frontiers | Frontiers in Animal Science

TYPE Original Research
PUBLISHED 18 February 2025
DOI 10.3389/fanim.2025.1481117

OPEN ACCESS

EDITED BY
Gerson Barreto Mourão,
University of São Paulo, Brazil

REVIEWED BY
Timothy D. Leeds,
United States Department of Agriculture (USDA), United States
Alexandre Wagner Silva Hilsdorf,
University of Mogi das Cruzes, Brazil

*CORRESPONDENCE
Martin Hubálek
mhubalek@frov.jcu.cz

†These authors have contributed equally to this work

RECEIVED 15 August 2024
ACCEPTED 28 January 2025
PUBLISHED 18 February 2025

CITATION
Flajšhans M, Kašpar V, Prchal M, Musatova I, Prášková E, Kašpar J and Hubálek M (2025) The assessment of the effects of genomic heterozygosity and sterility on the performance of triploid brook trout *Salvelinus fontinalis*. *Front. Anim. Sci.* 6:1481117. doi: 10.3389/fanim.2025.1481117

The assessment of the effects of genomic heterozygosity and sterility on the performance of triploid brook trout *Salvelinus fontinalis*

Martin Flajšhans[1†], Vojtěch Kašpar[1], Martin Prchal[1], Iryna Musatova[1], Eva Prášková[1], Jan Kašpar[2] and Martin Hubálek[1*†]

[1]Research Institute of Fish Culture and Hydrobiology, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Faculty of Fisheries and Protection of Waters, University of South Bohemia in Ceske Budejovice, Vodňany, Czechia, [2]Institute of Aquaculture and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Faculty of Fisheries and Protection of Waters, University of South Bohemia in Ceske Budejovice, České Budějovice, Czechia

This study analyses diploid and hydrostatic-pressure-induced triploid brook trout (Salvelinus fontinalis) sampled from a fish farm before (360 days post-hatch, d.p.h.) and during the normal time of sexual maturation for pan-sized market fish (555 and 667 d.p.h.). Biometric indices and slaughtering traits of examples of both ploidy levels were compared and their genomic heterozygosity and gonadal development were evaluated to assess the possible link between these two parameters and growth performance. At 555 d.p.h., triploids – irrespective of sex – had greater carcass yield than their diploid conspecifics. At 667 d.p.h., all triploids (females, males and intersex fish) were significantly larger and heavier and had far lower gonadosomatic indices than their diploid conspecifics; however, in terms of carcass and fillet yields these differences were not as notable. Delayed gonadal growth in triploids was confirmed. Microsatellite analysis at five polymorphic loci suggest that triploids could have higher levels of heterozygosity than their diploid counterparts, a trend in multilocus heterozygosity that was consistent in all three age groups. Nevertheless, the link between heterozygosity and body weight was inconclusive. Suppressed gonadal development seems to be more probable explanation for the improved growth performance of triploids. Remarkable occurrence of intersex fish in triploids at both 555 and 667 d.p.h suggests that intersexes can commonly arise from artificial triploidization in brook trout. External appearance, biometric indices and slaughtering traits of triploid intersex were highly similar to that of triploid females.

KEYWORDS

slaughtering traits, carcass yield, gonad histology, microsatellites, salmonids, triploidy

1 Introduction

Triploid organisms possess three chromosome sets in their somatic cells rather than the two sets commonly held by diploid organisms. In fish, triploidy can be induced artificially by physical shocks (pressure or temperature) that cause the retention of the second polar body; the optimal parameters for this type of treatment are species-specific (Benfey, 1999; Maxime, 2008; Piferrer et al., 2009). Optimized protocols yielding triploidization success near or equal to 100% have been developed for many commercially important salmonid species in which the production of triploids is of great interest for both economic and ecological reasons (e.g. Benfey and Sutterlin, 1984; O'Keefe and Benfey, 1999; Kozfkay et al., 2005; Preston et al., 2013). Among the features that attract the attention of salmonid farmers is the superior growth rate of triploid individuals, and greater growth in triploidized individuals than in common ploidy fish has been observed, for example, in Atlantic salmon (*Salmo salar*; O'Flynn et al., 1997; Fraser et al., 2022), brook trout (*Salvelinus fontinalis*; Boulanger, 1991; Uzunova, 2004), rainbow trout (*Oncorhynchus mykiss*; Thorgaard, 1986; Sheehan et al., 1999; Poontawee et al., 2007) and Atlantic salmon × brown trout hybrid (*Salmo salar* × *Salmo trutta*; Fraser et al., 2021, 2022), although contrasting results and no differences in growth between ploidies have also been reported in salmonids (Benfey et al., 1989; Withler et al., 1995; Bonnet et al., 1999; Aussanasuwannakul et al., 2011). Additional advantages include the prevention of sexual maturity that potentially improves flesh quality at harvest and better dressing out percentages due to less gonadal development (Gillet et al., 2001; Poontawee et al., 2007; Janhunen et al., 2019). Additionally, functional sterility, a general feature of triploid salmonids, can be advantageous as it precludes any impact on wild populations provoked by stocked or escaped farmed fish (Maxime, 2008; Piferrer et al., 2009; Benfey, 2016).

The initial hypothesis that triploids grow faster because they carry larger cells than diploids (the nucleus of each somatic cell contains 50% more DNA and cell volume increases to accommodate the larger nucleus) has been rejected since in fish the increase in cell size is compensated for by a concomitant decrease in cell number (Benfey, 1999; Gregory, 2001). Alternatively, the improved performance of triploid salmonids is often attributed to their sterility since, if less energy is required for gametogenesis, more energy will be available for somatic growth (Piferrer et al., 2009; Flajšhans et al., 2013). In favour of this hypothesis is the fact that, in studies reporting the growth superiority of triploid individuals, the difference between ploidies typically becomes evident around or after the time diploids became mature (e.g. Boulanger, 1991; O'Flynn et al., 1997; Sheehan et al., 1999; Uzunova, 2004; Poontawee et al., 2007). During the juvenile or immature phases, triploid salmonids usually only grew as much or less than their diploid counterparts – depending on the species and rearing conditions (e.g. Benfey and Sutterlin, 1984; Solar et al., 1984; Quillet et al., 1988; Withler et al., 1995; Uzunova, 2004). As well, whether or not triploids of a given salmonid species outperform diploids may depend on the sex composition of the populations examined. Gonadal development of triploid salmonids is age- and species-specific but seems to be largely suppressed in females, whereas gonads of triploid males often develop to the point of being fully functional as endocrine organs and spermiation is routinely observed (the spermatozoa are aneuploid and incapable of generating viable offspring; see review by Benfey, 1999, 2016; Maxime, 2008; Piferrer et al., 2009; Fraser et al., 2012). One may thus expect the negative consequences of sexual maturation – including a deceleration of somatic growth – to be prevented in triploid females rather than in males. For example, Uzunova (2004) observed that triploid females of brook trout were heavier and had higher SGR than diploid females at maturation, while triploid males were lighter than diploid males and did not differ from them in SGR. In this respect, it is important to note that some studies report growth advantage in triploids in all-female populations (Boulanger, 1991; Schafhauser-Smith and Benfey, 2001; Werner et al., 2008; Sheehan et al., 1999); by contrast, other studies base their comparisons on mixed-sex stocks (O'Flynn et al., 1997; Poontawee et al., 2007; Fraser et al., 2021, 2022).

Nevertheless, growth in triploids may be enhanced as a result of their genomic heterozygosity. In diploid salmonids several studies have documented positive relationships between growth performance and heterozygosity in, for example, Atlantic salmon (McCarthy et al., 2003) and rainbow trout (Danzmann et al., 1987; Ferguson, 1992). Owing to their trisomic mode of inheritance, triploid organisms can maintain three alleles at a single locus while only two alleles are present in diploids (Lindstrom, 1936). Consequently, they can theoretically be provided with higher levels of heterozygosity and potentially accrue associated fitness benefits from overdominance and reduced inbreeding (Otto and Whitton, 2000; Comai, 2005). However, although several authors have considered this to be possible in fish (e.g. Benfey, 1999; Piferrer et al., 2009; Fraser et al., 2012), the evidence of such phenomenon in salmonids remains inconclusive. For example, Leary et al. (1985) reported that the mean heterozygosity of triploid rainbow trout from two strains increased by 30% compared to meiotic gynogenotes, while Garner et al. (2008) observed no difference in heterozygosity between triploids and normal diploids in chinook salmon (*Oncorhynchus tshawytscha*), and no correlation between heterozygosity and any measure of performance such as growth rate. The effects of triploidy induced by second polar body retention on heterozygosity and the possible links with performance parameters have been more thoroughly examined in shellfish, although such studies also yield inconsistent results (e.g. Beaumont et al., 1995; Hawkins et al., 2000; Garnier-Géré et al., 2002; Wang et al., 2002; Sellars et al., 2009). If triploid fish are more heterozygous than diploid fish, one would expect them to suffer from lower levels of morphological abnormalities due to the positive effect of heterozygosity on developmental stability (Leary et al., 1984, 1985), but this does not seem to be the case as many studies have reported high prevalences of various types of deformities in triploid salmonids (e.g. O'Flynn et al., 1997; Madsen et al., 2000; Sadler et al., 2001; Fraser et al., 2013; Babaheydari et al., 2016). Nevertheless, leaving aside genetic factors, these deformities may be a side-effect of inducing treatment, of the specific requirements of triploids regarding nutrition, or of the interactions between these factors (Deschamps et al., 2014; Fjelldal et al., 2016; Glover et al.,

2020; Jagiełło et al., 2021). Finally, some uncertainty remains over the relative expression of the three chromosome sets due to their possible silencing or unequal expression following polyploidization (Adams et al., 2003).

