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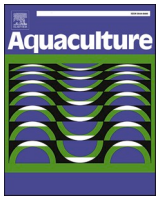

Effects of triploidy and probiotic therapies on juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) growth via a behavioural transcriptomics perspective

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ABSTRACT

Improving growth and product quality remains a key goal in aquaculture. Triploidization can result in improved flesh quality and larger body size as fish reach maturity by redirecting energy away from reproduction. Here, we investigated how triploidy and probiotic therapies influenced hatchery-reared Chinook salmon (*Oncorhynchus tshawytscha*) mass directly and via fish transcriptomic and behavioural profiles at the juvenile life stage, which may provide insight into factors associated with growth variation at early life stages. Specifically, we measured transcriptomic and behavioural profiles of juvenile Chinook salmon. Overall, triploid individuals had lower mass compared to diploid counterparts and triploidy was associated with mass through an interaction between *Shh* transcription and boldness/aggression. Further, probiotics did not directly impact mass, but instead interactions with p53 transcription were associated with mass variation. A few of the genes examined (*Auts2*, *CRF, IGF-1*, and *p53*) exhibited higher transcription levels which were associated with greater mass, independent of other variables. Ultimately, our study demonstrates the value of a behavioural transcriptomics approach for identifying relationships between gene transcription, behavioural profiles, and growth-related traits (e.g., mass) in hatchery-reared Chinook salmon.

1. Introduction

1.1. Growth and aquaculture productivity

Maximizing growth rates in finfish aquaculture is a primary goal of producers as it directly relates to productivity (De-Santis and Jerry, 2007; Gui and Zhu, 2012). In commercial salmonid aquaculture, fish farmers aim to produce many individuals of large size (i.e., biomass) quickly while maintaining flesh quality, at the lowest costs (Rørå et al., 2001). In addition to the selective choice of broodstock (Tymchuk et al., 2006) and controlling rearing density (Li et al., 2013) to maximize fish growth, other methods include triploidization of the stock (Rottmann et al., 1991; Benfey, 1999) and the use of specialized diets (Brizuela et al., 2001; Nayak, 2010; Gonçalves et al., 2011).

1.2. Triploidization: Benefits and drawbacks

Triploidization has sometimes been associated with improved growth on individuals in aquaculture (e.g., Atlantic salmon (*Salmo salar*), Fraser et al., 2021 and Black Sea Trout (*Salmo labrax*), Çimagil and Sonay, 2024). Since triploid individuals are functionally sterile, energy is redirected from reproduction to somatic growth, while maintaining high flesh quality (Tiwary et al., 2004). The elimination of sexual maturation can also increase production efficiency due to a larger window of time to harvest (Garner et al., 2008). Despite its promise, triploidization has had mixed results in improving the biomass of individuals, since no change in mass (Chinook salmon (*Oncorhynchus tshawytscha*), Garner et al., 2008; rainbow trout (*Oncorhynchus mykiss*), Scott et al., 2014) or even reduced mass, in some instances, have been reported (rainbow trout, Manor et al., 2014; Atlantic salmon, Hansen et al., 2015). Nevertheless, triploid individuals have been shown to have higher quality fillets due to preventing muscle deterioration that results from sexual maturation (Manor et al., 2014).

Drawbacks of triploidization treatments include reduced survival (Benfey, 2001; Ching et al., 2010; Fraser et al., 2022), compromised immune function/poorer disease resistance (Ching et al., 2010),

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increased sensitivity to poor environmental conditions (Scott et al., 2014; Hansen et al., 2015), and lowered responsiveness to environmental change in light, sound, and ammonia levels (Tiwary et al., 2004; Weiss and Zaniboni-Filho, 2009; Van De Pol et al., 2020). Additionally, some differences in gene expression have been found in triploids, including altered immune-response gene expression (e.g., upregulation of *NLRP3*, *LY9*, *PNMA1*, *MR1*, *PELI1*, and *NOTCH2*; downregulation of *NFIL3* and *NLRC4* in goldfish (*Carassius auratus*), Cai and Wei, 2023), upregulation of genes associated with growth (e.g., *GH*, *GHR*, *IGF-1* in crucian carp (*Carassius carassius*), Zhong et al., 2012), and with stress response (e.g., sacsin oxidative stress responsive 1b, 78 kDa glucose-related protein, heat shock protein 70, and hypoxia upregulated protein 1 precursor, *Clarias macrocephalus*, Chatchaiphan et al., 2017). Furthermore, triploidy has been found to result in a variety of behavioural differences when compared to diploid counterparts, including lowered aggression (e.g., Chinook salmon, Garner et al., 2008; Atlantic salmon, Carter et al., 1994), poorer foraging ability (e.g., Atlantic salmon, Fraser et al., 2012a; Fraser et al., 2012b; O'Keefe and Benfey, 1997; brook trout (*Salvelinus fontinalis*), O'Keefe and Benfey, 1997), lowered consumption (e.g., Chinook salmon, Garner et al., 2008; Atlantic salmon, Fraser et al., 2012a), lowered sensitivity to external stimuli (sweetfish (*Plecoglossus altivelis*), Aliah et al., 1990), and responsiveness (zebrafish (*Danio rerio*), Van De Pol et al., 2020).

1.3. Probiotics: Effects on growth and behaviour

While impacts of probiotics can depend on the species of bacteria and the host organism, probiotic diets have previously been used in aquaculture to improve a diverse suite of traits through altering the structure, function, and metabolism of the digestive tract of aquatic species (Balcázar et al., 2006) resulting in changes in: disease resistance (Aly et al., 2008; Liu et al., 2013; Ayyat et al., 2014; Butt and Volkoff, 2019), immune responses (He et al., 2013; Villamil et al., 2014), overall health (Ouwehand et al., 2002; Llewellyn et al., 2014; Butt and Volkoff, 2019), feed utilization (Irianto and Austin, 2002; Llewellyn et al., 2014; Nathanailides et al., 2021), stress responses (Llewellyn et al., 2014), and/or growth performance of fish (Irianto and Austin, 2002; Nayak, 2010; Llewellyn et al., 2014; Butt and Volkoff, 2019; Nathanailides et al., 2021). Specifically, probiotic administration can lead to growth enhancement through stimulating appetite and metabolic activity, improving nutrition by producing vitamins (Irianto and Austin, 2002), and through improved digestibility (Sahandi et al., 2019). Probiotic therapies can also stimulate gene expression in a variety of tissues such as intestinal, liver, and spleen, and have been shown to cause upregulation in genes associated with growth, such as intestinal tumor necrosis factor alpha (*TNF- α*), insulin-like growth factor (*IGF-1*), and growth hormone receptor (*GHR*) (El-kady et al., 2022; Shaheen et al., 2014).

Impacts of probiotics can furthermore extend to behavioural effects due to the enhanced release of gut microbiome metabolites from probiotic administration, stimulating enteroendocrine cells to produce hormones involved in digestion, food absorption and appetite and thus downstream consumptive behaviours (Gribble and Reimann, 2019). These effects are potentially manifested via stimulated areas within the brain responsible for feeding behaviours through appetite-regulating neuropeptides (Butt and Volkoff, 2019). As such, increased feeding rates and feed utilization can aid in explaining the increased growth benefits exhibited by probiotic therapies (Rohani et al., 2022).

1.4. Integrative behavioural-genomics approach and knowledge gaps

An individual's growth – as with any phenotypic trait – can be influenced by many factors, such as complex gene interactions, behaviours, and the environment (Benfey and Mitchell-Olds, 2009; Zuk and Balenger, 2014). For instance, behavioural phenotypes can potentially be related to variation of feeding rates/growth in fish (Castanheira et al., 2015; Huntingford and Adams, 2005). Specifically, highly responsive or reactionary fish tend to be more cautious in their environment, have lower consumption levels (Basic et al., 2012; Martins et al., 2011), and in turn have slower growth. Activity levels have also been positively associated with increases in feed intake and improvements in growth (Mas-Muñoz et al., 2011). Boldness and aggression have been related to the ability for fish to obtain more food (Krause, 1993) and improve growth (Brown et al., 2007; Lahti et al., 2001). Further, extremes of locomotion may be indicative of greater energy expenditure as swimming behaviours can be energetically costly (Rennie et al., 2005), which can result in reduced energy for somatic growth and thus lower masses. While studies have examined the link between behavioural phenotypes and underlying genetic drivers (Renn et al., 2008; Kim et al., 2015; Demin et al., 2020; Eastman et al., 2020; Sadoul et al., 2022), few have investigated how gene expression and behavioural profiles interact to explain growth performance (i.e., mass) in individuals who differ phenotypically. Moreover, the interactions between ploidy, probiotics, gene expression, behaviour, and growth in Chinook salmon are poorly understood. Since differential gene expression (De-Santis and Jerry, 2007; Mun et al., 2019), behaviour (Volpato and Fernandes, 1994; Huntingford and Adams, 2005), and the external environment (Viadero, 2005) can all influence mass, extending these interactions to performance traits may improve the power of the behavioural genomics approach in identifying integrated mechanisms driving mass variation.

In this study, we implement an integrative behavioural transcriptomics approach to determine the role of ploidy and probiotic therapy on mass of hatchery-reared juvenile Chinook salmon to better understand factors influencing growth. In a previous study (Frank et al., 2024), we performed a series of behavioural assays on diploid and triploid juvenile Chinook salmon fed either a probiotic or regular diet to determine the effects of ploidy and diet on the transcriptomic and behavioural profiles of fish. In this study, we are investigating how these profiles interact to affect variation in body size, given the individual's ploidy and diet. We predicted triploid individuals on a regular-feed diet would have greater mass relative to diploid counterparts; probiotic therapy would cause an increase in mass in both ploidies; and finally, transcriptomic and behavioural profiles may explain variation in growth. Overall, the combination of ploidy and probiotic treatments in aquaculture is expected to affect production metrics, and our work will contribute to our understanding on the mechanisms in salmon early-life stages.

2. Methods

2.1. Study species, breeding design, and husbandry

This study was performed at Yellow Island Aquaculture Ltd. (YIAL), an organic Chinook salmon farm on Quadra Island, British Columbia [50°07'59.124"N, 125°19'36.4392"W]. YIAL's domestic stock was initially bred in 1985 from individuals originating from crosses between Big Qualicum [49°23'37.954"N, 124°37'0.512″E] and Robertson Creek [49°20'6.835"N, 124°58'52.043"W] hatcheries on Vancouver Island, British Columbia (Lehnert et al., 2014). At YIAL, commercially produced Chinook salmon routinely undergo triploidization via hydrostatic pressure shock. In the current study, a full-factorial breeding design was used where eggs from eighteen captively-bred XX females were fertilized with milt from eighteen sex-reversed XX male Chinook salmon in six 3 × 3 crosses. Post-fertilization, eggs were assigned to either the diploid (2 N) or triploid (3 N) group. Diploid eggs were left untreated, while those assigned to the triploid group underwent hydrostatic pressure shock (Rottmann et al., 1991; Johnson et al., 2004; Shrimpton et al., 2012) for 5 min at 6.89×10^4kPa (10,000 psi), 30 min post-fertilization (Shrimpton et al., 2012), to prevent the ejection of the second polar body during the second meiotic division (Benfey, 1999), giving rise to triploid individuals. For this study, fertilization and triploidization occurred from October 19 to 22, 2018. Ploidy was not directly confirmed for this study;

however, in a parallel study at YIAL using the same methodology, a subset of fish ($n = 16$ per ploidy) was assessed and confirmed via flow cytometry, where triploid fish had significantly larger erythrocytes ($t_{30} = 4.390$, $p = 0.0001$) and higher DNA content ($t_{30} = 3.391$, $p = 0.0020$) than diploid fish (Cadonic et al., 2023).

