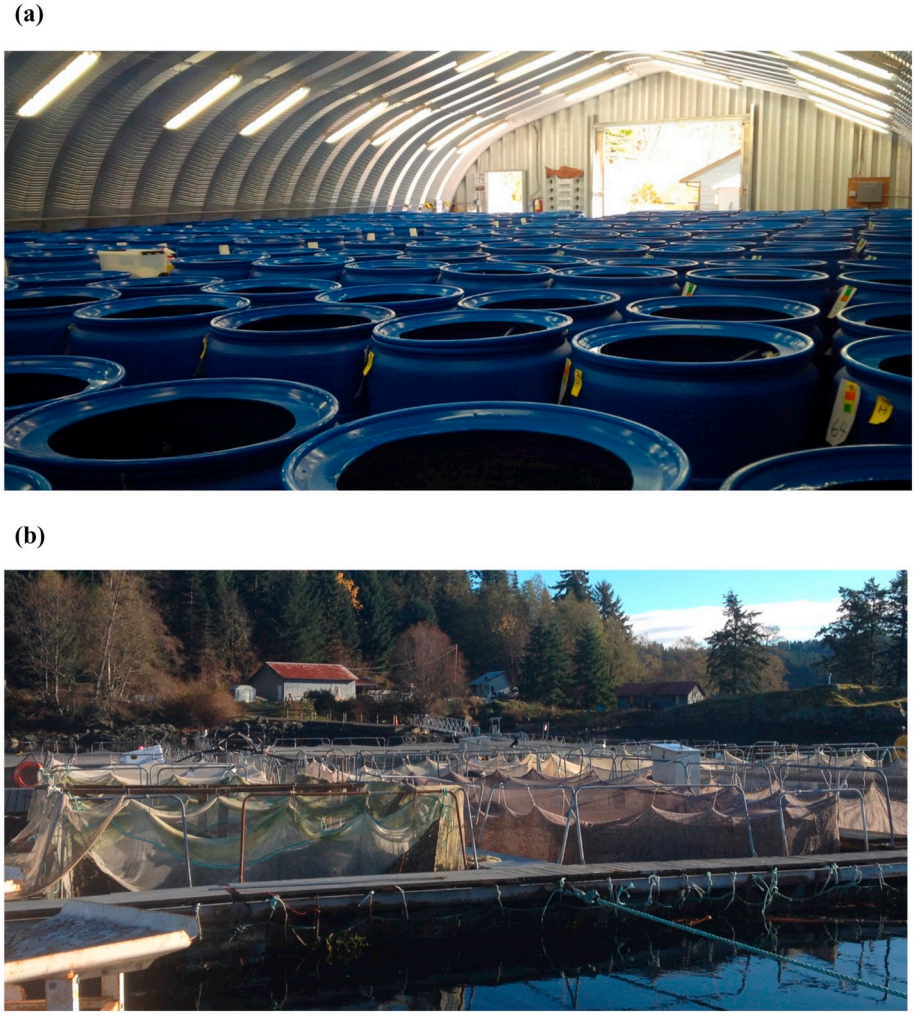

Fig. 2. (a) Freshwater rearing facility showing 200 L barrel layout, and (b) saltwater rearing facility demonstrating 5 m × 5 m × 5 m netcage layout.

(54/replicate): 108 fish × 80 families = 8640 tagged fish. Fish were allowed to recover in the troughs for 3 weeks at which point they were all vaccinated (July 7, 2014) for *Vibrio* in a bath vaccination using a commercial vaccine (Vibrogen 2: *Vibrio anguillarum-ordalii*; Novartis Animal Health Canada, Inc. Charlottetown, PEI), following standard hatchery practice. All post-tag and post-vaccination mortalities were recorded, and PIT tags were recovered. Fish remained in fresh water for a further month and on August 11–12, 2014 (40.5 wpf), individuals were transferred to saltwater netcages (dimensions: 5 m × 5 m × 5 m) with each stock (families combined by all fish PIT-tagged) split into two replicate netcages (16 netcages total; Fig. 2b). Fish were reared following standard aquaculture practices and fed ad libitum, by hand, 2–3 times per day. In May 2015 (80 wpf), all surviving fish were combined into a single saltwater netcage per stock (5 m × 5 m x 5 m depth).

2.3. Mass and survival

During the freshwater rearing stage, body mass was measured beginning April 22, 2014, when a random subset of 10–13 fish were removed from every tank twice monthly and individually weighed. Mass was recorded to the nearest 0.01 g, and fish were returned to their original tank. Mass was measured on five separate occasions during the freshwater stage, ending June 16, 2014, and spanning 24.5–32 wpf.