The objective of this study was to assess the suitability of triploidy induction without any deliberate manipulation of the sex ratio for the commercial production of brook trout, a salmonid species that is important in aquaculture and is farmed throughout the world. We compared the growth of farmed diploids and triploids before and during the normal time of sexual maturation, and then evaluated the biometric indices and slaughtering traits in these two ploidy levels. Finally, we studied the effect of triploidy on genomic heterozygosity and gonadal development and assessed the possible links between these two parameters and growth performance in brook trout.

2 Materials and methods

2.1 Experimental fish

The experimental protocol of the study underwent an ethical review process and was approved by the expert committee of the Institutional Animal Care and Use Committee (IACUC) at the University of South Bohemia (USB), according to the law on the protection of animals against cruelty (Act No. 246/1992 Coll.). Fish were obtained from the Annín trout farm belonging to the Klatovy Fishery (Klatovské rybářství a.s.), Czech Republic, where the commercial rearing of diploid and induced triploid brook trout took place. Briefly, triploidy was induced by our team for this company at the trout farm during the artificial reproduction of brook trout broodstock (3- to 4-year-old, 1.5–2.0 kg body weight, BW and 50–56 cm total length, TL) using a mobile hydrostatic pressure unit (producer SZDT servis, Lišov, Czech Republic) described by Flajšhans et al. (2020). The setup of hydrostatic pressure shock variables for brook trout was based on the methodological manual by Flajšhans et al. (2023) for salmonid fish as followed: fertilized eggs after 200 degree-minutes (°min) were transferred to a pressure chamber to be submitted to a hydrostatic pressure shock of 66 MPa (9 572.48 PSI) for 5 min. Each shock treated ca. 3 l of swollen eggs from one family comprising 9–10 females and 5–6 males. Altogether, four such batches were pressure shocked. At the same time, untreated fertilized eggs were incubated as control diploids. Eggs were incubated in 10-l Kannengieter jars at the fish farm until the eye-bud stage. Dead eggs were removed and living eggs were incubated in trays until hatching. Fertilization and hatching rates were estimated following Flajšhans et al. (2023). Alevin nursing in troughs and further rearing of juveniles in ponds and yearlings in channels was carried out at the fish farm following conventional aquaculture practice. Trout feed provided was by Skretting (Norway). Briefly, alevins in flow-through troughs at mean water temperature 8.5°C were fed starter feed Perla larva and Pro aqua brut *ad libitum*. Juveniles stocked by 100 000 fish per pond supplied with submountain riverine water with mean annual temperature 7.9°C were fed trout feed Pro aqua brut and later, from 2.5 mm pellet size, Optiline. The feeding scheme was as followed: 1 – 2.5 g fish were given 1 mm pellets with daily feed ratio 1.6% of the fish biomass weight (f.b.w.) and the 2.5 – 5 g fish were given 1.5 mm pellets with daily feed ratio 1.2% f.b.w. The 5 g to 20 g fish were fed with 1.8 mm pellets with daily feed ratio increasing continuously from 1 to 1.9% f.b.w. and 20 – 50 g fish were fed with 2.5 mm pellets with daily feed ratio increasing from 1.2 to 2.2% f.b.w. Yearlings at 360 days post hatching (d.p.h.) were transferred into sections of flow-through ongrowing channels by 25 000 fish each and fed 4 mm pellets with daily feed ratio increasing from 1.1 to 1.8% f.b.w. Finally, from 555 d.p.h. onwards, fish were fed 6 mm pellets with daily feed ratio from 0.8 to 1.6% f.b.w. Diploids and their triploid conspecifics were reared parallelly in the equal environmental conditions to minimize genotype by environmental effect. First of all, batches of 33 specimens of diploids and their induced triploid conspecifics were killed by CO_2 overdosage at 360 d.p.h. and checked for BW, TL, and sampled by fin-clipping for ploidy level and heterozygosity assessment. Beginning at 555 d.p.h., the fish farm commenced machine sorting of the brook trout into those that had already gained the requested market weight (≥ 350 g) and those that needed further rearing. This was the first term of random sampling the fish under study in order to reveal potential differences in slaughtering traits between the diploid brook trout and their induced triploid siblings. Rearing was terminated at 667 d.p.h. when all fish were harvested and sold, and this was the term of the final random sampling also.

2.2 Ploidy level assessment

In order to assess the ploidy level, the parent fish and both their putative triploid and control diploid progeny were sampled. Thirty live alevins were sampled from each hatched family, i.e. from each pressure shock and from the control. Both parent fish and yearlings (360-day-old fish), as well as the 555- and 667-day-old market-sized fish, were individually fin-clipped. Samples were individually frozen at -80°C in DMSO-citrate buffer (Vindelov et al., 1983) and processed following the protocol used by Hubálek and Flajšhans, 2021; the ploidy-level analysis was performed using a Partec CCA 1 flow cytometer (Partec GmBH, Germany) that determined the relative DNA content in the cell nuclei. For cell membrane lysis and nuclear staining, we used the kit CyStain UV precise T (Sysmex GmBH, Germany) containing 4′,6-diamidine-2-phenylindole (DAPI; excitation/emission maxima 358/461 nm) for nuclear DNA staining (Otto, 1994). Samples of adult fish of both sexes were used as a reference diploid standard. Samples were analyzed individually at a speed of 0.4 $\mu l.s^{-1}$.

2.3 Evaluation of slaughtering traits and biometric indices

The studied fish were processed following the standard aquaculture practice at the processing plant: fish were killed by a blow to the head and then bled by an incision in the gills as per local regulations. Immediately after bleeding, digital images were taken of each individual fish for later processing in another study. The TL, standard length (SL), head length (HL), maximum body height

(BH), maximum body width (MBW) and caudal peduncle length (CPL; all in mm; Supplementary Figure 1) were recorded manually using a measuring board and BW (in g) was recorded also. Sex (female, male) was visually assessed following Kazyak et al. (2013). Fat content in muscle (fat) expressed as a percentage was measured on whole, killed fish at 667 d.p.h. using a Fish Fatmeter FM 692 (Distell Ltd., UK). Due to the spread distribution of fat in muscle, the fat percentage for each individual was calculated as the mean of four repeated measurements on the left side of the fish (three above the lateral line and one below). Subsequently, the fish were gutted, filleted, sexed again by visual inspection of gonads (female, male) in order to attempt ranking the fish with juvenile appearance, and each part of the processed body (head, fillets, viscera, gonads, skin, skeleton with remnants, and fins) was weighed to the nearest 0.5 g. The percentage of processed body (the so-called carcass yield) and the yield of fillet with skin and without skin were calculated as the most important slaughtering traits:

Carcass yield (CY)

$$CY = (\textit{fillet weight} + \textit{skin weight} + \textit{weight of skeleton with head, remnants and fins})/BW \times 100$$

Fillet yield with skin (FYS)

$$FYS = (\textit{fillet weight} + \textit{skin weight})/BW \times 100$$

Fillet yield without skin (FY)

$$FY = (\textit{fillet weight}/BW) \times 100.$$

Biometric indices (in %) were later computed as followed:
Index of highbackedness (IH)

$$IH = BH/SL \times 100$$

Index of widebackedness (IW)

$$IW = MBW/SL \times 100$$

Index of head length (IHL)

$$IHL = HL/SL \times 100$$

Index of caudal peduncle (ICP)

$$ICP = CPL/SL \times 100$$

Gonadosomatic index (GSI)

$$GSI = \textit{gonad weight}/BW \times 100$$

Viscerosomatic index (VSI)

$$VSI = \textit{weight of viscera}/BW \times 100$$

Additionally, Fulton's condition factor (FC) was also computed:

$$FC = 10^5 \times BW/SL^3$$

2.4 Gonad histology

Gonads were dissected and fixed in Bouin's fluid. Histological slides were made following the standard paraffin technique. Tissue samples were collected from the mid-part of the gonad (if large) or whole gonad (if small). The 4.5-µm sections were stained with haematoxylin and eosin. Male and female gonads were microscopically examined with an Olympus BX50 (magnification ranging from 4× to 40×) for histopathological alterations (e.g. intersex). To evaluate the stage of male spermatogenesis, the testes were scored according to the criteria described in Blazer (2002), i.e. as pre-, early-, mid- or late spermatogenic. Female gonads were classified according to the most advanced stage of the oocytes present as described by Körner et al. (2007), i.e. as the stage of oogonia, that is, early or fully vitellogenic follicles. Samples that did not refer to any of the published developmental stage were scored as abnormal; samples exhibiting developmental stages of both sexes were scored as intersex.