Fifteen unique families (from a 3 × 3 and a 3 × 2 cross) were examined in this study, where crosses involving one female were omitted from a second 3 × 3 cross due to low survivorship of that female's offspring. When fish reached the exogenous feeding stage, they were removed from their incubation stacks to replicated rearing tanks (200*L*) between February 16 and 29, 2019, labelled with family ID (unique number assigned to each family) and treatment, with a total of 120 tanks examined (2 ploidies × 2 feed treatments X 15 families × 2 replicates per family). Fresh running water was supplied to rearing tanks from an underground well-water supply at an average temperature of 9.05 °C ± 0.485 SD, and dissolved oxygen of 9.07 mg/L ± 0.167 SD, with a consistent flow rate. Hatchery lights were set on a timer to align with the natural photoperiods on Quadra Island, British Columbia. Both triploid and diploid replicate families were assigned to their appropriate feed treatments (i.e., regular-feed or a probiotic additive-feed). Fish on the probiotic treatment began their diet treatment regime on April 5, 2019, when fish were six months post fertilization and could exogenously feed. Feed amounts were calculated according to manufacturer's instructions (Taplow Feeds, Chilliwack, BC, Canada), with daily amounts representing approximately 2% of the biomass in a tank to match a satiated growth promotion diet. Fish on a regular diet were given EWOS® Micro Crumble feed (1.2 mm in diameter), while the probiotic feed was prepared by mixing lyophilized *Lactobacillus, Bifidobacterium,* and *Lactococcus* powder from commercially available probiotics capsules (Jamieson brand, bacterial composition found in Table 1) (as described in Frank et al., 2024) with 10 mL of sodium alginate binder (made with 1 g Alginate powder mixed with 100 mL of water until dissolved) at a dosage of 10^9 cfu per kg of feed (one probiotic capsule per kg). The probiotic mixture was added to 100 g of feed and was prepared on a weekly basis and refrigerated between feedings. Fish were fed three times daily at 9 am, 1 pm, and 5 pm. Tanks were fed based on rearing densities and cleaned weekly. Tank density was not standardized to avoid overhandling the fish.

2.2. Sampling and mass measurements

At eight months post-fertilization, individuals ($n = 360$) were behaviourally assayed to characterize emergent behavioural phenotypes derived from principal component analyses (detailed in Frank et al., 2024). In brief, individual fish were subjected to a series of behavioural experiments – an open-field test, novel object test, predator response test, and a mirror test. The open-field test allowed for acclimation and exploration of the behavioural arenas to determine baseline activity levels and exploration tendencies. For the remaining tests, these were performed to examine responses to additional stimuli. The novel object test served as a new food being introduced (i.e., glass bead similar in colouration to food pellets, but larger in size). For the predator response test, a plush fish moved across the tanks to resemble the silhouette of a larger predatory fish. Finally, for the conspecific test, a mirror was added to the arena to stimulate a conspecific without the additional constraints introduced by secondary fish. The order housing barrels were sampled was randomized over the trial period each day, where housing barrels corresponded with the family number and treatment group; however, three individuals from the same housing barrel were tested and sampled simultaneously. Four behavioural profiles emerged, in which a fish was either behaviourally typed according to its responsiveness, activity, boldness/aggression, or laterality/locomotor extremes (i.e., body side preferences/reaching greater maximum velocities and locomotive distances). Upon completion of the behavioural assay, fish were then euthanized, weighed, and masses were recorded. Whole brain tissues were sampled for transcriptional analyses (Frank et al., 2024) and stored in RNAlater prior to extraction and quantification. In brief, RNA was extracted using TRIzol™ (ThermoFisher Scientific) following a protocol adapted from Wellband and Heath (2017), RNA was then converted to cDNA via high-capacity cDNA reverse transcription kits (ThermoFisher Scientific, Waltham, MA, USA). RNA integrity was tested with a subset of samples on a Bioanalyzer (Agilent Technologies, Mississauga, ON, Canada) to ensure RNA quality and all RNA integrity numbers ranged from 8.80 to 10. Transcriptional analyses were completed using the Taqman® OpenArray® chips (Applied Biosystems) designed in our previous work (Frank et al., 2024), which included a built-in technical replicate for each sample. Two endogenous control genes were used (EF-1a and ARP) to determine relative gene transcription of genes of interest. Twenty genes were verified as belonging to the following functional groups: Early Neural Development (*Shh*, *Auts2*, *Htr1aa*, and *DCX*), Long-term Neural Development (*NeuroD1*, *Crabp1a*, *Dpysl2*, *SNAP-25a*, *BDNF*, and *Dclk1*), Appetite/Metabolism (*POMC* and *NPY*), Growth (*IGF-1*, *p53*, *GH*, and *EGR1*), and Stress Response (*MR*, *GR2*, *CRF*, and *AVT*). Specific functions of genes and their relationship to growth and/or behaviour can be found in supplementary Table S1. Primer efficiencies were calculated using LinRegPCR (Ruijter et al., 2009) and transcriptional concentrations were corrected for using the geometric means of the endogenous controls, with the ΔΔCt values calculated for each gene of interest.

Table 1
Strains of bacteria and number of colony-forming units (CFU) in the Jamieson brand probiotics used in this study. Used as the probiotic feed treatment in juvenile Chinook salmon.

Bacterial Strain	Probiotic Complex in 10 Billion Colony-Forming Units (CFU)
Bifidobacterium animalis subsp. *lactis* (UABla-12)	2.0
Lactobacillus paracasei (UALpc-04)	1.5
Bifidobacterium breve (UABbr-11)	1.0
Lactobacillus gasseri (UALg-05)	1.0
Lactobacillus rhamnosus (UALr-06)	1.0
Lactobacillus rhamnosus (UALr-18)	1.0
Lactobacillus acidophilus (DDS®-1)	0.5
Lactobacillus plantarum (UALp-05)	0.5
Bifidobacterium longum subsp. *longum* (UABl-14)	0.39
Bifidobacterium bifidum (UABb-10)	0.3
Lactobacillus casei (UALc-03)	0.3
Lactobacillus reuteri (UALre-16)	0.3
Lactococcus lactis (UALI-08)	0.2
Bifidobacterium longum subsp. *infantis* (UABi-13)	0.01

2.3. Statistical analyses

To explore the integrated response of treatment, behavioural profiles, and transcriptional profiles on growth performance, GLMMs were conducted using a fish's mass (range: 1.86 g – 7.64 g) as the response variable. In these models, the effects of treatment, behavioural profiles, log-transformed single candidate gene transcription, and two-way interactions between behavioural profiles, candidate genes, and treatment (ploidy and feed) on mass were included as fixed effects. Treatments encompassed ploidy (categorical – diploid, triploid), feed (categorical – regular, probiotic), and their interactions. There was a total of four behavioural profiles (continuous scores) included: reactionary (range: 0.29–4.31), activity (range: 2.31–7.23), boldness/aggression (range: 1.48–10.20), and laterality/extremes of locomotor behaviour (range: 0.99–6.51). For these mass models, only a single gene (continuous, expression levels per gene) was included at a time to determine how each gene independently can impact the mass of individuals and interact with other variables of interest (i.e., treatment and behaviour) due to the multifaceted effects of genes. Covariates such as time of day (continuous, range: 9.4–15.53) and rearing density (continuous, range: 13–79

fish per barrel) were also included. GLMMs were run where Dam ID, Sire ID, family ID, and tank number (nested within family ID) were included as random effects. Twenty statistical models were run (one per gene), and we corrected for multiple hypothesis testing by grouping genes within their functional gene group and then using sequential Bonferroni method within each group. We took this statistical approach since gene function is not very well characterized in fish, and genes within a functional group can serve similar/overlapping functions, allowing greater confidence that general functions (i.e., neural development, metabolism/appetite, growth, and stress response) are tested. Quantile-quantile model residual plots and histograms of model residuals were visually assessed to ensure normality for all GLMMs.

Linear mixed modelling was performed in R version 4.2.2. Packages used included *dplyr* for data-manipulation (Wickham et al., 2023), *lme4* (Bates et al., 2015) for linear model creation, *ggplot2* (Wickham, 2016) and *ggeffects* (Lüdecke, 2018) for data and predicted model visualization, *interactions* for visualizing continuous-variable interactions in regression models (Long, 2024), and *pheatmap* for designing a heatmap of the mass-model results (Kolde, 2019).

3. Results

Raw *p*-values are reported in the text. Significance thresholds were determined using sequential Bonferroni corrections within gene functional groups (found in tables S2-S6).

3.1. Behavioural transcriptomics effects on mass

Mass was significantly influenced by an interaction between the transcription of the early neural development group gene *Shh* and boldness/aggression ($\beta = 0.59$, CI [0.29, 0.90], $t(118) = 3.5$, $p = 6.04 \times 10^{-4}$), regardless of treatment, indicating that the relationship between gene expression and mass depended on behavioural phenotype, as indicated in Fig. 1.

3.2. Ploidy and feed treatment effects on mass

Within the *Auts2* (early neural development gene) model, we found two significant effects: (1) an interaction between ploidy and gene transcription, and (2) ploidy. Ploidy and *Auts2* gene transcriptional levels interacted, with diploid individuals exhibiting greater mass with increasing *Auts2* transcription than triploid individuals ($\beta = -0.62$, CI [−1.17, −0.04], $t(154) = -2.03$, $p = 0.04$), indicated in Fig. 2. Ploidy was found to significantly affect mass as a main effect, with diploid individuals having greater mass (mean: 4.86 g) than triploid individuals (mean: 4.53 g) ($\beta = -1.64$, CI [−3.02, −0.17], $t(154) = -2.10$, $p = 0.037$), indicated in Fig. 3. Within the *p53* gene model (growth functional group), we found two significant treatment effects: (1) an interaction between ploidy and boldness/aggression, and (2) an interaction between feed type and gene transcription. Ploidy interacted with the boldness/aggression behaviour profile, where diploid individuals that were more bold/aggressive were heavier compared to triploid counterparts ($\beta = -0.38$, CI [−0.69, −0.07], $t(145) = -2.31$, $p = 0.023$), indicated in Fig. 2. Specifically, the estimated marginal slopes indicated a marginal change in mass for diploids ($\beta = 0.04 \pm 0.11$ SE), while triploids exhibit lower mass ($\beta = -0.34 \pm 0.13$) with increasing boldness/aggression.

Probiotic therapies (mean: 4.56 g) did not significantly impact mass directly compared to fish on a regular diet (mean: 4.84 g). However, feed type interacted with transcription of the *p53* gene ($\beta = -0.73$, CI [−1.37, −0.11], $t(145) = -2.13$, $p = 0.04$), indicated in Fig. 4, where individuals on a probiotic diet were heavier with increased *p53* transcription compared to individuals on a regular feed type.

3.3. Independent gene and Behaviour effects on mass

For all fish, regardless of treatment, gene transcription effects of four genes were positively related to mass: (1) early neural development gene *Auts2* ($\beta = 0.55$, CI [0.02, 1.03], $t(154) = 1.99$, $p = 0.048$), (2) growth gene *IGF-1* ($\beta = 0.60$, CI [0.14, 1.06], $t(165) = 2.39$, $p = 0.018$), (3) growth gene *p53* ($\beta = 1.04$, CI [0.41, 1.69], $t(145) = 2.98$, $p = 3.42 \times 10^{-3}$), and (4) the stress response gene *CRF* ($\beta = 0.81$, CI [0.28, 1.32], $t(145) = 2.87$, $p = 4.81 \times 10^{-3}$), indicated in Fig. 5. *Auts2*, *IGF-1*, *p53*, and *CRF* gene transcription were all associated with greater mass. No behavioural profiles analyzed were found to independently influence mass in any of the models (all *p*-values n.s. after Bonferroni correction to acceptance threshold) (Fig. 6).