After transfer to salt water, all fish from each netcage were seined and identified by PIT-tag, and a subset weighed (per pen minimum: 199, maximum: 928, median: 628) at three sampling times: November 2014 (54 wpf), February 2015 (67.5 wpf), and May 2015 (80 wpf). A final sampling was conducted in November 2015 (108 wpf), where all the surviving fish were weighed, individually PIT-tag identified, and all stocks combined into a single netcage (10 m × 10 m × 10 m depth).

The data from the sampling (mass and PIT tag ID) were used to estimate three performance parameters for all stocks: mean body size (mass) at age, survival and total biomass. As all the fish were fertilized and measured on the same day, body mass reflects cumulative growth (although see Supplementary Table S3 for specific growth rate estimates). Biomass was estimated using survival estimates within salt water only and saltwater body mass at the family level for each stock. Specifically, we calculated the proportion of fish that survived from fresh-saltwater transfer until May 2015 (80 weeks), calculated the average at the family level per netcage replicate, and then multiplied that proportion by the family-level mean body mass of the stock at that same sampling date.

2.4. Statistical analysis

2.4.1. Survival

Statistical analyses for survival during four rearing stages: incubation (post fertilization), freshwater (prior to tagging), transfer (between tagging, *Vibrio* vaccination and first sampling post saltwater transfer) and saltwater were completed using R version 3.2.4 (R Core Team 2016). Survival during incubation and freshwater rearing were summed per replicate cell and replicate tank, respectively (number dead and

number alive), and the *buildbinary* function in the package 'fullfact' was used to convert data to binary form (0 representing a dead fish and 1 representing a live one; Houde and Pitcher, 2016). Individual-level mortality (from PIT tag data) was used in subsequent rearing-stage analyses. To examine survival differences at each stage, generalized linear mixed models (GLMM) were fit using the glmer function for binary data with the logit link function. Incubation tray (tray ID), incubation well (well ID), tank ID, netcage ID, and family ID (nested within stock) were included in models as random effects, when appropriate for the stage. Log-likelihood ratio tests were used to compare model fit and test significance of the fixed (stock) and random effects. The 'lsmeans' package (Lenth, 2016) was used to assess pairwise differences between stocks with Tukey's post-hoc tests, and mixed models were fit using the 'lme4' package (Bates et al., 2015).

2.4.2. Body mass

Statistical analyses for body mass during the freshwater and saltwater stages were completed using JMP 12 (SAS Institute Inc. 2014). Model assumptions were assessed by graphical inspection: residuals versus fitted values were plotted to verify homogeneity, and quantile-quantile plots and histograms of the residuals were plotted to verify normality. Body mass within each of the sampling periods was analyzed in a mixed-model framework. Stock was a fixed effect in every analysis, and for the freshwater body size analyses, tank ID, replicate tank, and family were included as random effects with replicate tank nested within family and family nested within stock. Saltwater analyses included netcage ID (nested within population) as the random effect. The mass data were log transformed to remove data heterogeneity for the freshwater measurements only. Significant mean differences between stocks were examined using a Tukey's multiple comparison test.

2.4.3. Saltwater biomass

Saltwater biomass was calculated for each family within a given stock per netcage replicate. Analyses were similarly analyzed as for body mass, with stock as the sole fixed effect, and family ID (nested within stock) and netcage ID as random effects, both nested within stock. Significant mean differences between stocks were examined using a Tukey's multiple comparison test.

3. Results

3.1. Survival

There was substantial variation in survival across the hybrid and control cross stocks (Fig. 3; Table S4). We identified significant stock effects in the freshwater, transfer, and saltwater stages, but not during incubation. We identified significant sire (family) effects on survival only during the incubation stage. The Capilano stock had highest survival during incubation (68.5%), and Chilliwack the lowest (41.6%); with the latter differing significantly from Capilano in post hoc tests ($p < .04$). Survival during incubation for the domesticated YIAL stock was intermediate (57.4%). During the freshwater exogenous feeding stage, significant differences in survival persisted, although survival overall was considerably higher at this stage than during incubation for all stocks ($\chi^2 = 15.3$, df = 7, $p = .03$; Table 1). Although the domesticated YIAL stock displayed the highest survival during exogenous feeding (99.7%), this was only significantly greater than that for the Big Qualicum and Chilliwack stocks (98.5% and 98.4%, respectively; p-values < .036). The transition from fresh water to salt water caused significant mortality in the Nitinat stock compared to all other stocks except Quinsam and Capilano in post hoc tests (p-values < .01). During the saltwater grow-out stage, differences in stock survival after one year continued ($\chi^2 = 23.4$, df = 7, $p < .0003$; Table 1), with the Nitinat stock again demonstrating lowest survival in salt water (68%). Highest survival was experienced by the Chilliwack stock (90%), although this was not significantly different from the next three highest-surviving stocks in salt water: Quinsam (87.5%), Robertson Creek (86.8%) and YIAL, the fully domestic stock (83.8%) (Fig. 4).