2.5 Molecular genetic analysis

Genomic DNA was extracted from ethanol-fixed fin clips from 360-, 555- and 667-day-old fish using a HigherPurity™ Tissue DNA Purification Kit (Canvax, Spain) following the manufacturer's instructions. A commercially available kit STR Multiplex SALVident10 Kit (catalogue number STR0003a, Institute of Vertebrate Biology, Brno, Czech Republic), which consists of 10 microsatellite markers designed exclusively for brook trout (SALV1, SALV2, SALV3, SALV4, SALV5, SALV7, SALV8, SALV9, SALV10, SALV11), was utilized for the evaluation of genetic variation, according to the manufacturer's protocol. Amplifications were performed using the following PCR temperature profile: 94°C for 3 min, 30 cycles at 94°C for 45 s, 55°C for 90 s and 65°C for 60 s, followed by a final extension at 60°C for 30 min. Fragment analysis was conducted on ABI 3500 Genetic Analyzer (Applied Biosystems, USA) with a GeneScan LIZ 600 fluorescent size standard (Applied Biosystems, USA). Genotypes were resolved by eye using Geneious Prime 2019.0.4 bioinformatic software (Kearse et al., 2012).

The locus of individual fish was considered heterozygous when two or three different alleles were present. After this scoring, the monomorphic loci for the whole population were excluded and the multilocus heterozygosity (MLH) was calculated for each individual as the proportion of heterozygous loci to all examined loci. Apart from MLH, the average of the squared distances (in base pairs) between an individual's alleles at each locus was computed (=d^2, Goldstein et al., 1995; Coulson et al., 1998) and included as an alternative approach to the measurement of microsatellite variability. When three different alleles were observed at a given locus, d^2 was calculated using the two alleles separated by the greatest distance.

2.6 Statistics

The normality of the datasets was tested using the Shapiro-Wilk test. Homoskedasticity was evaluated using the F-test when comparing two populations (groups) or the Levene-test for more than two populations. The validity of the tests was assessed visually using a Q-Q plot of the fitted values against the residual values. Based on the testing of the assumptions, either parametric or nonparametric tests were selected in individual cases. All statistical analyses were performed using R statistical software (4.4.0), with an α of 0.05 predetermined as the significance level.

The BWs and TLs of diploids and triploids at 360 d.p.h. were compared using unpaired two-sample t-tests. Before the statistical analysis of the biometric indices and slaughtering traits at 555 and 667 d.p.h., the fish were sorted into groups based on their ploidy and sex ('ploidy-sex groups'). The ratio data (IH, IW, IHL, ICP, fat, GSI, CY, FYS, FY, VSI) defined in % were arcsine transformed. Subsequently, the BWs, TLs, SLs and FCs of different ploidy-sex groups were compared using one-way ANOVA followed by *post-hoc* Tukey's HSD test when significant. The effect of ploidy and sex on the remaining parameters was analyzed with ANCOVA, using SL as a covariate for IH, IW, IHL and ICP, and BW as a covariate for fat, CY, FYS, FY and VSI. In the case of significant ANCOVA results, a *post hoc* analysis was performed with a Bonferroni adjustment. When the assumption of data normality was not met, log transformation was applied to facilitate the analysis. In the case of GSI at 667 d.p.h, no transformation helped reach the normality assumption, so the Kruskal-Wallis test was run.

MLH and d^2 of diploids and triploids were compared using the Mann-Whitney U test for individual age groups (360, 555 and 667 d.p.h.) and for all age groups pooled. In order to assess the effect of ploidy on heterozygosity at individual loci, the numbers of diploid and triploid heterozygotes and homozygotes were compared at each locus using the chi-squared test, always within individual age groups and for all age groups pooled. To evaluate the effect of microsatellite variability on growth, several analyses were performed within individual age groups (360, 555 and 667 d.p.h.). The relationship between MLH or d^2 and BW was analyzed using Spearman's rank correlation irrespective of ploidy (diploid and triploid groups pooled) and within ploidy (diploid and triploid groups analyzed separately). Next, the BWs of heterozygotes and homozygotes at each locus were compared using two sample t-tests irrespective of ploidy. Fish were grouped based on the number of heterozygous loci (NHL), and the BWs of individuals from different NHL groups were analyzed using an ANOVA followed by *post-hoc* Tukey's HSD test. Subsequently, the BWs of heterozygotes possessing two and three different alleles at individual loci were compared with a t-test to assess whether triallelic individuals show better growth performance than diallelic ones.

Finally, the associations between gonadal development and growth were assessed. In both females and males at 555 and 667 d.p.h., fish were grouped based on observed stages of gonadal development and the BWs of these groups were compared using a t-test (when two different gonadal stages were present at a particular age for given sex) or an ANOVA (more than two stages). Additionally, at 667 d.p.h., the relationship between GSI and BW was analyzed using Spearman's rank correlation for different sex groups (females, males and intersex), in both cases irrespective of ploidy and within ploidy.

3 Results

3.1 Ploidy level assessment

The analysis of the ploidy level performed by the flow cytometric determination of the relative DNA content in the cell nuclei confirmed the diploid status of all 60 parent fish and all 120 control diploid alevins (30 samples from each of the four families). All the 120 putative triploid alevins were confirmed as triploids, thereby demonstrating the 100% success of the induction of triploidy with the hydrostatic pressure shock. Among the fish sampled 360 d.p.h., all 33 specimens of diploids and their induced triploid conspecifics were found to be diploid and triploid, respectively. Fish batches sampled 555 and 667 d.p.h. consisted of 43 diploids and 45 triploids, and 57 diploids and 56 triploids, respectively, all with their ploidy status confirmed cytometrically.

3.2 Biometric indices and slaughtering traits

The first screening of samples of the diploid yearlings at 360 d.p.h. and their induced triploid conspecifics did not reveal any significant difference in BW values (89.65 ± 39.25 and 103.15 ± 27.07g, respectively; t_{64} = -1.63, p = 0.109), although there were differences in TL values (199.00 ± 27.17 and 211.21 ± 17.65 mm, respectively; t_{64} = -2.16, p = 0.035). The values of the biometric indices and slaughtering traits of the individual ploidy-sex groups and the detailed results of their statistical comparisons are summarized in Table 1 for brook trout aged 555 d.p.h. and in Table 2 for brook trout aged 667 d.p.h. Figure 1 demonstrates the proportions and counts of fish based on sex determination using a histological assessment of gonad samples and ploidy levels at 555 and 667 d.p.h.

3.2.1 Fish at 555 d.p.h.

Individual ploidy-sex groups did not differ in BW, TL, SL, IW, IHL or FC. Significant differences between ploidy-sex groups were observed in the IH, ICP, CY, FYS, FY and VSI.

Compared to diploid females, triploid females had significantly higher values of CY (3.5%) but significantly lower IH (8.9%) and VSI (16.6%). Compared to diploid males, triploid males showed significantly higher CY (6.1%), FYS (5.7%) and FY (5.9%), as well as significantly lower IH (6.5%) and VSI (22.2%). Triploid intersexes did not differ from either triploid males or triploid females in any of the examined parameters. When considering only the differences within triploid groups, individuals of unidentified sex did not differ significantly from either females or intersexes in any parameter but did have

TABLE 1 Biometric indices and slaughtering traits of individual ploidy-sex groups of 555-day-old brook trout (*Salvelinus fontinalis*) and the results of their statistical comparisons.