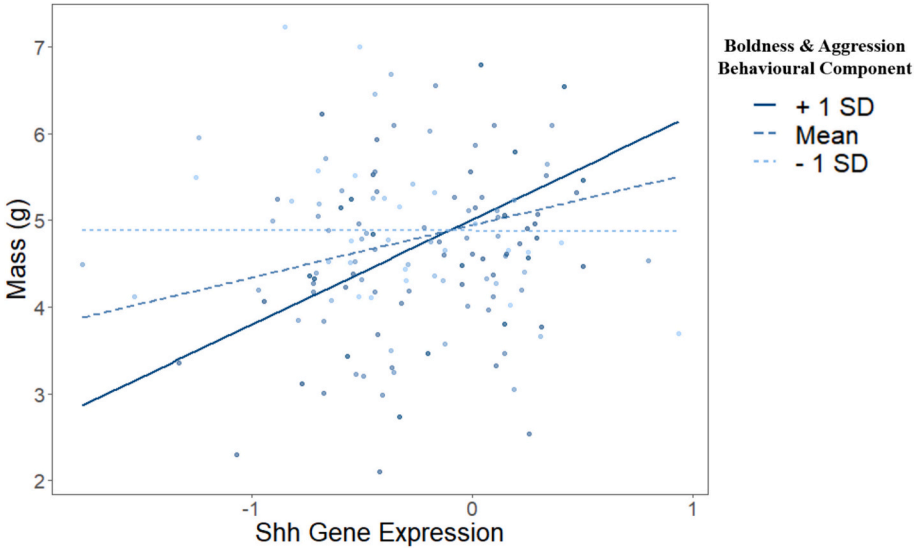

Fig. 1. Significant model predicted results of regression analysis of treatment, gene transcription, and behaviours on mass models. Behavioural transcriptomic interactions were found between *Shh* gene trascription and a bold/aggressive behavioural profile on mass of individuals. Fish displaying a more bold/aggressive behavioural profile (solid-dark blue line) were larger with increasing *Shh* transcription, while those that were less bold/aggressive (small dashed-light blue line) did not exhibit mass changes to increasing *Shh* transcription. Model-predicted relationships are plotted, with data points showing the predicted-model data. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

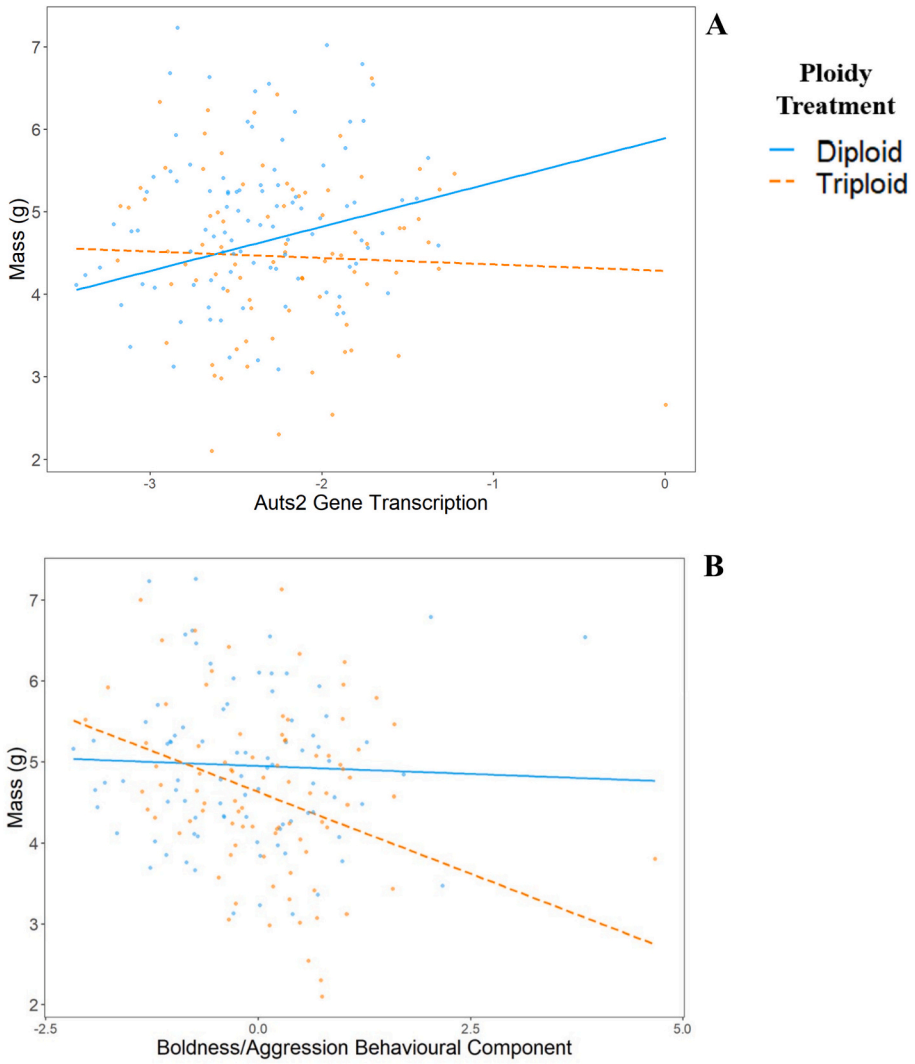

Fig. 2. Significant model-predicted results of regression analysis of treatment, relative gene transcription, and behaviours on mass. Interaction effects between ploidy and transcription, and ploidy and behavioural profiles. Diploid (blue solid) individuals exhibited greatest mass at high *Auts2* (early neural gene) transcription compared to triploid (orange dashed) individuals (A) ($n = 179$). Triploid individuals had lowest mass with a higly bold/aggressive profile in the *p53* mass model (B) ($n = 170$). Model-predicted relationships are plotted, with data points showing the predicted-model data. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.4. Additional variable effects on mass

Time of day significantly impacted mass in a consistent way (independent of treatment group) in the following three gene models: (1) long-term neural development gene *Dclk1* ($\beta = 0.09$, CI [0.02, 0.15], $t(202) = 2.44$, $p = 0.017$), (2) *p53* ($\beta = 0.11$, CI [0.03, 0.18], $t(145) = 2.59$, $p = 0.012$), and (3) *CRF* ($\beta = 0.12$, CI [0.04, 0.19], $t(145) = 2.92$, $p = 2.92 \times 10^{-3}$). Graphical representations of the relationship between time of day and mass can be found in supplementary materials Fig. S1. Heavier individuals were sampled later in the day. Tank-rearing densities had no effect on mass of individuals in any of the models (all p-values n.s. after Bonferroni correction to acceptance threshold). Random effects of sire impacted a single stress response gene: *MR* ($p = 0.03$). Neither dam nor family had a significant impact (all p-values n.s. after Bonferroni correction to acceptance threshold). A graphical summary of the significance values for all mass models can be found in supplementary materials Fig. S2. A summary of significant results from mass models across gene functional groups can be found in Table 2, and full results can be found in Tables S2-S6.

4. Discussion

In this study, we examined the effects of ploidy and probiotics – as a potential practice to improve triploid performance – on the growth of juvenile Chinook salmon via a behavioural transcriptomics approach. Both gene transcription and behaviour were incorporated into our body-mass models in recognition that behavioural traits often covary with gene transcription, indicating a transcriptomic constraint on organismal responses to variation in the environment (Rittschof and Hughes, 2018). When examining integrated effects on mass using a one-gene-at-a-time approach, we found a significant behavioural-transcriptomic relationship with mass independent of ploidy or probiotic diet (Shh x boldness/aggression). We found minimal direct effects of ploidy, although diploids were heavier than triploid individuals across our GLMMs, and no direct effects of probiotics on fish performance. No direct effects of behaviour on mass were observed either, but we did find an effect of ploidy x boldness/aggression on mass in the p53 model. Instead, we found an overall greater influence of transcriptional expression on mass, either independently, with higher expression of *Auts2*, *IGF-1*, *p53*, and *CRF* being positively associated with mass; or integrated with behaviour (see above) or treatment (ploidy x Auts2 and feed x p53 transcription).

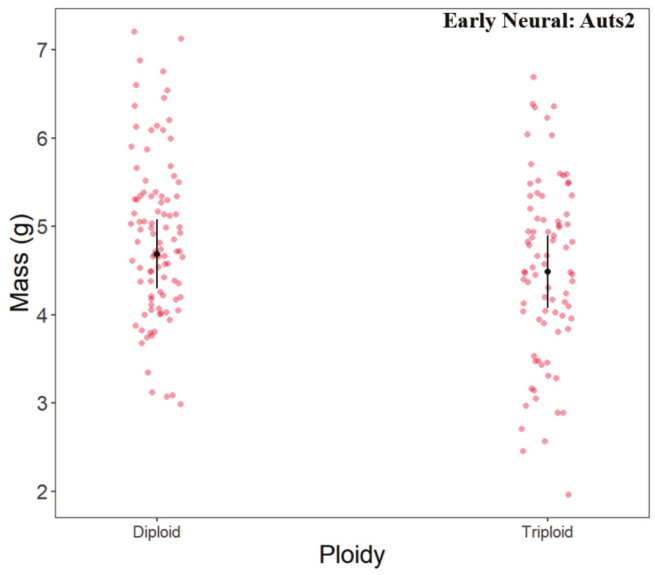

Fig. 3. Significant model-predicted results of regression analysis of treatment, gene transcription, and behaviours on mass. A significant effect of ploidy was found on mass through the *Auts2* (early neural) gene model (n = 179). Triploid individuals had lower mass compared to diploid counterparts. Model-predicted relationships are plotted, with data points showing the predicted -model data.

4.1. Behavioural transcriptomics effects on mass

Mass differences were associated with *Shh* transcription when interacting with boldness/aggression, in that fish with a highly bold/aggressive behavioural profile were generally more sensitive to differences in transcription of *Shh* than shy, non-aggressive individuals, and exhibited increased mass when coupled with high *Shh* transcription levels. *Shh* is important at various developmental stages (from embryogenesis to adult) and is necessary for the maintenance, proliferation, differentiation, and migration of neural stem cells (Sasai et al., 2019). *Shh* also plays a role in cell growth (Araújo et al., 2014), which may be related to its role in cellular growth processes, although this was not directly tested in this study. However, the exact mechanisms linking boldness/aggression behavioural types with Shh transcription are unknown and further research is necessary to explore this connection. Interestingly, Velando et al. (2017) found that the integration of transcriptional profiles (i.e., metabolic genes *pgc1a*, *cox4*, *sod2*, and *gpx1*) with activity phenotypes in three-spined sticklebacks (*Gasterosteus aculeatus*) reflected differential investment in growth during development (albeit sex-specific); a result similar to ours.