3.2. Body mass

There was substantial variation in body mass across the hybrid and control cross stocks through the experiment (Table 2). We identified significant stock effects on body mass at four of the five sampling times in fresh water (Fig. 5a), and at two of the four sampling events in salt water (Fig. 5b). Significant sire (family) effects on body mass were observed at all but one of the sampling events (30.5 wpf) and those effects were generally large. Random effects of freshwater rearing tank, replicates and saltwater netcages contributed < 10% on average of the overall variation in body size component estimates (Table S5).

3.3. Biomass

While both body size and survival are critical variables for commercial aquaculture, biomass, which reflects both growth and survival, has the greatest relevance for maximizing commercial purposes in a fluctuating price market. The substantial variation in both body mass and survival across the hybrid and control cross stocks led to considerable (and statistically significant) variation in saltwater biomass (Fig. 6, Table 3), with a 56% difference in biomass between the best and worst performing stocks (Table S6). Despite the substantial absolute variation in biomass, no hybrid groups exhibited statistically significant differences in growth relative the YIAL line in a Tukey's posthoc test, likely due to the domesticated stock falling in the middle of the distribution (Fig. 6).

4. Discussion

The incorporation of novel genetic material into captive or domesticated stocks to reverse the loss of genetic diversity is recognized as valuable across taxa (Chen, 2013; Whiteley et al., 2015). Indeed, the recognition of heterosis as a potential mechanism for performance enhancement has been described for diverse cultured aquatic species (e.g., Bryden et al., 2004; Emlen, 1991; Goyard et al., 2008; Hedgecock and Davis, 2007; Hulata, 2001). The primary goal of the current study was to create a breeding- and experimental design that would capitalize on outbreeding practices and systematically test for variation in growth (body size) and survival (and eventually biomass) among diverse hybrid crosses. Our ultimate aim was to identify optimal crosses with improved performance and survival for commercial harvest of Chinook salmon aquaculture in British Columbia, Canada. Our prediction was that some hybrid crosses would perform better than the presumably inbred YIAL domestic stock due to heterozygote advantage, the masking of deleterious recessives, or simply increased genetic variation. While some studies have shown heterosis in salmonids (e.g., Gharrett et al., 1999 - outside the scope of our study, which was to test for the effects of hybrids), others have failed to show evidence for significant hybrid vigour, and most show inconsistent results depending on the trait studied (Bryden et al., 2004; Cheng et al., 1987; Gjerde and Refstie, 1984; Iwamoto et al., 1986; McClelland et al., 2005) and/or among unrelated year classes (Hershberger et al., 1990). Our project is the first to systematically test for the effects of novel allele introduction and increased heterozygosity on growth and survival across multiple wild-source Chinook salmon populations. While our design does not allow specific partitioning of recessive deleterious allele masking versus genetic diversity effects, our data provide the best evidence to date for the potential for significant harvest gains in terms of growth and survival based on controlled outbreeding in salmon aquaculture. Our approach of screening multiple source populations as possible parental stocks, while controlling for confounding maternal effects and minimizing non-additive effects using an inbred-line (as demonstrated for the domesticated stock; Wellband et al., 2017) resulted in substantial