Parameter	Statistics	Diploid		Triploid			
		females	males	females	males	intersex	unidentified
BW (g)	$F_{5,82}$ = 2.22, p = 0.060	387.1 ± 48.6	425.7 ± 46.5	373.9 ± 39.7	404.4 ± 66.2	385.6 ± 60.3	386.5 ± 44.8
TL (mm)	$F_{5,82}$ = 1.82, p = 0.118	293.5 ± 13.8	303.3 ± 11.8	296.4 ± 9.4	302.3 ± 14.6	292.0 ± 18.7	297.1 ± 9.2
SL (mm)	$F_{5,82}$ = 1.66, p = 0.155	264.9 ± 13.8	274.5 ± 13.3	267.9 ± 9.0	271.8 ± 13.6	260.3 ± 17.2	268.6 ± 9.7
IH (%)	**$F_{5,81}$ = 5.73, p = 1×10^{-4}**	25.9 ± 1.3^{bc}	26.1 ± 1.1^{c}	23.6 ± 1.7^{a}	24.4 ± 1.8^{ab}	26.0 ± 1.7^{abc}	25.1 ± 2.1^{abc}
IW (%)	$F_{5,81}$ = 0.27, p = 0.929	14.2 ± 0.7	14.1 ± 1.0	13.9 ± 0.9	14.1 ± 0.9	14.6 ± 0.3	14.0 ± 1.3
IHL (%)	$F_{5,81}$ = 2.26, p = 0.056	18.4 ± 1.2	19.2 ± 1.0	19.1 ± 1.2	18.5 ± 1.3	18.8 ± 1.8	18.0 ± 1.7
ICP (%)	**$F_{5,81}$ = 3.73, p = 4×10^{-3}**	19.0 ± 1.4^{ab}	18.7 ± 1.1^{ab}	19.9 ± 1.4^{b}	18.1 ± 1.0^{a}	18.5 ± 0.8^{ab}	18.6 ± 1.2^{ab}
FC	$F_{5,82}$ = 1.29, p = 0.278	2.1 ± 0.2	2.1 ± 0.2	1.9 ± 0.2	2.0 ± 0.2	2.2 ± 0.2	2.0 ± 0.2
CY (%)	**$F_{5,81}$ = 26.65, p = 8×10^{-16}**	79.9 ± 2.0^{a}	79.8 ± 1.9^{a}	82.7 ± 1.5^{b}	84.7 ± 1.4^{c}	82.7 ± 1.9^{abc}	84.1 ± 1.6^{bc}
FYS (%)	**$F_{5,81}$ = 9.82, p = 2×10^{-7}**	57.5 ± 2.1^{ab}	56.5 ± 1.6^{a}	58.2 ± 1.7^{bc}	59.7 ± 1.5^{c}	58.1 ± 1.5^{abc}	57.7 ± 1.8^{ab}
FY (%)	**$F_{5,81}$ = 10.76, p = 6×10^{-8}**	51.6 ± 2.1^{b}	50.6 ± 1.4^{a}	52.2 ± 1.6^{bc}	53.6 ± 1.6^{c}	51.8 ± 1.6^{abc}	51.6 ± 1.7^{ab}
VSI (%)	**$F_{5,81}$ = 25.50, p = 2×10^{-15}**	16.9 ± 1.2^{b}	16.7 ± 1.6^{b}	14.1 ± 1.3^{a}	13.0 ± 1.1^{a}	14.6 ± 1.1^{ab}	13.8 ± 1.4^{a}

The values are expressed as mean ± SD. Statistically significant tests ($p < 0.05$) are highlighted in bold font. Lowercase letters denote significant differences ($p < 0.05$) between individual ploidy-sex groups. BW, body weight; TL, total length; SL, standard length; IH, index of highbackedness; IW, index of widebackedness; IHL, index of head length; ICPL, index of caudal peduncle; FC, Fulton's condition factor; CY, carcass yield; FYS, fillet yield with skin; FY, fillet yield without skin; VSI, viscerosomatic index.

significantly lower FYS and FY than males (3.4 and 3.7%, respectively). It was notable that the CY of all diploid groups was significantly lower than the CY of all triploid groups, the exception being the triploid intersexes. Irrespective of sex, the CY of triploids on average exceeded the CY of diploids by 4.6%. By contrast, all diploid groups had significantly higher VSI than triploid groups, except for triploid intersexes. Independently of sex, the VSI of triploids was reduced by 17.4%.

TABLE 2 Biometric indices and slaughtering traits of individual ploidy-sex groups of 667-day-old brook trout (*Salvelinus fontinalis*) and the results of their statistical comparisons.

Parameter	Statistics	Diploid		Triploid		
		females	males	females	males	intersex
BW (g)	**$F_{4,108}$ = 18.63, p = 1×10^{-11}**	397.1 ± 106.4^{a}	394.3 ± 106.4^{a}	691.9 ± 95.6^{b}	566.4 ± 138.4^{b}	639.3 ± 162.3^{b}
TL (mm)	**$F_{4,108}$ = 22.50, p = 2×10^{-13}**	309 ± 22.1^{a}	312.7 ± 26.2^{a}	360.0 ± 16.0^{b}	343.6 ± 22.4^{b}	361.4 ± 18.6^{b}
SL (mm)	**$F_{4,108}$ = 17.71, p = 3×10^{-11}**	274.7 ± 21.7^{a}	279.3 ± 25.1^{a}	323.3 ± 16.0^{b}	304.0 ± 20.7^{b}	319.9 ± 21.2^{b}
IH (%)	**$F_{4,107}$ = 10.23, p = 5×10^{-7}**	23.6 ± 2.1^{a}	25.5 ± 2.1^{ab}	25.3 ± 1.9^{ab}	27.2 ± 2.7^{b}	24.0 ± 1.6^{a}
IW (%)	**$F_{4,107}$ = 3.51, p = 0.010**	14.4 ± 1.2^{b}	13.4 ± 1.0^{a}	15.5 ± 0.7^{ab}	14.4 ± 1.2^{ab}	14.0 ± 1.2^{ab}
IHL (%)	**$F_{4,107}$ = 20.97, p = 9×10^{-13}**	19.2 ± 1.1^{a}	21.3 ± 1.7^{b}	19.3 ± 1.1^{ab}	22.0 ± 2.1^{c}	18.9 ± 1.1^{a}
ICP (%)	**$F_{4,107}$ = 7.11, p = 4×10^{-5}**	17.3 ± 2.1^{ab}	18.6 ± 1.9^{b}	19.7 ± 1.1^{ab}	16.8 ± 1.9^{a}	19.5 ± 1.6^{b}
FC	**$F_{4,108}$ = 3.39, p = 0.012**	1.9 ± 0.3^{ab}	1.8 ± 0.2^{a}	2.0 ± 0.2^{ab}	2.0 ± 0.2^{b}	1.9 ± 0.2 ^{ab}
fat (%)	**$F_{4,107}$ = 30.42, p < 2×10^{-16}**	4.4 ± 0.9^{b}	2.7 ± 0.7^{a}	5.0 ± 0.9^{b}	2.7 ± 1.2^{a}	5.1 ± 1.4^{b}
GSI (%)	**H_4 = 91.28, p < 2×10^{-16}**	10.86 ± 3.56^{d}	3.63 ± 1.32^{c}	0.07 ± 0.03^{a}	0.70 ± 0.31^{b}	0.05 ± 0.01^{a}
CY (%)	**$F_{4,107}$ = 66.85, p < 2×10^{-16}**	77.1 ± 2.9^{a}	83.7 ± 2.7^{b}	83.3 ± 1.9^{b}	87.8 ± 2.2^{c}	85.7 ± 2.1^{bc}
FYS (%)	**$F_{4,107}$ = 14.33, p = 2×10^{-8}**	47.6 ± 3.0^{a}	50.7 ± 3.4^{b}	58.3 ± 1.0^{bc}	53.6 ± 2.8^{b}	59.3 ± 2.1^{c}
FY (%)	**$F_{4,107}$ = 11.26, p = 5×10^{-7}**	41.3 ± 3.2^{a}	43.6 ± 3.4^{ab}	53.2 ± 1.1^{bc}	45.4 ± 3.9^{a}	53.5 ± 2.1^{c}
VSI (%)	**$F_{4,107}$ = 120.79, p < 2×10^{-16}**	23.0 ± 2.6^{c}	15.2 ± 1.9^{b}	14.1 ± 1.1^{b}	10.3 ± 2.2^{a}	13.6 ± 1.9^{b}

The values are expressed as mean ± SD. Statistically significant tests ($p < 0.05$) are highlighted in bold font. Lowercase letters denote significant differences ($p < 0.05$) between individual ploidy-sex groups. BW, body weight; TL, total length; SL, standard length; IH, index of highbackedness; IW, index of widebackedness; IHL, index of head length; ICP, index of caudal peduncle; FC, Fulton's condition factor; GSI, gonadosomatic index; CY, carcass yield; FYS, fillet yield with skin; FY, fillet yield without skin; VSI, viscerosomatic index.