4.2. Main effects of ploidy and probiotics on mass

Contrary to our predictions, we found no overall or consistent direct effect of ploidy or probiotic treatment on fish performance in our mass models (which included fish behaviour and gene transcription factors). This is surprising, given our expectations that both ploidy (Rottmann et al., 1991; Benfey, 1999) and probiotics (Brizuela et al., 2001; Nayak, 2010; Gonçalves et al., 2011) can improve growth/mass of an individual. Triploidization may not have produced the expected mass impacts which could indicate that these individuals do not experience the same effects of triploidy as other species (e.g., Atlantic salmon, *Salmo salar*, Carter et al., 1994; rainbow trout, *Oncorhynchus mykiss*, Cleveland and Weber, 2013). Other studies have also found no effect of triploidy on growth of fish (brook trout, *Salvelinus fontinalis*, O'Keefe and Benfey, 1999; zebrafish, Van De Pol et al., 2020). Only in one mass model with the *Auts2* gene (essential in early neurodevelopment) did we find diploid individuals to be significantly greater in mass (average difference of 0.33 g) than their triploid counterparts. Similarly, Withler et al.'s (1995) research did find triploid coho salmon to be significantly smaller at the pre-smolt stage; however, this difference between the two ploidy groups did not persist when they were measured again as post-smolts, and coho salmon, regardless of ploidy, maintained similar growth rates (Withler et al., 1998). Further, Devlin et al.'s (2001) work with transgenic (i.e., microinjected with growth hormone gene construct) rainbow trout demonstrated that both domestic strains, regardless of transgene presence, did not vary significantly in growth performance. One cannot rule out that the growth advantage expected by triploids, regardless of diet, may not be present during earlier life stages, but rather when individuals normally reach maturity and begin to heavily invest energy in reproduction (Tiwary et al., 2004). A longer-term study

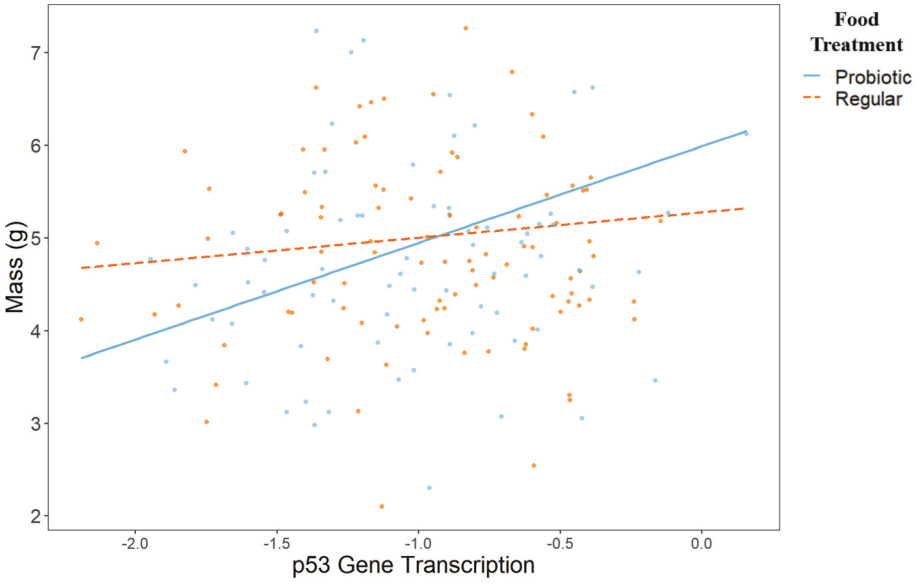

Fig. 4. Significant model-predicted results of regression analysis of treatment, gene transcription, and behaviours on mass models. Interaction effects of probiotics and *p53* gene expression (growth gene model, n = 170). Individuals on a probiotic diet (blue, solid line) were heavier than regular fed individuals at high *p53* transcription levels. Model-predicted relationships are plotted, with data points showing the predicted-model data. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

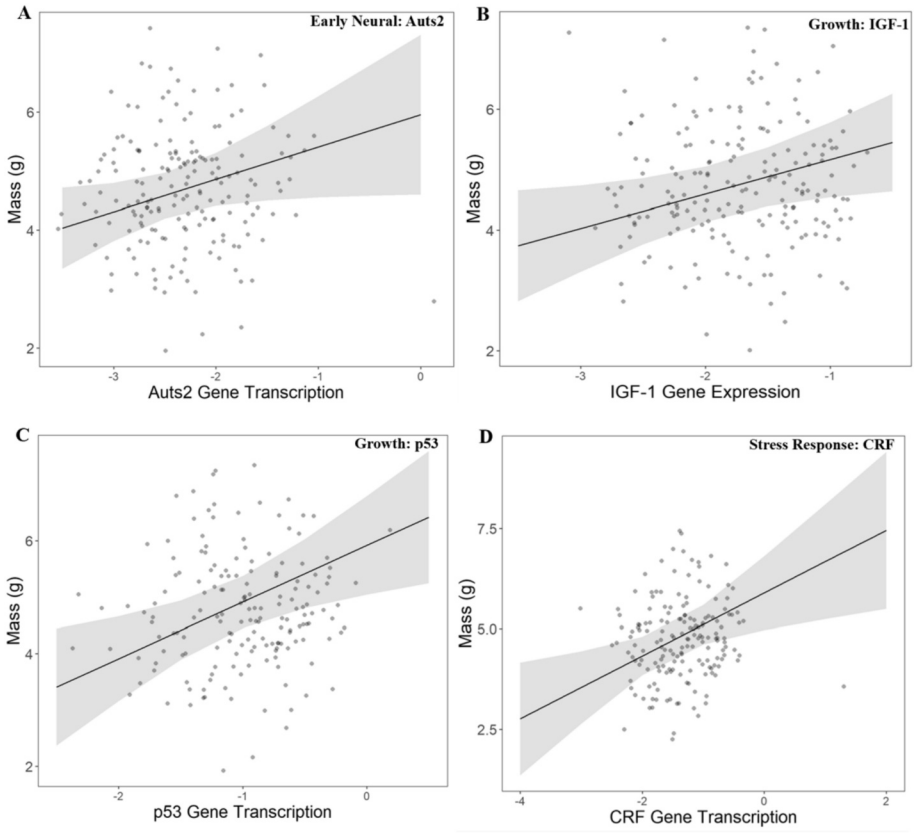

Fig. 5. Significant model-predicted results of regression analysis of treatment, gene transcription, and behaviours on mass models. Gene expression levels with a direct significant relationship with mass. Individuals with increased *Auts2* (A) (n = 179), *IGF-1* (B) (*n* = 190), *p53* (C) (n = 170) and *CRF* (D) (n = 170) transcription all exhibited increases in mass. Model-predicted relationships are plotted, data points show the predicted-model data.

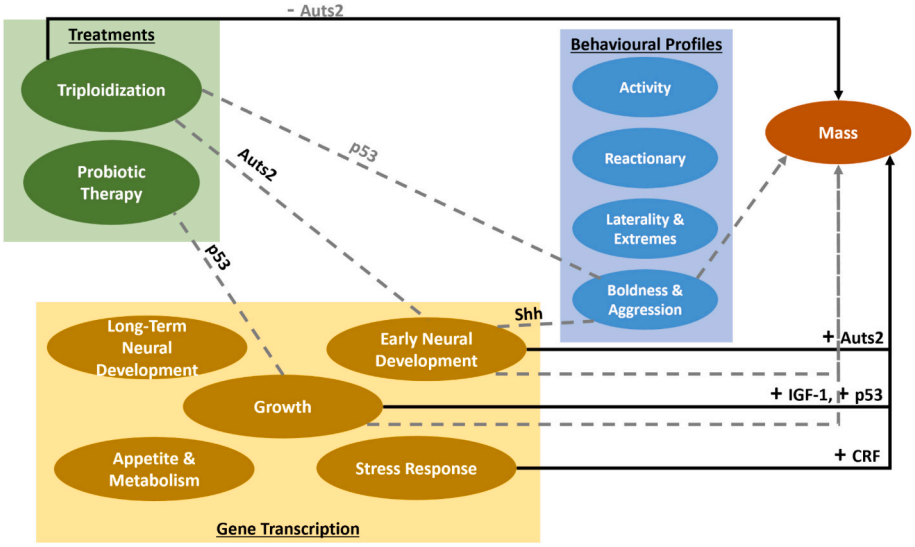

Fig. 6. Summary diagram of findings (significant interactions), incorporating gene transcriptional profiles, behavioural profiles and mass results. Time of day and rearing tank density not included in this summary. Significant interactions of direct effects indicated by solid (black) lines with directionality indicated, and interactions between variables indicated by dashed lines (grey). Final effect of interactions is always on mass output. Where genes are directly involved, genes are indicated in black text, while genes included in the mass model, but not directly driving the impact are indicated in grey.

would be required to test this possibility. Since we did not directly validate triploidization, it is possible that some of the triploid fish may be diploid. However, given the success of triploidization of YIAL in previous studies conducted over more than twenty years (e.g., Shrimpton et al., 2012; Ching et al., 2010; Johnson et al., 2004), this would be few (if any) individuals, and act in a manner that eliminated ploidy effects found here.

Longer-term probiotic therapies may be necessary, as our probiotic mixture did not provide a growth benefit during early-life stages that would ultimately enhance biomass. We only observed an impact on mass

Table 2
Summary of significant results from mass GLMMs with gene transcription as an explanatory variable, with t-values and significance values (indicated in parentheses) for each variable included in the model indicated. Adjusted accepted significance values indicated separately for each gene model, and data reported here indicates significant values after Bonferroni-Holm corrections.

Gene Model	Variable	t-value	Significance
	Early Neural Development Genes		
Shh ($p < 0.017$)	Gene*Boldness/Aggression	3.53	$6.04e^{-4}$
Auts2 ($p < 0.05$)	Ploidy	−2.10	0.037
	Gene	1.99	0.038
	Ploidy*Gene	−2.03	0.04
	Long-Term Neural Development		
Dclk1 ($p < 0.05$)	Time of Day	2.44	0.027
	Growth		
IGF-1 ($p < 0.025$)	Gene	2.39	0.018
p53 ($p < 0.05$)	Gene	2.98	0.003
	Time of Day	2.59	0.012
	Ploidy*Boldness/Aggression	−2.31	0.023
	Feed*Gene	−2.13	0.04
	Stress Response		
CRF ($p < 0.017$)	Gene	2.87	0.004
	Time of Day	2.92	0.005

through transcriptional interactions. Probiotics have been found to benefit the host, although effects on the host organism can depend on both the bacterial composition and the host administered (Sumon et al., 2022). Multi-strain probiotics have been proven to be more beneficial than single strain, but strains can be incompatible (Puvanasundrum et al., 2021) and a possible mechanism for a lack of growth benefits, however, this has not been explored in our study. The encapsulation method used for administration of probiotics (i.e., sodium alginate) may have deterred fish from consuming the feed, negating expected growth effects, but this requires further examination. Our research did not directly confirm the success of probiotic colonization within the gut for the individuals studied here. In parallel studies at YIAL and using the same system, probiotics were confirmed to influence the composition of the gut microbiota in juvenile Chinook salmon compared to individuals on a regular feed (Sadeghi, 2022; Frank et al., 2024; Soto-Dávila et al., 2024). In another parallel study, five of the twelve bacterial species (as metabarcoding could not differentiate between strains) contained in the probiotic mixture were found in elevated levels within the gut at the individual level; meanwhile, all were elevated at the family level (Bai, 2026). Furthermore, these probiotics were successful for improving long-term survival of these same families of hatchery-reared Chinook salmon (Soto-Dávila et al., 2024). Previous work (Frank et al., 2024) found an impact of our probiotic mixture on metabolism/appetite gene transcription; thus, it would be interesting for future research to explore how these probiotics directly impact feeding behaviours and downstream mass differences. Because specific impacts of probiotics are dependent on the strain(s) of bacteria (Ouwehand et al., 2002; Das et al., 2008; Shi et al., 2016), dosage concentration of probiotics administered (Nayak, 2010; Butt and Volkoff, 2019), and environmental factors (e.g., temperature, water pH, and oxygen concentration) (Das et al., 2008), these elements should also be accounted for when selecting a probiotic to use in an aquaculture setting. The selected probiotic formulation was chosen for its practical applications in hatcheries including ease of use and cost-effectiveness. Since these probiotics were formulated for human consumption, there may be variability in success for different species of fish, which should be explored in future studies. Future research could also focus on other diet additives (e.g., antibiotics, prebiotics, or acidifiers) which have been useful in altering fish performance/quality (Dawood et al., 2015; Ng and Koh, 2016; Dawood et al., 2018).