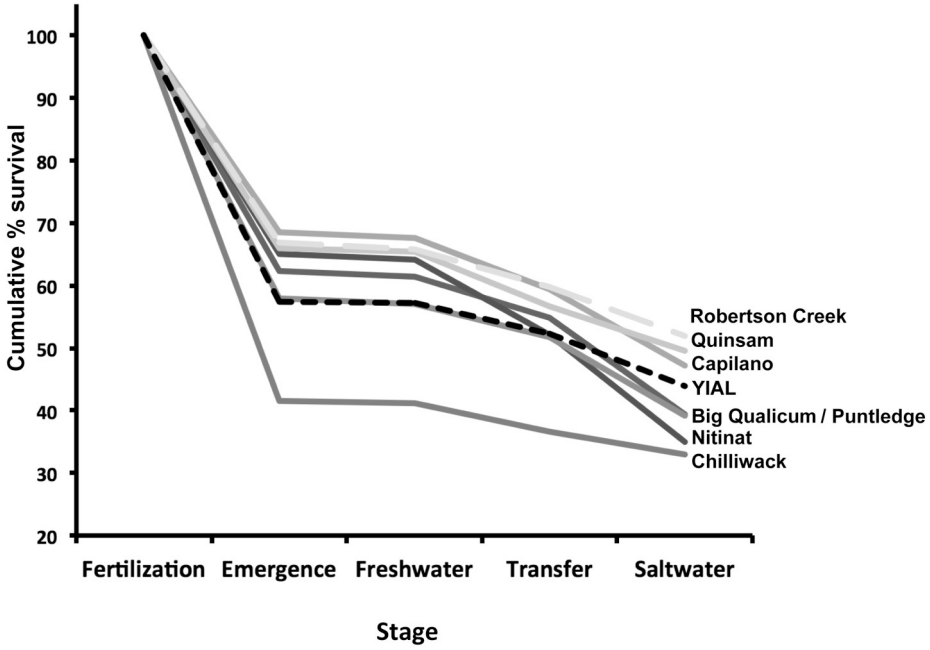

Fig. 3. Total cumulative percent survival for all stocks across all families through incubation, freshwater, and saltwater phases. Values derived from actual survival data at each stage.

variation among the eight crosses for both survival and body size in the fresh- and saltwater rearing stages.

We identified widespread stock and family effects on survival and growth (body size) from the egg stage to approximately two years post fertilization. Such differences, observed despite having common maternal genetic and non-genetic contribution and common rearing environments, likely reflect genetic or epigenetic effects inherited primarily from the sires. Indeed, we observed highly significant family (sire) effects nested within stocks for most of our growth measures and for incubation survival, likely indicative of substantial additive genetic variation components. While it is possible that the cryopreservation may have affected survival during the incubation stage (de Mello et al., 2017), we assume it to have only a short-term effect on fertilization (Labbe et al., 2001) and that any effects would be distributed equally across all offspring (Martínez-Páramo et al., 2009) adding to the variance among offspring but uniformly across sires and populations. It is also possible that sire by dam interactions (non-additive effects) may have contributed to variation in incubation survival – i.e., that each sire might interact with the inbred female genome differently - such effects should vary for each offspring. However, the elegance of our breeding design is that it essentially uses only a single dam (mixed highly inbred females), with most sire-dam interaction effects captured as residual variance. Previous studies have reported relatively high additive genetic variance for growth parameters in salmonids (Falica et al., 2017; Sae-Lim et al., 2015), although heritabilities for survival tend to be low within individual stocks (e.g., Garcia de Leaniz et al., 2007; Withler et al., 1987). The high additive genetic (sire) variance observed in this study indicates strong potential for further selection improvements for growth. The consistent among-stock effects observed for both saltwater survival and growth throughout rearing indicates meaningful variation across stocks for these important production traits. Given the relatively limited geographical distribution of our source stocks, it is perhaps surprising we found such high stock variance components for both growth and survival under farmed conditions. This is particularly true

Table 1
Means ± SE (sample size) for percent survival for seven hybrid and one domesticated stocks during different rearing stages. Data presented in table are based on % survival per freshwater or saltwater replicate, averaged. Statistics were performed on binary response data (0, 1) per individual. Post-hoc comparisons performed with Tukey HSD when stock was a significant fixed effect at either the 5% or 10% level (italicized). Statistical differences denoted by non-matching superscripts ($p < .05$).