3.2.2 Fish at 667 d.p.h.

In all the 14 parameters, statistically significant differences between individual ploidy-sex groups were observed.

The BW, TL and SL of all triploid groups were significantly different from both diploid groups: irrespective of sex, the BW, TL and SL of triploids were 59.9, 14.2 and 14.0% higher than in diploids, respectively. Compared to diploid females, triploid females had significantly higher values of CY (8.0%), FYS (22.5%) and FY (28.8%) but significantly lower values of GSI (99.4%) and VSI (38.7%). Triploid males exhibited significantly higher IHL (3.2%), FC (11.1%) and CY (4.9%) than diploid males but lower values for ICP (9.7%), GSI (80.1%) and VSI (32.2%). Triploid intersexes tended to show similar values of biometric indices and slaughtering traits as triploid females, and these two ploidy-sex groups did not differ significantly in any of these parameters. Conversely, triploid intersexes differed from triploid males in many parameters having higher ICP (16.1%), fat content (88.9%), FYS (10.6%), FY (17.8%) and VSI (32.0%) but lower IH (11.8%), IHL (14.1%) and GSI (92.9%).

3.3 Heterozygosity and allelic distances

The loci SALV1, SALV2, SALV3, SALV7 and SALV8 were monomorphic (monoallelic) for all age groups and so were excluded from the analyses. The heterozygosity of the five remaining loci that were polymorphic (SALV4, SALV5, SALV9, SALV10 and SALV 11), the MLH and d^2 are summarized in Table 3. Detailed results of all statistical comparisons of heterozygosity, MLH and d^2 are provided in the Supplementary Material (Supplementary Table 1).

Although the MLH did not differ significantly between diploid and triploid brook trout at 360 d.p.h., at both 555 and 667 d.p.h. triploids exhibited higher levels of MLH than diploids. When all these age groups were pooled, the difference between ploidies was significant, with triploids having 24.2% higher MLH than diploids. The comparison of the number of heterozygotes at individual loci revealed that in some age groups triploids were significantly more heterozygous than diploids at SALV4 (667 d.p.h., all groups pooled), SALV9 and SALV10 (555 and 667 d.p.h., all groups pooled). Furthermore, triploids exhibited higher values of d^2 than diploids at 667 d.p.h., although the difference in d^2 between ploidies was not significant at either 360 or 555 d.p.h. With all age groups pooled, d^2 differed significantly between ploidies and the d^2 of triploids was 19.3% higher than the d^2 of diploids.

3.4 Associations between heterozygosity and growth

When the ploidy level was not taken into account, the statistical evaluation of the relationship between MLH and BW revealed no significant association in the 360 and 555 d.p.h. age groups (rs_{64} = 0.08, p = 0.532 and rs_{86} = -0.20, p = 0.060, respectively) but a positive association at 667 d.p.h. (rs_{111} = 0.37, p = 6×10^{-5}). The analyses of the relationship between d^2 and BW – irrespective of ploidy – revealed no significant association at 360 d.p.h. (rs_{64} = 0.08, p = 0.506), a negative association at 555 d.p.h. (rs_{86} = -0.37, p = 4×10^{-4}) and a positive association at 665 d.p.h. (rs_{111} = 0.22, p = 0.021). When analogical analyses were performed separately for each ploidy from the individual age groups, the only significant relationship was detected in triploids aged 555 d.p.h. whose d^2 was negatively associated with BW (rs_{43} = -0.41, p = 0.005), while the remaining five correlations with d^2 and all six correlations with MLH were non-significant (see Supplementary Table 2 for detailed results).

The BWs of homozygotes and heterozygotes at each of the five polymorphic loci and the results of their statistical comparisons are given in Table 4. Heterozygotes at loci SALV4 were significantly heavier than homozygotes at 360 and 667 d.p.h., as was the case also of locus SALV9 in the 667 d.p.h. age group. Conversely, at 555 d.p.h. the heterozygotes at loci SALV5 and SALV10 were significantly lighter than the homozygotes at the same loci. The remaining 10 comparisons of heterozygote and homozygote BWs were non-significant.

TABLE 3 The heterozygosity at five polymorphic microsatellite loci (SALV4, SALV5, SALV9, SALV10, SALV11), multilocus heterozygosity (MLH) and squared allelic distance (d^2) for diploid and triploid brook trout (*Salvelinus fontinalis*) at 360, 555 and 667 days post-hatching and for diploids and triploids of all age groups pooled.

Age group	Ploidy	Heterozygosity per locus					MLH	d^2
		SALV4	SALV5	SALV9	SALV10	SALV11		
360 d.p.h.	diploid	0.576	0.697	0.485	0.848	0.212	0.564 ± 0.190	98.0 ± 76.6
	triploid	0.788	0.697	0.576	0.848	0.182	0.618 ± 0.161	98.1 ± 52.2
555 d.p.h.	diploid	0.814	0.698	0.465[a]	0.651[a]	0.116	0.549 ± 0.191[a]	99.8 ± 58.3
	triploid	0.889	0.600	0.756[b]	0.889[b]	0.289	0.684 ± 0.157[b]	121.0 ± 60.5
667 d.p.h.	diploid	0.614[a]	0.596	0.544[a]	0.754[a]	0.193	0.540 ± 0.207[a]	93.0 ± 61.2[a]
	triploid	0.893[b]	0.750	0.821[b]	0.911[b]	0.214	0.718 ± 0.142[b]	121.0 ± 57.6[b]
All groups pooled	diploid	0.669[a]	0.654	0.504[a]	0.744[a]	0.173	0.549 ± 0.196[a]	96.4 ± 64.1[a]
	triploid	0.866[b]	0.687	0.739[b]	0.888[b]	0.231	0.682 ± 0.156[b]	115.0 ± 57.8[b]

MLH and d^2 values are expressed as mean ± SD. Statistical analyses were performed for individual age groups and for all age groups pooled; lowercase letters denote significant differences ($p < 0.05$) in MLH, d^2 and the numbers of heterozygotes at given locus between diploids and triploids.

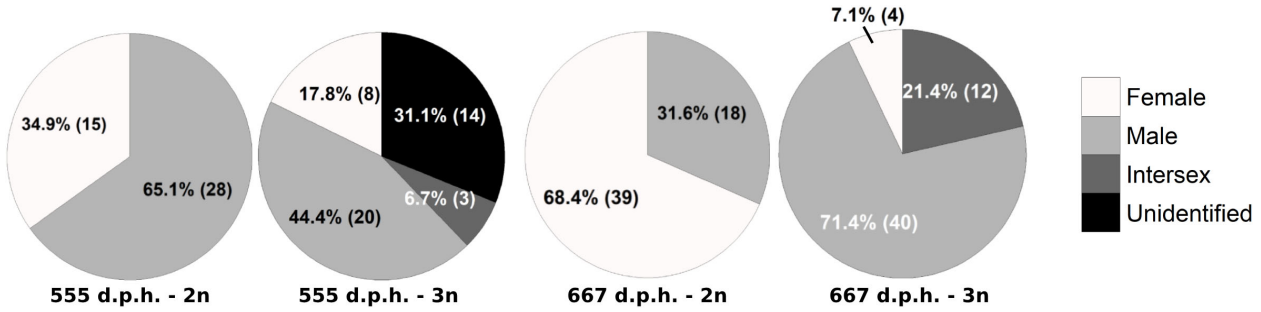

FIGURE 1
Sex ratios in diploid (2n) and triploid (3n) brook trout (*Salvelinus fontinalis*) at 555 and 667 days post-hatching determined by histological assessment.

The BWs of the individuals possessing different numbers of heterozygous loci (NHL) are summarized in Table 5. In the different age groups, the NHL ranged from 1 to 5 (360 and 667 d.p.h.) or from 0 to 5 (555 d.p.h.). NHL groups represented by a single individual within the respective age group were excluded from statistical analysis (including NHL 0 and 5 at 555 d.p.h. and NHL 5 at 667 d.p.h.). The analyses with these adjusted datasets revealed no differences in the BW between individual NHL groups at either 360 or 555 d.p.h. ($F_{4,61}$ = 0.50, p = 0.753 and $F_{3,82}$ = 1.87, p = 0.141, respectively) but significant differences at 667 d.p.h. ($F_{3,108}$ = 4.9, p = 0.003). In the latter case, the individuals possessing four heterozygous loci were significantly heavier than individuals with two heterozygous loci, while the BW of fish with one and three heterozygous loci did not differ from NHL groups 4 and 2.