4.3. Gene transcription effects on mass

4.3.1. Direct effects

The few direct effects of gene transcription variation on fish performance originated from the early neural development, growth, and stress response functional groups. We had expected a greater number of genes to be associated with growth, given that several genes (e.g., *GH*, *EGR-1*, *IGF-1*, *IGFBP2b*, *p53*) have been shown to be directly linked with growth in fish (Bhaskaran et al., 1999; Zhou et al., 2008; Rajan et al., 2011; Duclot and Kabbaj, 2017; Bertucci et al., 2019; Khan, 2019), including with an altered diet (Amin et al., 2019; Kari et al., 2022). Our unexpected results may be due to our multi-variable models combined with our conservative correction for multiple-hypothesis testing, as previous studies examined one, or at most a few, genes independently, using conservative false discovery rate corrections that can contribute to Type II error as a result of multiple-hypothesis testing. Nonetheless, after correction, we still found four genes that were related to the mass of fish, where an increase in the transcription of *Auts2*, *IGF-1*, *p53*, and *CRF* genes were associated with heavier individuals.

Auts2 expression is critical for appropriate neurodevelopment, and under-expression can result in growth/feeding problems, body/skeletal abnormalities, and neurological disorders (Oksenberg and Ahituv, 2013). In terms of function, the *Auts2* gene has not previously been linked to size or mass of fish. However, this gene does play a role in the activation of transcription and regulation of neural development through participation in neuronal migration/neurite extensions (Colson et al., 2019) and is highly expressed in neural areas of high cognitive functions (Bedogni et al., 2010). The role of *Auts2* in cognitive abilities (Colson et al., 2019) may translate to the coping ability of animals, where greater cognitive abilities may allow for better coping with their environment (Broom, 2010). It is important to note, that the function of this gene is not fully understood (Oksenberg and Ahituv, 2013), and more research on the relationship between *Auts2* and body size is required.

Up-regulation of *IGF-1* is expected to increase mass due to its involvement in growth as it stimulates muscle cell proliferation, differentiation, and hypertrophy (i.e., enlargement of muscle tissues), while inhibiting muscle atrophy (Glass, 2003; Glass, 2005); and it has previously been shown to be positively related to growth rate in Atlantic salmon (*Salmo salar*) (Solberg et al., 2012).

While our results did not show transcription of the *GH* gene to directly impact mass (as reported in Trainor and Hofmann, 2006, 2007), another growth gene (i.e., *p53*) was an important variable in explaining the interaction between ploidy treatments, aggressive behavioural profiles, and mass. Increased *p53* expression is associated with increases in mass in *Drosophila*, possibly due to the increased maintenance of healthy cells ensuring increased organismal growth (Mollereau and Ma, 2014).

Lastly, our *CRF*-gene result is challenging to explain, given CRF's main role is in organismal stress response (Bernier, 2006), where up-regulation of the *CRF* gene has been previously found to suppress feed intake (Volkoff and Peter, 2006) and appetite during stress (Denver, 2009; Conde-Sieira et al., 2018). However, *CRF* gene expression also has a role in the regulation of food intake in fish, and increased *CRF* expression has been found to lead to increased metabolic rate (Rothwell, 1990), and positively related to growth rates (e.g., brown trout, *Salmo trutta*, Álvarez and Nicieza, 2005; sockeye salmon, *Oncorhynchus nerka*, Jobling, 1981), and can be used to stimulate foraging via cortisol following a stressful event. Nevertheless, excess cortisol can result in poorer growth (Bernier et al., 2004). However, the relationship between CRF expression and growth is complex and context dependent, requiring further investigation.

We did not find support for metabolism and appetite genes being directly associated with mass, despite their known activation in neural tissues (Ahi et al., 2022). However, appetite is controlled through multiple systems (Bernier and Craig, 2005), and the candidate genes we selected may not be reflective of these additional processes.

4.3.2. Interactive effects with treatment

We also found, in certain instances, genes from the early-neural development and growth functional groups to interact with treatment to influence mass. Again, the role of increased *Auts2* expression on mass is still not fully understood (Oksenberg and Ahituv, 2013); however, with regards to ploidy, our results may indicate that diploid individuals have an increased sensitivity to changing *Auts2* transcription levels since there is a relatively greater increase in mass with increasing *Auts2* transcription than when compared to triploids. Next, probiotics were found to interact with growth gene *p53*, where individuals on a probiotic diet had increased mass at high levels of *p53* gene transcription. Alternatively, those on a regular diet were less responsive to changes in this gene's transcription and subsequent mass increases. Probiotics have previously been found to increase growth performance (Dawood and Koshio, 2016), and in our study, this pattern suggests an association between probiotic treatment and gene expression effects on mass, although causality cannot be inferred. As *p53* transcription was furthermore associated with mass independent of food treatments, it is plausible that the probiotic therapy is enhancing the existing mass impact of increased *p53* expression.

4.4. Behavioural effects on mass

Unexpectedly, we found no independent behavioural effects on mass. Instead, although not universal, boldness/aggression was associated with mass through an interaction effect with ploidy. In the *p53* mass model, triploid individuals had greater mass when exhibiting a shy or non-aggressive behavioural profile, compared to aggressive triploid counterparts, possibly due to lower investment in this energetically costly behaviour (Dehnen et al., 2022). Diploids did not experience the same change in mass as triploids in relation to variation across a bold or aggressive behavioural axis, suggesting that these behaviours may not impose the same energetic constraints for diploid fish or may be a byproduct of less variation exhibited by triploid fish. Long-term implications of a stronger relationship between mass and boldness/aggression in triploids remains to be studied.

4.5. Additional effects on mass

Finally, time of day was correlated with mass differences (*Dclk1*, *p53*, and *CRF*-gene models) where individuals sampled later in the day had a greater mass than those sampled at earlier time points. This finding could be reflective of the time points in which individuals were fed, as the first feeding in a day occurred prior to trials. Fish sampled first would have been examined immediately after a regular nighttime period of fasting. However, one of the feed periods occurred during the behavioural trials, but the results did not reflect an increase in mass around 1 pm or shortly following this feed time.

We found no significant maternal effects on body mass across the tested models; however, these effects would predominantly be detected in early egg/larval (Berg et al., 2001). Maternal effects would likely not persist into the fry life stage, since maternal effects decline with developmental stage (i.e., prevalent during the egg and alevin stage in Chinook salmon) (Heath et al., 1999; Venney et al., 2019). Sire effects, in turn, have been shown to be prevalent later in life (Falica and Higgs, 2013). We found sire effects on mass (only in the stress response gene MR), which could be indicative of the paternal genetic contribution on offspring size at the 8-month postfertilization stage. Lastly, there was no significant family effect indicating no presence of an interaction between dam and sire on mass. These findings may be a result of few individuals bred for this study (i.e., three females crossed with three males, and two females crossed with three males) for a total of 15 unique families. The goal of this study was not to explore the role of genetic architecture on our variables, but rather to correct for any confounding genetic effects.

5. Conclusions

With our study, we examined mass of juvenile Chinook salmon with respect to treatment, behavioural profiles, gene transcription, and the interactions between these variables. Our statistical approach incorporating a single gene in each model and performing sequential Bonferroni corrections allowed for us to examine possible interactions of interest between behavioural profiles and gene transcription. Mass was positively associated with the interaction between *Shh* gene transcription and boldness/aggression. In terms of our treatments, mass was negatively associated with the interactions between triploidy and both *Auts2* transcription and boldness/aggression while being positively associated with an interaction between probiotics and *p53* transcription. Finally, mass was positively associated with gene transcription of *Auts2*, *IGF-1*, *p53*, and *CRF*. Combining transcriptional and behavioural profiles to determine how these variables interact in terms of fish mass or growth with respect to ploidy and feed treatments is a relatively uncommon approach in aquaculture. Our results were contrary to our expectations, however, probiotic effects on mass suggest our probiotic supplement used – that contained species of *Bifidobacterium*, *Lactobacillus*, and *Lactococcus* may not increase mass in hatchery-reared juvenile Chinook salmon despite their confirmed effect on gut microbiota (Nayak, 2010).

Nevertheless, our study underscores the relevance of behavioural transcriptomics as an analytical approach with practical application in providing mechanistic information about whole-animal phenotype (i.e., mass in this study). Behavioural transcriptomics, as applied here, highlights important relationships and interactions that remain to be described. While we did not find overall direct impacts of ploidy or probiotics on mass, our findings suggest that growth variation may be associated with interactions between gene transcription and behavioural traits, rather than broad treatment-level influences. These results emphasize the importance of considering the interplay between ploidy, feed, gene expression, and behaviours, which aquaculture managers should account for when selecting treatment options to enhance biomass.

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Relationships

There are no additional relationships to disclose.

Patents and intellectual property

There are no patents to disclose.

Other activities

There are no additional activities to disclose.

CRediT authorship contribution statement

Chelsea E. Frank: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis. **Daniel D. Heath:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Christina A.D. Semeniuk:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Ethics approval

The experiment conducted here was conducted in accordance with the University of Windsor's Animal Care Committee and the Canadian Council of Animal Care to ensure ethical research practices for the experiment outlined here.

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Declaration of competing interest

None.

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Data availability

Data will be made available on request.

References

Ahi, E.P., Brunel, M., Tsakoumis, E., Chen, J., Schmitz, M., 2022. Appetite regulating genes in zebrafish gut; a gene expression study. PLoS One 17 (7), e0255201. https://doi.org/10.1371/journal.pone.0255201.

Aliah, R.S., Yamaoka, K., Inada, Y., Taniguchi, N., 1990. Effects of triploidy on tissue structure of some organs in ayu. Nippon Suisan Gakkaishi 56 (4), 569–575. https://doi.org/10.2331/suisan.56.569.

Álvarez, D., Nicieza, A.G., 2005. Is metabolic rate a reliable predictor of growth and survival of brown trout (*Salmo trutta*) in the wild? Can. J. Fish. Aquat. Sci. 62, 643–649. https://doi.org/10.1139/F04-223.

Aly, S.M., Abdel-Galil Ahmed, Y., Abdel-Aziz Ghareeb, A., Mohamed, M.F., 2008. Studies on *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of *Tilapia nilotica* (*Oreochromis niloticus*) to challenge infections. Fish Shellfish Immunol. 25 (1–2), 128–136. https://doi.org/10.1016/j.fsi.2008.03.013.

Amin, A., Asely, A.E., El-Naby, A.S.A., Samir, F., El-Ashram, A., Sudhakaran, R., Dawood, M.A.O., 2019. Growth performance, intestinal histomorphology and growth-related gene expression in response to dietary *Ziziphus mauritiana* in Nile tilapia (*Oreochromis niloticus*). Aquaculture 512, 734301. https://doi.org/10.1016/j.aquaculture.2019.734301.

Araújo, G.L.L., Araújo, A.M., Schroeder, T., Tort, A.B.L., Costa, M.R., 2014. Sonic hedgehog signaling regulates mode of cell division of early cerebral cortex progenitors and increases astrogliogenesis. Front. Cell. Neurosci. 8, 77. https://doi.org/10.3389/fncel.2014.00077.

Ayyat, M.S., Labib, H.M., Mahmoud, H., 2014. A probiotic cocktail as a growth promoter in Nile Tilapia (*Oreochromis niloticus*). J. Appl. Aquac. 26 (3), 208–215. https://doi.org/10.1080/10454438.2014.934164.