Stock	Incubation	Freshwater	Transfer	Saltwater
YIAL	57.4 ± 5.53 ^{a,b} (4178)	99.7 ± 0.10 × 10^{-3} ^{a} (2387)	91.4 ± 3.06 ^{a} (1084)	83.8 ± 0.012 ^{a,c,d} (990)
Robertson Creek	66.9 ± 3.01 ^{a,b} (4827)	98.7 ± 0.32 × 10^{-3} ^{a,b,c} (2306)	90.8 ± 3.08 ^{a} (1080)	86.6 ± 0.011 ^{a,c} (979)
Quinsam	66.0 ± 2.76 ^{a,b} (4642)	99.2 ± 0.16 × 10^{-3} ^{a,b,c} (2399)	86.4 ± 3.05 ^{b} (1096)	87.2 ± 0.011 ^{a,c} (948)
Puntledge	58.0 ± 3.13 ^{a,b} (4893)	98.6 ± 0.45 × 10^{-3} ^{a,b,c} (2303)	90.6 ± 3.26 ^{a} (1099)	75.8 ± 0.014 ^{a,b} (997)
Nitinat	65.0 ± 3.74 ^{a,b} (4772)	98.8 ± 0.26 × 10^{-3} ^{a,b,c} (2265)	81.3 ± 3.08 ^{b} (1122)	68.1 ± 0.015 ^{b} (921)
Chilliwack	41.6 ± 4.87 ^{a} (4234)	98.4 ± 0.37 × 10^{-3} ^{b} (1658)	84.1 ± 4.47 ^{a} (812)	90.1 ± 0.011 ^{c} (729)
Capilano	68.5 ± 2.60 ^{b} (4095)	98.8 ± 0.41 × 10^{-3} ^{a,b,c} (1448)	86.3 ± 4.13 ^{b} (812)	78.7 ± 0.015 ^{a,b} (713)
Big Qualicum	62.4 ± 2.28 ^{a,b} (3988)	98.5 ± 0.31 × 10^{-3} ^{c} (2072)	89.3 ± 3.06 ^{a} (980)	72.1 ± 0.015 ^{b,d,e} (875)

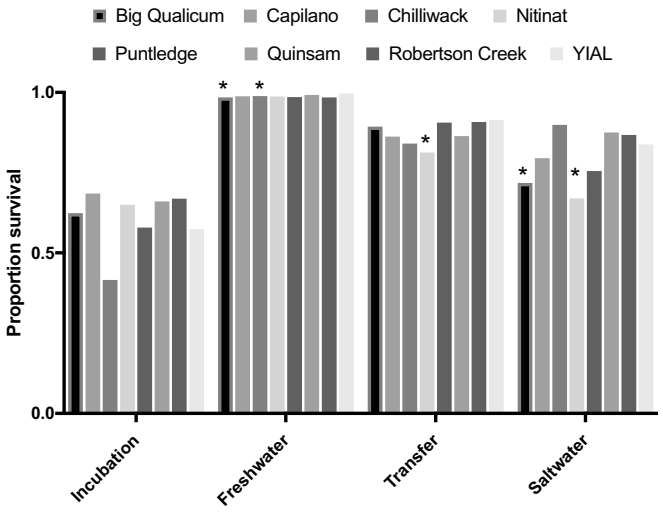

Fig. 4. Mean proportion survival (means across all families within stock) through incubation, freshwater, and saltwater phases for the seven hybrid cross stocks. Asterisks denote significant differences from the YIAL (domestic X domestic) crosses when used as the reference level in analyses.

given that life history and genetic divergence is thought to be driven by geographic proximity (and hence evolutionary history of re-colonization; Waples et al., 2004). However, many studies have shown strong evidence for local adaptation among Pacific salmon populations (e.g., Evans and Neff, 2009; Evans et al., 2010; Fraser et al., 2011; Heath et al., 2006), and the stock differences we observed may reflect those evolved differences - in particular, responses and performance under novel conditions. Regardless of the nature of the stock and family effects we observed, our data indicate that performance improvement is possible through specific hybrid crossing, as well as selection for performance traits among those sires within the hybrid stocks.

Survival is a critically limiting factor for commercial salmon aquaculture, with the cost of mortality rising as the fish age and grow. Loss of fish at any stage incurs not only lost production costs (which increase cumulatively), but also lost opportunity costs (which also increase as the fish reach larger sizes). Our analysis shows the incubation stage to