Three alleles (A, B, C) were detected in some triploid individuals from all age groups at loci SALV4, SALV5 and SALV 10. The BWs of diallelic (e.g. ABB, AAC) and triallelic (e.g. ABC) heterozygotes at individual loci and the results of their statistical comparisons are shown in Table 6. Of all the eight comparisons performed, only one analysis revealed a significant difference in BW between diallelic and triallelic individuals: diallelic individuals at loci SALV5 were significantly heavier than triallelic at 555 d.p.h.

TABLE 4 Bodyweight (BW) and counts (N) of homozygotes and heterozygotes at each of the five polymorphic loci.

Age group	Locus	Homozygot		Heterozygot		Statistics
		BW (g)	N	BW (g)	N	
360 d.p.h	SALV4	84.1 ± 36.7^{a}	21	102.1 ± 31.7^{b}	45	**t_{64} = -2.0, p = 0.045**
	SALV5	92.3 ± 38.9	20	98.2 ± 32.2	46	t_{64} = -0.6, p = 0.529
	SALV9	104.8 ± 35.3	31	88.9 ± 31.7	35	t_{64} = 1.9, p = 0.058
	SALV10	96.0 ± 19.9	10	96.5 ± 36.2	56	t_{64} = -0.04, p = 0.969
	SALV11	94.7 ± 34.7	53	103.2 ± 32.3	13	t_{64} = -0.8, p = 0.427
555 d.p.h.	SALV4	405.5 ± 53.1	13	401.3 ± 53.9	75	t_{86} = 0.3, p = 0.798
	SALV5	417.5 ± 57.7^{b}	31	393.5 ± 49.5^{a}	57	**t_{86} = 2.0, p = 0.0498**
	SALV9	402.7 ± 42.1	34	401.5 ± 59.9	54	t_{86} = 0.1, p = 0.915
	SALV10	427.6 ± 36.8^{b}	20	394.4 ± 55.4^{a}	68	**t_{86} = 2.7, p = 0.009**
	SALV11	397.3 ± 50.6	70	420.2 ± 61.8	18	t_{86} = -1.6, p = 0.106
667 d.p.h.	SALV4	408.9 ± 124.1^{a}	28	520.4 ± 160.9^{b}	85	**t_{111} = -34.35, p = 1×10^{-3}**
	SALV5	497.9 ± 189.4	37	490.2 ± 144.2	76	t_{111} = -0.07, p = 0.945
	SALV9	445.2 ± 139.3^{a}	36	515.0 ± 164.4^{b}	77	**t_{111} = -2.2, p = 0.030**
	SALV10	445.3 ± 127.7	19	502.3 ± 164.2	94	t_{111} = -1.2, p = 0.238
	SALV11	481.6 ± 147.3	90	536.3 ± 198.4	23	t_{111} = -1.5, p = 0.144

The data are for brook trout (*Salvelinus fontinalis*) sampled at 360, 555 and 667 days post-hatching; the ploidy level was not considered (both diploids and triploids). BWs are expressed as mean ± SD. Statistical analysis was performed separately for each age group; lowercase letters denote significant differences (p < 0.05) in BWs between homozygotes and heterozygotes. Statistically significant tests (p < 0.05) are highlighted in bold font.

10.3389/fanim.2025.1481117

TABLE 5 Bodyweight (BW) and counts (N) of the individuals possessing different numbers of heterozygous loci (NHL).

Age group	NHL	BW (g)	N
360 d.p.h	1	75.7 ± 40.2	4
	2	101.3 ± 43.2	13
	3	95.2 ± 27.5	33
	4	99.7 ± 41.2	14
	5	101.8 ± 5.2	2
555 d.p.h.	0	430	1
	1	448.4 ± 39.6	4
	2	407.7 ± 41.8	15
	3	402.5 ± 51.3	35
	4	388.2 ± 56.6	32
	5	523	1
667 d.p.h.	1	406.4 ± 70.0^{ab}	8
	2	419.2 ± 130.4^{a}	23
	3	479.2 ± 173.4^{ab}	28
	4	546.1 ± 157.4^{b}	53
	5	428.5	1

The data are for brook trout (*Salvelinus fontinalis*) sampled 360, 555 and 667 days post-hatching; the ploidy level was not considered (both diploids and triploids). Total number of polymorphic microsatellite loci examined = 5. BWs are expressed as mean ± SD. Statistical analyses were performed separately for each age group; lowercase letters denote significant differences ($p < 0.05$) in BW between individual NHL groups. NHL groups represented by a single individual within the respective age group were excluded from statistical analyses.

3.5 Associations between gonadal development and growth

The proportions and counts of diploid and triploid fish with gonads at different developmental stages detected by the histological assessment are given in Figure 2 for females and Figure 3 for males.

3.5.1 Gonadal stages versus body weight

At the age of 555 d.p.h., all diploid females possessed vitellogenic ovaries, while the majority of triploid females (87.5%) had gonads at the stage of oogonia. The BWs of females with vitellogenic ovaries (387.1 ± 48.6g) and oogonia (363.9 ± 30.1g) did not differ significantly ($t_{20} = -1.15$, $p = 0.262$). Almost all diploid females (97.4%) also had vitellogenic ovaries at 667 d.p.h., while most triploid females (75.0%) possessed pre-vitellogenic ovaries. The BWs of females with pre-vitellogenic and vitellogenic ovaries (619.9 ± 152.6 and 396.0 ± 107.6g, respectively) were significantly different ($t_{40} = 3.82$, $p = 5\times10^{-4}$).

At the age of 555 d.p.h., early spermatogenic testes were the most prevalent stage of gonadal development in both diploid (92.9%) and triploid (85.0%) males. Pre-spermatogenic testes were observed in the other males of both ploidies. The difference between the BWs of males with pre-spermatogenic testes (404.7 ± 83.6g) and early spermatogenic testes (418.2 ± 53.0g) was not significant ($t_{46} = -0.51$, $p = 0.614$). At 667 d.p.h., mid-spermatogenic testes and late spermatogenic testes were the prevalent gonadal stages in diploid males (50.0 and 44.4%, respectively), while triploid males still possessed early spermatogenic testes (67.5%) or pre-spermatogenic testes (32.5%). Significant differences in BW were observed between individuals at different gonadal stages ($F_{3,54} = 8.13$, $p = 1\times10^{-11}$): males with early spermatogenic testes were significantly heavier than males with mid-spermatogenic and late spermatogenic testes (587.9 ± 139.1g vs. 368.5 ± 122.4 and 421.0 ± 93.1g, respectively); males with pre-spermatogenic testes did not differ from the three remaining groups in their BW (514.0 ± 129.3g).

3.5.2 Gonadosomatic index vs. body weight

When the ploidy level was ignored, the statistical analysis of the 667 d.p.h. age group revealed a significant negative association between GSI and BW in females ($rs_{41} = -0.33$, $p = 0.032$), males ($rs_{56} = -0.42$, $p = 0.002$) and intersexes ($rs_{10} = -0.76$, $p = 0.006$). However, when the ploidy level was taken into consideration, the

TABLE 6 The bodyweight (BW) and counts (N) of diallelic (e.g. ABB, AAC) and triallelic (e.g. ABC) heterozygotes at individual loci.

Age group	Locus	Diallelic individual		Triallelic individual		Statistics
		BW (g)	N	BW (g)	N	
360 d.p.h.	SALV4	100.2 ± 33.1	39	114.7 ± 16.9	6	$t_{43} = -1.04$, $p = 0.302$
	SALV5	98.3 ± 32.5	45	94.1	1	–
	SALV10	95.5 ± 36.8	51	106.7 ± 32.2	5	$t_{54} = -0.66$, $p = 0.511$
555 d.p.h.	SALV4	398.5 ± 51.6	65	419.8 ± 66.6	10	$t_{73} = -1.17$, $p = 0.248$
	SALV5	397.9 ± 48.2^{b}	53	334.8 ± 24.5^{a}	4	**$t_{55} = 2.6$, $p = 0.013$**
	SALV10	397.2 ± 56.6	58	378.3 ± 47.3	10	$t_{66} = 0.97$, $p = 0.335$
667 d.p.h.	SALV4	517.3 ± 165.9	73	539.2 ± 130.9	12	$t_{83} = -0.43$, $p = 0.665$
	SALV5	487.7 ± 148.2	71	526.0 ± 63.5	5	$t_{74} = -0.57$, $p = 0.569$
	SALV10	489.6 ± 169.4	81	581.4 ± 98.6	13	$t_{92} = -1.90$, $p = 0.061$

The data are for brook trout (*Salvelinus fontinalis*) sampled 360, 555 and 667 days post-hatching, ploidy level is not considered (both diploids and triploids). BWs are expressed as mean ± SD. Statistical analyses were performed separately for each age group; lowercase letters denote significant differences ($p < 0.05$) in BWs between diallelic and triallelic heterozygotes. Statistically significant tests ($p < 0.05$) are highlighted in bold font.