Bai, E., 2026. The Effects of Triploidization and Probiotics on the Gut Microbiome of Chinook salmon (*Oncorhynchus tshawytscha*) fry. [Unpublished MSc. Thesis, University of Windsor]..

Balcázar, J.L., Decamp, O., Vendrell, D., De Blas, I., Ruiz-Zarzuela, I., 2006. Health and nutritional properties of probiotics in fish and shellfish. Microb. Ecol. Health Dis. 18 (2), 65–70. https://doi.org/10.1080/08910600600799497.

Basic, D., Winberg, S., Schjolden, J., Krogdahl, Å., Höglund, E., 2012. Context-dependent responses to novelty in rainbow trout (*Oncorhynchus mykiss*), selected for high and low post-stress cortisol responsiveness. Physiol. Behav. 105 (5), 1175–1181. https://doi.org/10.1016/j.physbeh.2011.12.021.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67 (1), 1–48. https://doi.org/10.18637/jss.v067.i01.

Bedogni, F., Hodge, R.D., Nelson, B.R., Frederick, E.A., Shiba, N., Daza, R.A., Hevner, R. F., 2010. Autism susceptibility candidate 2 (Auts2) encodes a nuclear protein expressed in developing brain regions implicated in autism neuropathy. Gene Expr. Patterns 10 (2020), 9–15. https://doi.org/10.1016/j.gep.2009.11.005.

Benfey, P.N., Mitchell-Olds, T., 2009. From genotype to phenotype: systems biology meets natural variation. Science 320 (5875), 495–497. https://doi.org/10.1126/science.1153716.

Benfey, T.J., 1999. The physiology and behavior of triploid fishes. Rev. Fish. Sci. 7 (1), 39–67. https://doi.org/10.1080/10641269991319162.

Benfey, T.J., 2001. Use of sterile triploid Atlantic salmon (*Salmo salar* L.) for aquaculture in New Brunswick, Canada. ICES J. Mar. Sci. 58, 525–529. https://doi.org/10.1006/jmsc.2000.1019.

Berg, O.K., Hendry, P., Svendsen, B., Bech, C., Arnekleiv, J.V., Lohrmann, A., 2001. Maternal provisioning of offspring and the use of those resources during ontogeny: variation within and between Atlantic Salmon families. Funct. Ecol. 15 (1), 13–23. https://doi.org/10.1046/j.1365-2435.2001.00473.x.

Bernier, N.J., 2006. The corticotropin-releasing factor system as a mediator of the appetite-suppressing effects of stress in fish. Gen. Comp. Endocrinol. 146 (2006), 45–55. https://doi.org/10.1016/j.ygcen.2005.11.016.

Bernier, N.J., Craig, P.M., 2005. CRF-related peptides contribute to stress response and regulation of appetite in hypoxic rainbow trout. Am. J. Phys. Regul. Integr. Comp. Phys. 289 (4 58–4), 982–990. https://doi.org/10.1152/ajpregu.00668.2004.

Bernier, N.J., Bedard, N., Peter, R.E., 2004. Effects of cortisol on food intake, growth, and forebrain neuropeptide Y and corticotropin-releasing factor gene expression in goldfish. Gen. Comp. Endocrinol. 135 (2), 230–240. https://doi.org/10.1016/j.ygcen.2003.09.016.

Bertucci, J.I., Blanco, A.M., Sundarrajan, L., Rajeswari, J.J., Velasco, C., Unniappan, S., 2019. Nutrient regulation of endocrine factors influencing feeding and growth in fish. Front. Endocrinol. 10, 83. https://doi.org/10.3389/fendo.2019.00083.

Bhaskaran, A., May, D., Rand-Weaver, M., Tyler, C.R., 1999. Fish p53 as a possible biomarker for genotoxins in the aquatic environment. Environ. Mol. Mutagen. 33 (3), 177–184.

Brizuela, M.A., Serrano, P., Ferez, Y., 2001. Studies on probiotics properties of two lactobacillus strains. Braz. Arch. Biol. Technol. 44 (1), 95–99. https://doi.org/10.1590/S1516-89132001000100013.

Broom, D.M., 2010. Cognitive ability and awareness in domestic animals and decisions about obligations to animals. Appl. Anim. Behav. Sci. 126, 1–11. https://doi.org/10.1016/j.applanim.2010.05.001.

Brown, C., Burgess, F., Braithwaite, V.A., 2007. Heritable and experiential effects on boldness in a tropical poecilid. Behav. Ecol. Sociobiol. 62, 237–243. https://doi.org/10.1007/s00265-007-0458-3.

Butt, R.L., Volkoff, H., 2019. Gut microbiota and energy homeostasis in fish. Front. Endocrinol. 10, 9. https://doi.org/10.3389/fendo.2019.00009.

Cadonic, I.G., Heath, J.W., Dixon, B., Craig, P.M., 2023. Diploid and triploid Chinook salmon (*Oncorhynchus tshawytscha*) have altered microRNA responses in immune tissues after infection wit *Vibrio anguillarum*. Comp. Biochem. Physiol. D Genomics Proteomics 48, 101121. https://doi.org/10.1016/j.cbd.2023.101121.

Cai, Y., Wei, K., 2023. Comparative analysis of intestinal microbiota composition and transcriptome in diploid and triploid *Carassius auratus*. BMC Microbiol. 23 (1), 1. https://doi.org/10.1186/s12866-022-02709-5.

Carter, C.G., McCarthy, I.D., Houlihan, D.F., Johnstone, R., Walsingham, M.V., Mitchell, A.I., 1994. Food consumption, feeding behaviour, and growth of triploid and diploid Atlantic salmon, *Salmo salar L.*, parr. Can. J. Zool. 72, 609–617. https://doi.org/10.1139/z94-083.

Castanheira, M.F., Conceição, L.E.C., Millot, S., Rey, S., Bégout, M., Damsgård, B., Kristiansen, T., Höglund, E., Øverli, Ø., Martins, C.I.M., 2015. Coping styles in farmed fish: consequences for aquaculture. Rev. Aquac. 9 (1), 23–41. https://doi.org/10.1111/raq.12100.

Chatchaiphan, S., Srisapoome, P., Kim, J., Devlin, R.H., Na-nakorn, U., 2017. De novo transcriptome characterization and growth-related gene expression profiling of diploid and triploid bighead catfish (*Clarias macrocephalus*). Mar. Biotechnol. 19, 36–48. https://doi.org/10.1007/s10126-017-9730-3.

Ching, B., Jamieson, S., Heath, J.W., Heath, D.D., Hubberstey, A., 2010. Transcriptional differences between triploid and diploid Chinook salmon (*Oncorhynchus tshawytscha*) during live *vibrio anguillarum* challenge. Heredity 104 (2), 224–234. https://doi.org/10.1038/hdy.2009.108.

Çimagil, R., Sonay, F.D., 2024. Comparison of growth performance and smoltification of triploid and diploid black sea trout (*Salmo labrax*) in freshwater and seawater. Turk. J. Fish. Aquat. Sci. 25 (4), TRJFAS26908. https://doi.org/10.4194/TRJFAS26908.

Cleveland, B.M., Weber, G.M., 2013. Effects of triploidy on growth and protein degradation in skeletal muscle during recovery from feed deprivation in juvenile rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. -Part A Mol. Integr. Physiol. 166 (1), 128–137. https://doi.org/10.1016/j.cbpa.2013.05.017.

Colson, V., Cousture, M., Damasceno, D., Valotaire, C., Nguyen, T., Le Cam, A., Bobe, J., 2019. Maternal temperature exposure impairs emotional and cognitive responses and triggers dysregulation of neurodevelopment genes in fish. PeerJ 7, e6338. https://doi.org/10.7717/peerj.6338.

Conde-Sieira, M., Chivite, M., Míguez, J.M., Soengas, J.L., 2018. Stress effects on the mechanisms regulating appetite in teleost fish. Front. Endocrinol. 9, 1–8. https://doi.org/10.3389/fendo.2018.00631.

Das, S., Ward, L.R., Burke, C., 2008. Prospects of using marine actinobacteria as probiotics in aquaculture. Appl. Microbiol. Biotechnol. 81 (3), 419–429. https://doi.org/10.1007/s00253-008-1731-8.

Dawood, M.A.O., Koshio, S., 2016. Recent advances in the role of probiotics and prebiotics in carp aquaculture: a review. Aquaculture 454, 243–251. https://doi.org/10.1016/j.aquaculture.2015.12.033.

Dawood, M.A.O., Koshio, S., Ishikawa, M., Yokoyama, S., El Basuini, M.F., Hossain, M.S., Nhu, T.H., Moss, A.S., Dossou, S., Wei, H., 2015. Dietary supplementation of β-glucan improves growth performance, the innate immune response and stress resistance of red sea bream, *Pagrus major*. Aquac. Nutr. 23 (1), 148–159. https://doi.org/10.1111/anu.12376.

Dawood, M.A.O., Koshio, S., Esteban, M.Á., 2018. Beneficial roles of feed additives as immunostimulants in aquaculture: a review. Rev. Aquac. 10, 950–974. https://doi.org/10.1111/raq.12209.

Dehnen, T., Papageorgiou, D., Nyaguthii, B., Cherono, W., Penndorf, J., Boogert, N.J., Farine, D.R., 2022. Costs dictate strategic investment in dominance interactions. Philos. Trans. R. Soc. B 377 (1845), 20200447. https://doi.org/10.1098/rstb.2020.0447.

Demin, K.A., Lakstygal, A.M., Volgin, A.D., de Abreu, M.S., Genario, R., Alpyshov, E.T., Serikuly, N., Wang, D., Wang, J., Yan, D., Wang, M., Yang, L., Hu, G., Bytov, M.,

Zabegalov, K.N., Zhdanov, A., Harvey, B.H., Costa, F., Rosemberg, D.B., Leonard, B. E., Fontana, B.D., Cleal, M., Parker, M.O., Wang, J., Song, C., Amstislavskaya, T.G., Kalueff, A.V., 2020. Cross-species analyses of intraspecies behavioral differences in mammals and fish. Neuroscience 429, 33–45. https://doi.org/10.1016/j.neuroscience.2019.12.035.

Denver, R.J., 2009. Stress hormones mediate environment-genotype interactions during amphibian development. Gen. Comp. Endocrinol. 164 (1), 20–31. https://doi.org/10.1016/j.ygcen.2009.04.016.

De-Santis, C., Jerry, D.R., 2007. Candidate growth genes in finfish — where should we be looking? Aquaculture 272 (1), 22–38. https://doi.org/10.1016/j.aquaculture.2007.08.036.

Devlin, R.H., Biagi, C.A., Yesaki, T.Y., Smailus, D.E., Byatt, J.C., 2001. Growth of domesticated transgenic fish. Nature 409, 781–782.

Duclot, F., Kabbaj, M., 2017. The role of early growth response 1 (*EGR1*) in brain plasticity and neuropsychiatric disorders. Front. Behav. Neurosci. 11, 1–20. https://doi.org/10.3389/fnbeh.2017.00035.

Eastman, G., Valiño, G., Radío, S., Young, R.L., Quintana, L., Zakon, H.H., Hofman, H.A., Sotelo-Silveria, J., Silva, A., 2020. Brain transcriptomics of agonistic behaviour in the weakly electric fish *Gymnotus omarorum*, a wild teleost model of non-breeding aggression. Sci. Rep. 10 (1), 1–11. https://doi.org/10.1038/s41598-020-66494-9.

El-kady, A.A., Magouz, F.I., Mahmoud, S.A., Abdel-rahim, M.M., 2022. The effects of some commercial probiotics as water additive on water quality, fish performance, blood biochemical parameters, expression of growth and immune-related genes, and histology of Nile tilapia (*Oreochromis niloticus*). Aquaculture 546, 737249. https://doi.org/10.1016/j.aquaculture.2021.737249.