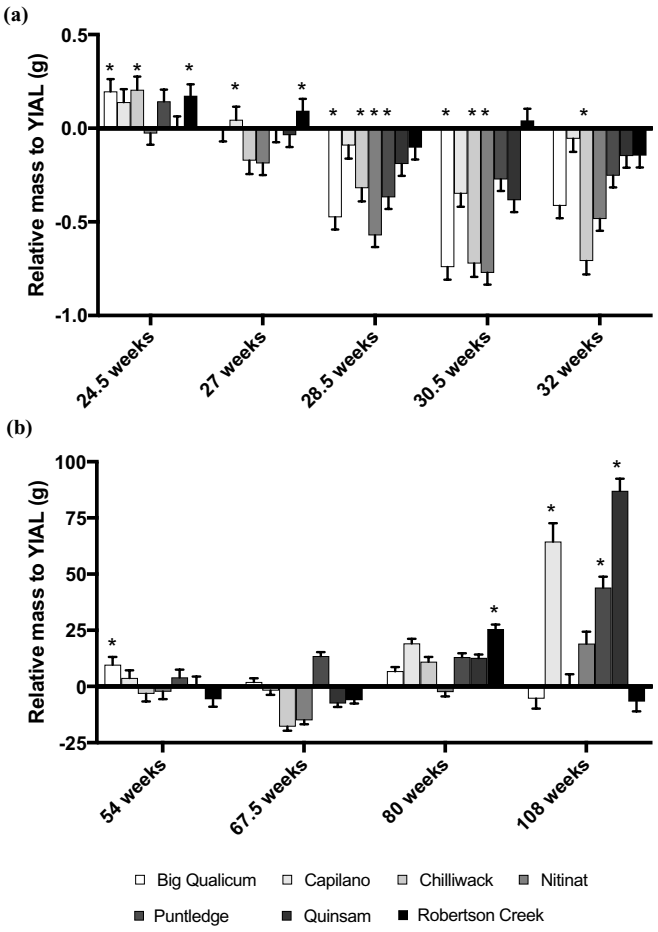

Fig. 5. Mean body mass (± S.E.) across individuals (across all families) through (a) freshwater, and (b) saltwater phases for the seven hybrid cross stocks, normalized to the YIAL body mass (by subtraction of the YIAL mean value). Asterisks denote significant differences from the YIAL (domestic X domestic) crosses when used as the reference level in analyses.

Table 2
Means ± SE (sample size) for body mass (g) for seven hybrid- and one domesticated stocks throughout rearing. Post-hoc comparisons performed with Tukey HSD when stock was a significant fixed effect at either the 5% or 10% level (italicized). Statistical differences denoted by non-matching superscripts ($p < .05$). wpf = weeks post fertilization.

Sampling time (wpf)	Sampling environment								
	Freshwater					Saltwater			
	24.5	27	28.5	30.5	32	54	67.5	80	108
YIAL	1.10 ± 0.02 (208)^{b,c,d}	1.95 ± 0.04 (200)^{a,b}	3.13 ± 0.05 (200) ^{a}	4.05 ± 0.09 (200) ^{a,b}	5.45 ± 0.07 (200) ^{a}	53.7 ± 0.77 (202) ^{b,c}	137.1 ± 0.94 (928)	182.8 ± 1.87 (826)	415.5 ± 6.81 (200) ^{c}
Robertson Creek	1.27 ± 0.02 (211)^{a}	2.04 ± 0.04 (200)^{a}	3.03 ± 0.06 (200) ^{a,b}	4.09 ± 0.09 (200) ^{a}	5.31 ± 0.09 (200) ^{a,b}	48.3 ± 0.78 (201) ^{c}	131.2 ± 0.97 (926)	208.3 ± 2.15 (529)	408.8 ± 8.21 (121) ^{c}
Quinsam	1.10 ± 0.02 (206)^{c,d}	1.92 ± 0.04 (200) ^{a,b}	2.94 ± 0.06 (200) ^{a,b}	3.67 ± 0.09 (200) ^{b,c,d,e}	5.31 ± 0.08 (200) ^{a,b}	55.2 ± 0.78 (200) ^{b,c}	129.6 ± 0.90 (905)	195.4 ± 1.78 (827)	502.5 ± 14.6 (80) ^{a}
Puntledge	1.24 ± 0.02 (206)^{a,b,c}	1.94 ± 0.04 (200) ^{a,b}	2.76 ± 0.06 (200) ^{b,c}	3.78 ± 0.09 (200) ^{a,b,c}	5.20 ± 0.09 (200) ^{a,b}	58.3 ± 0.78 (200) ^{a,b}	150.7 ± 1.24 (750)	195.8 ± 2.16 (755)	459.4 ± 11.8 (99) ^{a,b}
Nitinat	1.07 ± 0.02 (212)^{d}	1.77 ± 0.03 (199) ^{b}	2.56 ± 0.05 (200) ^{c}	3.30 ± 0.08 (200) ^{d,e}	4.97 ± 0.09 (200) ^{a,b}	51.9 ± 0.78 (199) ^{b,c}	122.1 ± 1.0 (807)	180.3 ± 2.31 (627)	434.5 ± 13.1 (82) ^{b,c}
Chilliwack	1.31 ± 0.03 (161)^{a}	1.78 ± 0.04 (159) ^{b}	2.81 ± 0.07 (160) ^{b,c}	3.33 ± 0.11 (156) ^{c,d,e}	4.74 ± 0.08 (156) ^{b}	50.7 ± 0.78 (201) ^{b,c}	119.3 ± 1.28 (682)	193.7 ± 2.43 (492)	416.2 ± 10.6 (108) ^{c}
Capilano	1.24 ± 0.03 (165)^{a,b}	2.00 ± 0.04 (160) ^{a}	3.04 ± 0.07 (160) ^{a,b}	3.70 ± 0.10 (160) ^{a,b,c,d}	5.40 ± 0.09 (160) ^{a,b}	56.6 ± 0.77 (205) ^{a,b}	135.3 ± 1.19 (653)	201.9 ± 2.42 (560)	479.9 ± 16.0 (34) ^{a,b}
Big Qualicum	1.30 ± 0.03 (184)^{a}	1.95 ± 0.5 (180) ^{a,b}	2.66 ± 0.07 (180) ^{c}	3.31 ± 0.10 (180) ^{e}	5.04 ± 0.09 (180) ^{a,b}	63.0 ± 0.77 (203) ^{a}	138.9 ± 1.20 (778)	189.5 ± 2.48 (630)	410.2 ± 12.5 (113) ^{c}