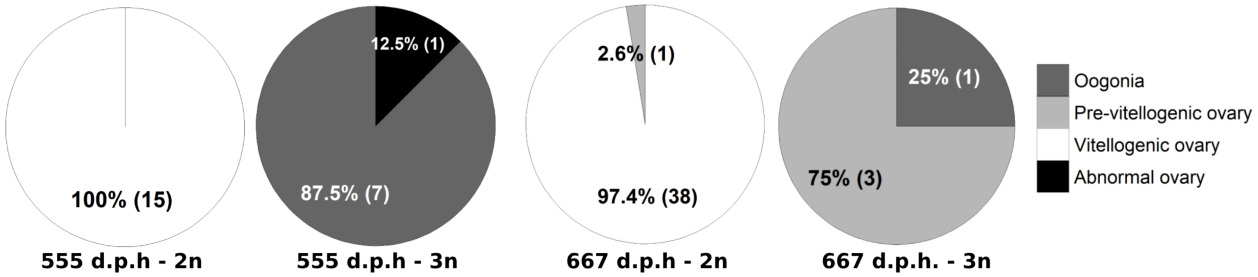

FIGURE 2
The proportions and counts (in brackets) of diploid (2n) and triploid (3n) females of brook trout (*Salvelinus fontinalis*) with gonads at individual developmental stages. Data based on histological assessment at 555 and 667 days post-hatching.

associations between GSI and BW were not significant between either diploid females (rs_{37} = -0.11, p =0.500), diploid males (rs_{18} = -0.07, p = 0.754), triploid females (rs_2 = 0.8, p =0.333) or triploid males (rs_{38} = -0.07, p = 0.677).

4 Discussion

It is generally considered that triploids could grow faster than diploids due to their higher overall genomic heterozygosity and the diversion of energy from gonadal to somatic growth (Piferrer et al., 2009). According to these authors, the former phenomenon manifests itself throughout the entire lifespan of the triploids while the latter first comes into effect at sexual maturation. The latter may also contribute to explaining the results of this study: in fish sampled at 360 d.p.h. (yearlings), triploids were on average 12 mm longer than the diploids but without exhibiting any difference in weight growth. Accordingly, the batches of diploid and triploid fish at 555 d.p.h. did not differ either in length or weight growth. O'Keefe and Benfey (1999) studied the comparative growth and food consumption of young diploid and triploid brook trout (from BWs of 42 to 258 g) and found no difference in growth or food consumption in either the separated or mixed stocks of fish at both ploidy levels. Budy et al. (2012) evaluated the performance of diploid and triploid brook trout in ponds and found neither length nor weight growth differences in juvenile fish at both ploidy levels; Schafhauser-Smith and Benfey (2001) report that differences between diploid and triploid brook trout in weight growth only become significant during and after maturation. A similar study to ours was performed by Werner et al. (2008) on all-female pan-sized farmed triploids of another salmonid species, the rainbow trout, at ages of 66 and 75 weeks (i.e. 462 and 525 days) after fertilization, with no BW difference between younger diploids and triploids but differences in fillet weight and yield. No maturation-related impact on growth was detected due to small gonads – as we also found in our study – despite the histologically determined higher developmental degree of ovaries and testes of diploids compared to triploids. A conclusion similar to our findings, i.e. that sexually mature diploids have lower CY than triploids or immature diploids, has been reported by Janhunen et al. (2019) in another study of rainbow trout.

Results for the older triploid brook trout in our study are best compared to those of Boulanger (1991); Schafhauser-Smith and Benfey (2001) and Uzunova (2004) since the other relevant studies mentioned above were terminated at younger fish ages. Uzunova (2004) compared different ploidy-sex groups in brook trout and found that during the spawning period triploid males had lower BW, dressed weight and TL than diploid males, while the opposite occurred in diploid and triploid females. However, contrary to these findings, our study found greater weight and length growth, and CY (de facto dressed weight in %) in triploid males but similar conclusions for diploid and triploid females. Boulanger (1991) studied all-female populations of brook trout and concluded that triploid growth continued unabated for three consecutive years while diploids even lost weight during spawning, resulting in triploid females being almost twice the weight of diploids at the

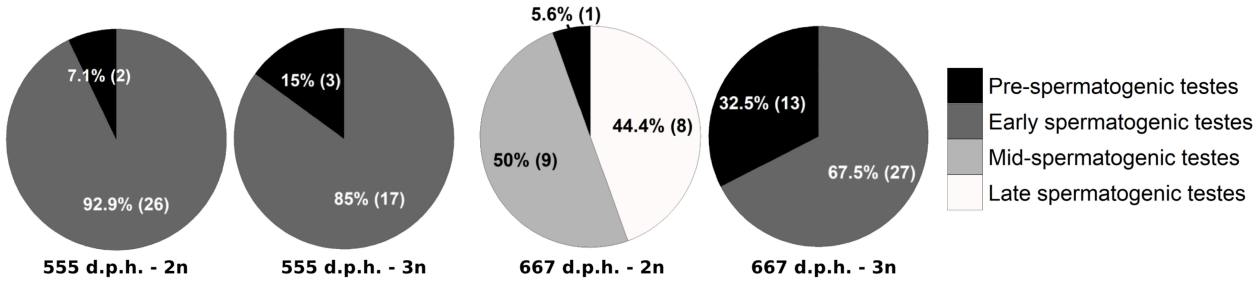

FIGURE 3
The proportions and counts (in brackets) of diploid (2n) and triploid (3n) males of brook trout (*Salvelinus fontinalis*) with gonads at individual developmental stages. Data based on histological assessment at 555 and 667 days post-hatching.

end of the trial. In accordance with our results, these authors mention that the average gutting loss in triploids is approximately two thirds to that of diploids. The study by Schafhauser-Smith and Benfey (2001) was conducted on all-female brook trout (up to the age of 3+ years) and also reported that triploids were heavier than diploids for most of the study period, although the statistically significant differences were mainly observed during maturation and the spawning of diploids. In all the previously mentioned studies (including ours), enhanced somatic growth in triploid females was accompanied by the observation of very small, poorly developed gonads and a mean GSI never exceeded 0.17%. This value of mean GSI was observed in 3+-year fish by Schafhauser-Smith and Benfey (2001) who are the only authors to find mature-stage oocytes in triploid brook trout, albeit only in three out of 19 females at age 3+ that collectively produced only 72 oocytes. Unsurprisingly, the lowest GSI of this study was found in triploid females (0.07%) with oogonia and/or pre-vitellogenic ovaries but also for intersex fish (0.05%), a group that was very similar to triploid females in terms of biometric indices and slaughtering traits. A 10-fold higher GSI was detected in triploid males with pre- and early spermatogenic testes, yet five times higher for diploid males with mostly mid- and late spermatogenic testes, and highest (15.5-fold) for diploid females with vitellogenic ovaries. It is worth noting that the five-fold difference between GSI of diploid and triploid males could explain the contradictory results in the somatic growth between our study and the study by Uzunova (2004). These authors examined brook trout at an almost similar age (22 months post hatching) to our trout but surprisingly reported that diploid males did not differ in GSI from triploids. Although the reason behind these inconsistencies in the GSI of triploid males remains unclear and may be related to the different breeds of brook trout used in these two studies, this example demonstrates that suppressed gonadal development in triploids is the essential prerequisite for and main cause of their better growth. Our results agree with this hypothesis regarding the relationship between GSI and BW: when diploids and triploids from the 667 d.p.h. age group were pooled, we observed significant negative correlation between GSI and BW in both females and males, and a similar trend in triploid intersex. As well, our comparison of the BWs of 667 d.p.h.-individuals with different gonadal stages showed that in both males and females, fish at earlier stages tend to have rather higher BW than fish at more advanced stages. Nevertheless, it is worth highlighting the fact that ploidy was ignored in this comparison (diploids and triploids pooled) and that the earlier stages were represented almost solely by triploids that were generally heavier. Apart from higher BW and CY in triploid brook trout at 667 d.p.h, our study also detected higher fillet yields in triploid than in diploid females, a finding that has not yet been replicated in brook trout but has been confirmed for another salmonid species, the rainbow trout (Werner et al., 2008; Janhunen et al., 2019; Everson et al., 2021).