Falica, B.K., Higgs, D.M., 2013. Paternal genetic effects on offspring swimming performance vary with age of juvenile Chinook Salmon, *Oncorhynchus tshawytscha*. Evol. Biol. 40 (3), 355–365. https://doi.org/10.1007/s11692-012-9217-0.

Frank, C.E., Sadeghi, J., Heath, D.D., Semeniuk, C.A.D., 2024. Behavioral transcriptomic effects of triploidy and probiotic therapy (*Bifidobacterium*, *Lactobacillus*, and *Lactococcus* mixture) on juvenile Chinook salmon (*Oncorhynchus tshawytscha*). Genes Brain Behav. 2024 (23), e12898. https://doi.org/10.1111/gbb.12898.

Fraser, T.W.K., Fjelldal, P.G., Skjæraasen, J.E., Hansen, T., Mayer, I., 2012a. Triploidy alters brain morphology in pre-smolt Atlantic salmon *Salmo salar*: possible implications for behaviour. J. Fish Biol. 81 (7), 2199–2212. https://doi.org/10.1111/j.1095-8649.2012.03479.x.

Fraser, T.W.K., Fjelldal, P.G., Hansen, T., Mayer, I., 2012b. Welfare considerations of triploid fish. Rev. Fish. Sci. 20 (4), 192–211. https://doi.org/10.1080/10641262.2012.700817.

Fraser, T.W.K., Lerøy, H., Hansen, T.J., Skjæraasen, J.E., Tronci, V., Pedrosa, C.P., Fjelldal, P.G., Nilsen, T.O., 2021. Triploid Atlantic salmon and triploid Atlantic salmon x brown trout hybrids have better freshwater and early seawater growth than diploid counterparts. Aquaculture 540, 736698. https://doi.org/10.1016/j.aquaculture.2021.736698.

Fraser, T.W.K., Hansen, T.J., Remø, S.C., Fjelldal, P.G., 2022. Triploidy effects growth, life history strategies, and bone health in Arctic char (*Salvelinus alpinus*), but does not impact cataract incidence. Aquaculture 547, 737465. https://doi.org/10.1016/j.aquaculture.2021.737465.

Garner, S.R., Madison, B.N., Bernier, N.J., Neff, B.D., 2008. Juvenile growth and aggression in diploid and triploid Chinook salmon *Oncorhynchus tshawytscha* (Walbaum). J. Fish Biol. 73 (1), 169–185. https://doi.org/10.1111/j.1095-8649.2008.01923.x.

Glass, D.J., 2003. Molecular mechanisms modulating muscle mass. Trends Mol. Med. 9 (8), 344–350. https://doi.org/10.1016/S1471-4914(03)00138-2.

Glass, D.J., 2005. Skeletal muscle hypertrophy and atrophy signaling pathways. Int. J. Biochem. Cell Biol. 37 (10 SPEC. ISS), 1974–1984. https://doi.org/10.1016/j.biocel.2005.04.018.

Gonçalves, A.T., Maita, M., Futami, K., Endo, M., Katagiri, T., 2011. Effects of a probiotic bacterial Lactobacillus rhamnosus dietary supplement on the crowding stress response of juvenile Nile tilapia *Oreochromis niloticus*. Fish. Sci. 77 (4), 633–642. https://doi.org/10.1007/s12562-011-0367-2.

Gribble, F.M., Reimann, F., 2019. Function and mechanisms of enteroendocrine cells and gut hormones in metabolism. Nat. Rev. Endocrinol. 15 (4), 226–237. https://doi.org/10.1038/s41574-019-0168-8.

Gui, J.F., Zhu, Z.Y., 2012. Molecular basis and genetic improvement of economically important traits in aquaculture animals. Hydrobiology 57 (15), 1751–1760. https://doi.org/10.1007/s11434-012-5213-0.

Hansen, T.J., Olsen, R.E., Stien, L., Oppedal, F., Torgersen, T., Breck, O., Remen, M., Vågseth, T., Fjelldal, P.G., 2015. Effect of water oxygen level on performance of diploid and triploid Atlantic salmon post-smolts reared at high temperature. Aquaculture 435, 354–360. https://doi.org/10.1016/j.aquaculture.2014.10.017.

He, S., Zhang, Y., Xu, L., Yang, Y., Marubashi, T., Zhou, Z., Yao, B., 2013. Effects of dietary *Bacillus subtilis* C-3102 on the production, intestinal cytokine expression and autochthonous bacteria of hybrid tilapia *Oreochromis niloticus* ♀ × *Oreochromis aureus* ♂. Aquaculture 412-413 (2013), 125–130. https://doi.org/10.1016/j.aquaculture.2013.06.028.

Heath, D.D., Fox, C.W., Heath, J.W., 1999. Maternal effects on offspring size: variation through early development of Chinook Salmon. Evolution 53 (5), 1605–1611.

Huntingford, F., Adams, C., 2005. Behavioural syndromes in farmed fish: implications for production and welfare. Behaviour 142 (9), 1207–1221. https://doi.org/10.1163/156853905774539382.

Irianto, A., Austin, B., 2002. Probiotics in aquaculture. J. Fish Dis. 25, 633–642.

Jobling, M., 1981. The influences of feeding on the metabolic rate of fishes: a short review. J. Fish Biol. 18, 385–400.

Johnson, R.M., Shrimpton, J.M., Heath, J.W., Heath, D.D., 2004. Family, induction methodology and interaction effects on the performance of diploid and triploid Chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 234 (1), 123–142. https://doi.org/10.1016/j.aquaculture.2004.01.024.

Kari, Z.A., Kabir, M.A., Dawood, M.A.O., Razab, M.K.A.R., Ariff, N.S.N.A., Sarkar, T., Pati, S., Edinur, H.A., Mat, K., Ismail, T.A., Wei, L.S., 2022. Effect of fish meal substitution with fermented soy pulp on growth performance, amino acid profile, and immune-related gene expression of African catfish (*Clarias gariepinus*). Aquaculture 546, 737418. https://doi.org/10.1016/j.aquaculture.2021.737418.

Khan, S., 2019. IGFBP-2 Signaling in the brain: from brain development to higher order brain functions. Front. Endocrinol. 10, 1–14. https://doi.org/10.3389/fendo.2019.00822.

Kim, J.H., Leggatt, R.A., Chan, M., Volkoff, H., Devlin, R.H., 2015. Effects of chronic growth hormone overexpression on appetite-regulating brain gene expression in coho salmon. Mol. Cell. Endocrinol. 413, 178–188. https://doi.org/10.1016/j.mce.2015.06.024.

Kolde, R., 2019. pheatmap: Pretty Heatmaps. R package version 1.0.12. https://CRAN.R-project.org/package=pheatmap.

Krause, J., 1993. Positioning behaviour in fish shoals: a cost-benefit analysis. J. Fish Biol. 43, 309–314. https://doi.org/10.1111/j.1095-8649.1993.tb01194.x.

Lahti, K., Laurila, A., Enberg, K., Piironen, J., 2001. Variation in aggressive behaviour and growth rate between populations and migratory forms in the brown trout, *Salmo trutta*. Anim. Behav. 62, 935–944. https://doi.org/10.1006/anbe.2001.1821.

Lehnert, S., Love, O.P., Pitcher, T.E., Higgs, D.M., Heath, D.D., 2014. Multigenerational outbreeding effects in Chinook salmon (*Oncorhynchus tshawytscha*). Genetica 142, 281–293. https://doi.org/10.1007/s10709-014-9774-5.

Li, X., Liu, Y., Blancheton, J.P., 2013. Effect of stocking density on performances of juvenile turbot (Scophthalmus maximus) in recirculating aquaculture systems. Chin. J. Oceanol. Limnol. 31 (3), 514–522. https://doi.org/10.1007/s00343-013-2205-0.

Liu, W., Ren, P., He, S., Xu, L., Yang, Y., Gu, Z., Zhou, Z., 2013. Comparison of adhesive gut bacteria composition, immunity, and disease resistance in juvenile hybrid tilapia fed two different Lactobacillus strains. Fish Shellfish Immunol. 35 (1), 54–62. https://doi.org/10.1016/j.fsi.2013.04.010.

Llewellyn, M.S., Boutin, S., Hoseinifar, S.H., Derome, N., 2014. Teleost microbiomes: the state of the art in their characterization, manipulation and importance in aquaculture and fisheries. Front. Microbiol. 5, 1–17. https://doi.org/10.3389/fmicb.2014.00207.

Long, J.A., 2024. interactions: Comprehensive, User-Friendly Toolkit for Probing Interactions. R package version 1.2.0. https://doi.org/10.32614/CRAN.package.interactions.

Lüdecke, D., 2018. ggeffects: tidy data frames of marginal effects from regression models. J. Open Source Softw. 3 (26), 772. https://doi.org/10.21105/joss.00772.

Manor, M.L., Weber, G.M., Cleveland, B.M., Kenney, P.B., 2014. Effects of feeding level and sexual maturation on fatty acid composition of energy stores in diploid and triploid rainbow trout (*Oncorhynchus mykiss*). Aquaculture 435 (2015), 178–186. https://doi.org/10.1016/j.aquaculture.2013.09.023.

Martins, C.I., Silva, P.I.M., Conceição, L.E.C., Costas, B., Höglund, E., Øverli, Ø., Schrama, J.W., 2011. Linking fearfulness and coping styles in fish. PLoS One 6 (11), e28084. https://doi.org/10.1371/journal.pone.0028084.

Mas-Muñoz, J., Komen, H., Schneider, O., Visch, S.W., Schrama, J.W., 2011. Feeding behaviour, swimming activity and boldness explain variation in feed intake and growth of sole (*Solea solea*) reared in captivity. PLoS One 6 (6), e21393. https://doi.org/10.1371/journal.pone.0021393.

Mollereau, B., Ma, D., 2014. The p53 control of apoptosis and proliferation: lessons from Drosophila. Apoptosis 19 (10), 1421–1429. https://doi.org/10.1007/s10495-014-1035-7.

Mun, S.H., You, J.H., Oh, H.J., Lee, C.H., Baek, H.J., Lee, Y., Kwon, J.Y., 2019. Expression patterns of growth related genes in juvenile red spotted grouper (*Epinephelus akaara*) with different growth performance after size grading. Dev. Reprod. 23 (1), 35–42.

Nathanailides, C., Kolygas, M., Choremi, K., Mavraganis, T., Gouva, E., Vidalis, K., Athanassopoulou, F., 2021. Probiotics have the potential to significantly mitigate the environmental impact of freshwater fish farms. Fishes 6 (4), 76.

Nayak, S.K., 2010. Probiotics and immunity: a fish perspective. Fish Shellfish Immunol. 29 (1), 2–14. https://doi.org/10.1016/j.fsi.2010.02.017.

Ng, W.K., Koh, C.B., 2016. The utilization and mode of action of organic acids in the feeds of cultured aquatic animals. Rev. Aquac. 9 (4), 342–368. https://doi.org/10.1111/raq.12141.

O'Keefe, R.A., Benfey, T.J., 1997. The feeding response of diploid and triploid Atlantic salmon and brook trout. J. Fish Biol. 51 (5), 989–997. https://doi.org/10.1006/jfbi.1997.0505.

O'Keefe, R.A., Benfey, T.J., 1999. Comparative growth and food consumption of diploid and triploid brook trout (*Salvelinus fontinalis*) monitored by radiography. Aquaculture 175 (1–2), 111–120. https://doi.org/10.1016/S0044-8486(99)00038-1.