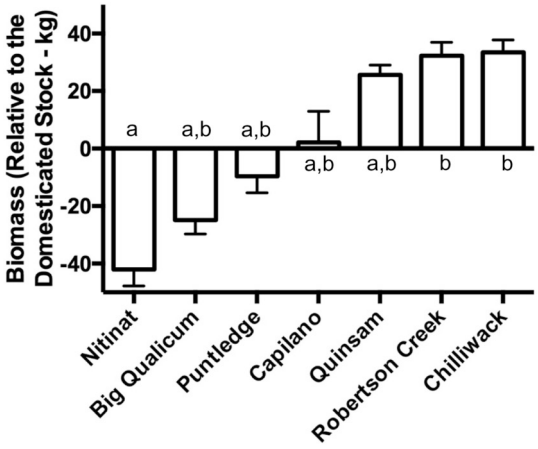

Fig. 6. Mean saltwater biomass estimates (± SE) for the seven hybrid cross stocks, normalized to the mean YIAL biomass. Biomass is calculated using family-level proportion survival per netcage replicate for each stock in saltwater multiplied by the average family-level mass per stock (May 2015). Post-hoc comparisons performed with Tukey HSD. Statistical differences denoted by non-matching superscripts ($p < .05$) from Table 3. Note: no stock is significantly different in biomass from YIAL.

Table 3
Means ± SE (sample size) for biomass of each stock calculated at the family level per netcage replicate for seven hybrid and one domesticated stocks during saltwater grow-out cycle. Biomass is the product of proportion saltwater survival and mass (g). Post-hoc comparisons performed with Tukey HSD. Statistical differences denoted by non-matching superscripts ($p < .05$).

Stock	Saltwater biomass
YIAL	139.2 ± 6.0 ^{a,b} (20)
Robertson Creek	171.5 ± 4.7 ^{a} (20)
Quinsam	164.8 ± 3.4 ^{a,b} (20)
Puntledge	129.5 ± 5.7 ^{a,b} (22)
Nitinat	97.1 ± 5.8 ^{b} (20)
Chilliwack	172.6 ± 4.4 ^{a} (18)
Capilano	141.3 ± 10.9 ^{a,b} (20)
Big Qualicum	114.3 ± 4.9 ^{a,b} (18)

be most susceptible, with no stock survival higher than 68.5% (due to genetic and possible non-genetic effects of fertilization success), while the post-incubation freshwater stage had exceptionally high survival rates (> 98%). Additionally, the pattern of survival across grow-out varies substantially among the 7 hybrid crosses. For instance, hybrids from the Nitinat stock suffered most during the transition stage from fresh water to salt water, when fish were tagged, vaccinated, and transferred, suggestive of Nitinat fish being sensitive to the handling necessary at this stage. The Chilliwack hybrid stock had lowest survival until the grow-out phase, where they recovered and performed as well as the domesticated stock (along with Quinsam and Robertson Creek stocks). Overall, however, when assessing cumulative survival (Fig. 3), the Chilliwack hybrid stock would be considered to be the poorest performer based on this metric (37% cumulative survival), and hybrids of the Quinsam and Robertson Creek cross stocks the greatest (57.3% and 57.1% respectively), with the domesticated (YIAL x YIAL) stock performing in between (48.0%).