Oksenberg, N., Ahituv, N., 2013. The role of AUTS2 in neurodevelopment and human evolution. Trends Genet. 29 (10), 600–608. https://doi.org/10.1016/j.tig.2013.08.001.

Ouwehand, A.C., Salminen, S., Isolauri, E., 2002. Probiotics: an overview of beneficial effects. Anton. Leeuw. Int. J. Gen. Mol. Microbiol. 82 (1–4), 279–289. https://doi.org/10.1023/A:1020620607611.

Puvanasundrum, P., Chong, C.M., Sabri, S., Yusoff, S., Karim, M., 2021. Multi-strain probiotics: functions, effectiveness and formulations for aquaculture applications. Aquacult. Rep. 21, 100905. https://doi.org/10.1016/j.aqrep.2021.100905.

Rajan, K.E., Ganesh, A., Dharaneedharan, S., Radhakrishnan, K., 2011. Spatial learning-induced egr-1 expression in telencephalon of goldfish *Carassius auratus*. Fish Physiol. Biochem. 37, 153–159. https://doi.org/10.1007/s10695-010-9425-4.

Renn, S.C.P., Aubin-Horth, N., Hofmann, H.A., 2008. Fish and chips: functional genomics of social plasticity in an African cichlid fish. J. Exp. Biol. 211 (18), 3041–3056. https://doi.org/10.1242/jeb.018242.

Rennie, M.D., Collins, N.C., Shuter, B.J., Rajotte, J.W., Couture, P., 2005. A comparison of methods for estimating activity costs of wild fish populations: more active fish observed to grow slower. Fish. Aquat. Sci. 62 (4), 725–959. https://doi.org/10.1139/f05-052.

Rittschof, C.C., Hughes, K.A., 2018. Advancing behavioural genomics by considering timescale. Nat. Commun. 9, 489. https://doi.org/10.1038/s41467-018-02971-0.

Rohani, M.F., Islam, S.M., Hossain, M.K., Ferdous, Z., Siddik, M.A., Nuruzzaman, M., Padeniya, U., Brown, C., 2022. Probiotics, prebiotics and synbiotics improved the functionality of aquafeed: upgrading growth, reproduction, immunity and disease resistance in fish. Fish Shellfish Immunol. 120, 569–589. https://doi.org/10.1016/j.fsi.2021.12.037.

Rørå, A.M.B., Mørkøre, T., Einen, O., 2001. Primary processing (evisceration and filleting). In: Farmed Fish Quality. Blackwell, UK, pp. 249–260.

Rothwell, N.J., 1990. Central effects of CRF on metabolism and energy balance. Neurosci. Biobehav. Rev. 14, 263–271. https://doi.org/10.1016/s0149-7634(05)80037-5.

Rottmann, R.W., Shireman, J.V., Chapman, F.A., 1991. Induction and verification of Triploidy in fish. Southern Regional Aquaculture Center, p. 421.

Ruijter, J.M., Ramakers, C., Hoogaars, W.M.H., Karlen, Y., Bakker, O., van den Hoff, M.J.B., Moorman, A.F.M., 2009. Amplification efficiency: linking baseline and bias in the analysis of quatitative PCR data. Nucleic Acids Res. 37 (6). https://doi.org/10.1093/nar/gkp045.

Sadeghi, J., 2022. Endogenous and Exogenous Factors Driving Bacterial Community Composition in Aquatic Ecosystems. [PhD Thesis, University of Windsor].

Sadoul, B., Alfonso, S., Goold, C., Pratlong, M., Rialle, S., Geffroy, B., Bégout, M.L., 2022. Transcriptomic profiles of consistent risk-taking behaviour across time and contexts in European sea bass. Proc. R. Soc. B Biol. Sci. 289 (1975), 20220399. https://doi.org/10.1098/rspb.2022.0399.

Sahandi, J., Jafaryan, H., Soltani, M., Ebrahimi, P., 2019. The use of two Bifidobacterium strains enhanced growth performance and nutrient utilization of rainbow trout (*Oncorhynchus mykiss*) fry. Probiotics Antimicrobial Proteins 11 (3), 966–972. https://doi.org/10.1007/s12602-018-9455-2.

Sasai, N., Toriyama, M., Kondo, T., 2019. Hedgehog signal and genetic disorders. Front. Genet. 10, 1103. https://doi.org/10.3389/fgene.2019.01103.

Scott, M.A., Dhillon, R.S., Schulte, P.M., Richards, J.G., 2014. Physiology and performance of wild and domestic strains of diploid and triploid rainbow trout (*Oncorhynchus mykiss*) in response to environmental challenges. Can. J. Fish. Aquat. Sci. 72 (1), 125–134. https://doi.org/10.1139/cjfas-2013-0450.

Shaheen, A., Eissa, N., Abou-ElGheit, E., Yao, H., Wang, H., 2014. Effect of probiotic on growth performance and growth-regulated genes in yellow perch (*Perca flavescens*). Glob. J. Fish. Aquacult. Res. 1 (2), 1–15. https://doi.org/10.13140/2.1.4325.2803.

Shi, L.H., Balakrishnan, K., Thiagarajah, K., Mohd Ismail, N.I., Yin, O.S., 2016. Beneficial properties of probiotics. Trop. Life Sci. Res. 27 (2), 73–90. https://doi.org/10.21315/tlsr2016.27.2.6.

Shrimpton, J.M., Heath, J.W., Devlin, R.H., Heath, D.D., 2012. Effect of triploidy on growth and ionoregulatory performance in ocean-type Chinook salmon: a quantitative genetics approach. Aquaculture 362-363, 248–254. https://doi.org/10.1016/j.aquaculture.2011.05.010.

Solberg, M.F., Kvamme, B.O., Nilsen, F., Glover, K.A., 2012. Effects of environmental stress on mRNA expression levels of seven genes related to oxidative stress and growth in Atlantic salmon *Salmo salar L.* of farmed, hybrid and wild origin. BMC. Res. Notes 5, 672. https://doi.org/10.1186/1756-0500-5-672.

Soto-Dávila, M., Webb, R.A., Rodríguez-Ramos, T., McDonald, G., Dang, X., Heath, J.W., Reid, G., Dixon, B., 2024. Effect of dietary supplementation of probiotic on growth, survival, and immune-related biomarkers in Chinook salmon (*Oncorhynchus tshawytscha*) challenged with *Vibrio anguillarum*. Aquaculture 583, 740582. https://doi.org/10.1016/j.aquaculture.2024.740582.

Sumon, M.A.A., Sumon, T.A., Hussain, M.A., Lee, S., Jang, W.J., Sharifuzzaman, S.M., Brown, C.L., Lee, E., Hasan, T., 2022. Single and multi-strain probiotics supplementation in commercially prominent finfish aquaculture: review of the current knowledge. J. Microbiol. Biotechnol. 32 (6), 681–698. https://doi.org/10.4014/jmb.2202.02032.

Tiwary, B.K., Kirubagaran, R., Ray, A.K., 2004. The biology of triploid fish. Rev. Fish Biol. Fish. 14 (4), 391–402. https://doi.org/10.1007/s11160-004-8361-8.

Trainor, B.C., Hofmann, H.A., 2006. Somatostatin regulates aggressive behavior in an African cichlid fish. Endocrinology 147 (11), 5119–5125. https://doi.org/10.1210/en.2006-0511.

Trainor, B.C., Hofmann, H.A., 2007. Somatostatin and somatostatin receptor gene expression in dominant and subordinate males of an African cichlid fish. Behav. Brain Res. 179 (2), 314–320. https://doi.org/10.1016/j.bbr.2007.02.014.

Tymchuk, W.E., Biagi, C., Withler, R., Robert, H., 2006. Growth and behavioural consequences of introgression of a domesticated aquaculture genotype into a native strain of coho salmon. Trans. Am. Fish. Soc. 134, 442–455. https://doi.org/10.1577/T05-181.1.

Van De Pol, I.L.E., Flik, G., Verberk, W.C.E.P., 2020. Triploidy in zebrafish larvae: effects on gene expression, cell size and cell number, growth, development and swimming performance. PLoS One 15 (3), 1–20. https://doi.org/10.1371/journal.pone.0229468.

Velando, A., Costa, M.M., Kim, S.Y., 2017. Sex-specific phenotypes and metabolism related gene expression in juvenile sticklebacks. Behav. Ecol. 28 (6), 1553–1563. https://doi.org/10.1093/beheco/arx129.

Venney, C.J., Love, O.P., Drown, E.J., Heath, D.D., 2019. DNA methylation profiles suggest intergenerational transfer of maternal effects. Mol. Biol. Evol. 37 (2), 540–548. https://doi.org/10.1093/molbev/msz244.

Viadero, R.C., 2005. Factors affecting fish and growth production. In: Encyclopedia of Water: Science Technology, and Society, 3, pp. 129–133. https://doi.org/10.1002/047147844x.sw241.

Villamil, L., Reyes, C., Martínez-Silva, M.A., 2014. In vivo and in vitro assessment of *Lactobacillus acidophilus* as probiotic for tilapia (*Oreochromis niloticus*, *Perciformes: Cichlidae*) culture improvement. Aquac. Res. 45 (7), 1116–1125. https://doi.org/10.1111/are.12051.

Volkoff, Helene, Peter, R.E., 2006. Feeding behavior of fish and its control. Zebrafish 3 (2), 131–140.

Volpato, G.L., Fernandes, M.O., 1994. Social control of growth in fish. Braz. J. Med. Biol. Res. 27, 797–810.

Weiss, L.A., Zaniboni-Filho, E., 2009. Survival of diploid and triploid *Rhamdia quelen* juveniles in different ammonia concentrations. Aquaculture 298 (1–2), 153–156. https://doi.org/10.1016/j.aquaculture.2009.10.010.

Wellband, K.W., Heath, D.D., 2017. Plasticity in gene transcription explains the differential performance of two invasive fish species. Evol. Appl. 10 (6), 563–576. https://doi.org/10.1111/eva.12463.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.

Wickham, H., François, R., Henry, L., Müller, K., Vaughan, D., 2023. dplyr: A Grammar of Data Manipulation. R package version 1.1.4. https://CRAN.R-project.org/package=dplyr.

Withler, R.E., Beacham, T.D., Solar, I.I., Donaldson, E.M., 1995. Freshwater growth, smolting, and marine survival and growth of diploid and triploid coho salmon (*Oncorhynchus kisutch*). Aquaculture 136, 91–107. https://doi.org/10.1016/0044-8486(95)01306-X.

Withler, R.E., Clarke, W.C., Blackburn, J., Baker, I., 1998. Effect of triploidy on growth and survival of pre-smolt and post-smolt coho salomn (*Oncorhynchus kisutch*). Aquaculture 168, 413–422. https://doi.org/10.1016/S0044-8486(98)00367-6.

Zhong, H., Zhou, Y., Liu, S., Tao, M., Long, Y., Liu, Z., Zhang, C., Duan, W., Hu, J., Song, C., Liu, Y., 2012. Elevated expressions of GH/IGF axis genes in triploid crucian carp. Gen. Comp. Endocrinol. 178 (2), 291–300. https://doi.org/10.1016/j.ygcen.2012.06.006.

Zhou, J., Li, W., Kamei, H., Duan, C., 2008. Duplication of the IGFBP-2 gene in teleost fish: protein structure and functionality conservation and gene expression divergence. PLoS One 3 (12), e3926. https://doi.org/10.1371/journal.pone.0003926.

Zuk, M., Balenger, S.L., 2014. Behavioral ecology and genomics: new directions, or just a more detailed map? Behav. Ecol. 25 (6), 1277–1282. https://doi.org/10.1093/beheco/aru.