The efficiency of commercial grow-out of anadromous salmon is dependent on both growth rate and body size, as these factors reduce time to harvest and increase the value of the fish per kilogram, respectively. When compared with the (YIAL X YIAL) control crosses, the hybrid crosses generally grew faster early in freshwater rearing, but substantially slower later in freshwater rearing (Fig. 5). Interestingly, the hybrid cross stocks generally performed similarly to the (YIAL X YIAL) control crosses early in the saltwater grow-out phase, but by the final sampling (108 weeks post-fertilization), three of seven hybrid cross stocks significantly exhibited larger mean body mass than the YIAL domestic control cross (Fig. 5). Our analysis further showed variation in both growth rate and body size within stocks over time; and that body size was not consistently driving variation in survival across stocks or developmental stages. Specifically, at the final sampling, the largest body-sized hybrid stock was Quinsam River; however, this stock did not display the largest mass consistently at any of the previous sampling times. In fact, hybrids from Robertson Creek were the largest at three out of the nine sampling periods, yet were the smallest stock by the end sampling date. Regardless, both Quinsam River and Robertson Creek hybrid-cross stocks displayed the highest cumulative survival despite these body mass differences (Fig. 3).

The integration of both survival and growth rates yields biomass, an important measure of aquaculture productivity. When we compare total biomass yield expected from a standardized 100 fish transferred from fresh- to salt water, we find three hybrid stocks resulted with higher biomass than the pure domestic YIAL stock, while three do not perform as well (Fig. 6). However, these differences in comparison to the YIAL domesticated stock were not statistically significant overall, likely due to high variation in biomass estimates resulting from variation in both survival and growth. Despite these results, estimates of final biomass are nevertheless valuable for assessing culture performance, as they reflect the variation in the cost of mortalities across the grow-out cycle – losses early in life represent minor costs to the producers, while older, larger salmon are more valuable. However, because these biomass calculations assume an equal number of fish (i.e., 100) transferred to salt water to assess relative production during the grow-out phase, factoring pre-transfer survival will further have an impact on final harvest. As such, this project goes beyond simple measures of biomass in an attempt to identify the highest performing stock; indeed, the elegance of the breeding design coupled with the aquaculture facility allows for the performance of individuals, families, and stocks in both fresh- and saltwater stages to be measured.

Here we created hybrid crosses between an inbred line of domestic Chinook salmon and males from seven populations distributed around Vancouver Island and lower-mainland British Columbia and males from the domestic line. Our detailed and long-term survival and size monitoring allowed us to systematically assess cross differences in culture performance. Overall, we identified substantial variation among the crosses for performance, indicative of potential for performance increases resulting from outcrossing with wild-sourced stocks. Interestingly, our inbred maternal line, when crossed with domestic production stock sires (YIAL X YIAL) did not appear to exhibit reduced performance metrics relative to the other hybrid stocks as might be expected due to inbreeding depression. Since all stocks were treated equally from cryopreservation of milt to saltwater grow-out, and heterozygote advantage is a relative effect – the performance of the YIAL domesticated stock remained average overall. It is possible that the YIAL X YIAL crosses experienced some genetic gains resulting from crossing the highly inbred females with production males. Alternatively, most deleterious genes may have been selected out since the domesticated stock has been selected over multiple generations for the captive environment at the aquaculture facility, and would presumably be generally better adapted to these captive conditions. Nonetheless, we identified two parental stock populations, Quinsam River and Robertson Creek, that when crossed with the YIAL inbred line produced hybrids that are promising for commercial harvesting, as they

most consistently performed best, including outperforming the domesticated stock. We also sought to quantify variation among the crosses as evidence for future performance gain potential, and on-going research (e.g., disease resistance, flesh quality, conversion efficiency, adaptive behavioural-physiological phenotypes) is exploring in more detail the costs and benefits of the specific crosses created here. Ultimately, future factorial cross experiments using specific populations may identify one or more parental stocks whose hybrids are promising for harvest production, or perhaps a broader evaluation of crosses from more divergent source populations may need to be evaluated to better define variance components. Nevertheless, the present study provides a controlled and systematic long-term evaluation of culture performance of hybrid lines of Chinook salmon. Because F1 hybrid production stock is challenging to maintain (pure-type parental broodstocks must persist, or cryogenically stored milt must be preserved), F1 hybrid production stock can be incorporated into a commercial aquaculture business plan, as further crossing (or back-crossing) would likely yield unpredictable results. Thus, like hybrid maize, the F1 hybrid cross would be, by definition, proprietary as further breeding of these hybrids would lead to unpredictable outcomes in the F2 and further crosses. Our industry partner could thus market the production stock for harvest without risking propagation at other facilities.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2019.734255.

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