In all batches of randomly sampled market-sized diploid brook trout and their induced triploid siblings, the sex ratio was somewhat skewed from 1:1. In diploid and triploid fish sampled 555 d.p.h. the ratio was 1.8:1 and 2.49:1 in favour of males, respectively. The gonads of the triploids were very small and only histologically distinguishable from adipose tissue, so the GSI was not computed. Moreover, in addition to the histologically determined triploid male/female sex, the batch also contained three intersex fish and almost one third of all fish had histologically indifferent gonads but with female external appearances. At this age the sex ratio is probably not yet affected by the initial sorting of market-sized fish performed by the company, as there was no weight or length difference between the sexes. On the other hand, among diploid fish randomly sampled 667 d.p.h., the ratio was found to be 2.16:1 in favour of females but vice versa in triploids (2.5:1). However, the proportion of triploid fish with female external appearances consisting of histologically determined females and intersex was approximately 1:3. Although skewed sex ratios have been described previously in triploid fish (e.g. Okomoda et al., 2020), shrimps (Li et al., 2003; Pongtippatee et al., 2012) and molluscs (Brake et al., 2004), these ratios did not change simply within age categories, so it might be expected that the sex ratio of the studied fish were most probably affected by the random sampling of all batches.

The results of our microsatellite analysis of the five polymorphic loci suggest that triploids can be provided with higher levels of heterozygosity than their diploid counterparts, a trend in MLH that was consistent in all three age groups of brook trout, although the difference between ploidies was not significant for the youngest group examined (360 d.p.h.), a group with smaller sample size than the other age classes. When all age groups were pooled, the MLH of triploids was 24% higher and significant differences in heterozygosity were detected at three out of five loci, thereby further supporting the proposed hypothesis. A similar conclusion – that triploids are more heterozygous than diploids – has been reported by other studies such as Leary et al. (1985), who focused on the heterozygosity and developmental stability in triploids and meiotic gynogenotes of rainbow trout, and studies comparing heterozygosity between second polar body-induced triploids and normal diploids in shellfish (e.g. common mussel *Mytilus edulis* – Beaumont et al., 1995; Pacific oyster *Crassostrea gigas* – Hawkins et al., 2000; Wang et al., 2002; Chinese shrimp *Fenneropenaeus Chinensis* – Wang et al., 2008). In their study of the Chinook salmon, Garner et al. (2008) did not detect any significant difference in MLH between diploids and triploids although these authors did hypothesize that triploidy had failed to increase heterozygosity because of its already high level in the diploid population (MLH > 0.90). This suggests that the magnitude and/or detectability of triploidy-induced change in heterozygosity depends, among other factors, greatly on the genetic background of the population under study. In this respect, it is also of note that Garner et al. (2008) failed to detect any difference in d^2 between ploidies, while our triploids exhibited significantly higher values for this parameter than diploids when the 667 d.p.h. group and pooled age groups were examined.

Unlike the trends in heterozygosity between our diploid and triploid brook trout, the trends in the effects of heterozygosity upon growth performance were inconsistent. When diploid and triploid groups were pooled, the correlation between MLH and BW was significant in only one of the three age groups examined (667 d.p.h.); moreover, the correlation coefficient of 0.37 suggests a rather weak association in this case. There was no significant

correlation between MLH and BW within ploidies. In terms of the association between d^2 and BW, we obtained contradictory results: a negative correlation at 555 d.p.h. but a positive correlation at 667 d.p.h. Likewise, we derived conflicting results from the comparison of BW between heterozygotes and homozygotes at individual loci: in most cases, their BWs did not differ but on occasions heterozygotes were either lighter (SALV5,10 at 555 d.p.h.) or heavier (SALV4 at 360 d.p.h. and SALV4,9 at 667 d.p.h.) than homozygotes. Finally, we observed significant differences in BW between individuals possessing different NHL only in the oldest age group (667 d.p.h.), when fish with four heterozygous loci were heavier than fish with two. The link between heterozygosity and d^2 and performance in both diploid and triploid fish was studied by Garner et al. (2008), who found no significant relationship between MLH or d^2 and specific growth rates or feeding rates in juvenile Chinook salmon. Some studies focusing solely on diploid salmonids have reported a positive correlation between heterozygosity and length and weight, for example in adult Atlantic salmon (Blanco et al., 1998), pink salmon fry (*Oncorhynchus gorbuscha*, Kartavtsev, 1992, 1998) and juvenile rainbow trout (Danzmann et al., 1987, 1988). On the other hand, Ferguson (1990, 1992) reported negative correlation between heterozygosity and fork length/BW in juvenile and mature diploids of rainbow trout; like our results for 360 and 555 d.p.h. age groups of brook trout, Koljonen (1986) failed to find any significant relationship between heterozygosity and BW (and TL) in adult diploid rainbow trout. In addition, Ferguson (1992) observed that fish age affected both the strength and direction of the association, and mentioned the biasing effect of individual sexual maturation on growth as highly probable cause. Although Ferguson´s study was conducted on diploid fish exclusively, we consider that the individual differences in the allocation of energy resources into somatic and reproductive tissue skewed the heterozygosity-growth associations also in our study with diploids and triploids, resulting in weak correlations or inconsistent trends when ploidy was either considered or not considered in statistical evaluation. For clarity, it should be mentioned that more studies including triploids and focusing on heterozygosity-performance associations have been conducted in shellfish, some of which have reported positive trends. For example, Wang et al. (2002) reported in Pacific oyster strong positive correlation between meat weight and MLH in diploids, second polar body-induced triploids, and diploid-tetraploid mating-produced triploids. Using diploids, first and second polar body-induced triploids of the same species, Hawkins et al. (2000) observed significant positive correlations between MLH and physiological traits including filtration rate, absorption efficiency and net energy balance. Beaumont et al. (1995) reported a significant positive correlation between MLH and shell length in diploids of common mussels, which was absent or much more weakly expressed in triploid cohorts obtained from first and second polar body retention. Overall, we conclude that, even though heterozygosity and/or d^2 can be enhanced in brook trout by triploidization, we cannot provide any clear evidence that this contributes to better growth. Given that the positive effects of these measures of genetic variability on BW were detected almost solely in the oldest of all the three age groups examined when both ploidies were pooled, we suggest that the results could be biased by other factors, e.g. supressed gonadal development in triploids. At the age of 667 d.p.h., the triploids had higher heterozygosity, d^2 and BW than diploids, so the positive correlation might be a coincidence. In this respect, it is noteworthy that the examination of more polymorphic loci might help the search for possible relationships, even though half (5) of *a priori* planned loci turned out to be monomorphic in our case.

As Janhunen et al. (2019) conclude, the performance of triploids and diploids during the growth period is an important issue that determines whether or not the potential benefits of triploid trout will manifest themselves at the beginning and end of the sorting for the requested market size (pan-size). At the beginning of sorting, in pan-size brook trout 555 d.p.h. triploids – regardless of sex – had higher carcass yield than their diploid conspecifics. At the end or rearing period, 667 d.p.h., all triploids (females, males and intersex fish) were significantly larger and heavier with far lower gonadosomatic index than their diploid conspecifics; nevertheless, differences were not so marked in terms of carcass and fillet yields. Delayed gonadal growth in triploids was confirmed as was expected. Microsatellite analysis revealed that triploids are provided with considerably higher levels of heterozygosity than diploids, although the link between heterozygosity and bodyweight was inconclusive. Suppressed gonadal development seems to be a more probable explanation for the improved growth performance of triploids. Aside from this, triploid intersex with external appearance, biometric indices and slaughtering traits that are highly similar to that of triploid females can commonly arise from artificial triploidization in brook trout and their occurrence may contribute to mistakes in external sexing. As the environmental conditions provided for diploids and triploids were equal, reasons for the significant occurrence of intersex among triploids might be hypothetically affected by various genetic factors such as disturbed recombination, meiosis or expression patterns of genes controlling the gonad development and might be an interesting topic of next closely targeted research. Concluding this study, induction of triploidy in brook trout in a farm-scale using the mobile hydrostatic pressure unit and farming the triploids till live weight over 550 g was found technically feasible till 667 days post hatching.

Data availability statement

Datasets are available on request: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by Ethics Committee for the Protection of Animals in Research of the University of South

Bohemia in České Budějovice. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

MF: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft. VK: Conceptualization, Investigation, Methodology, Writing – review & editing. MP: Formal analysis, Investigation, Writing – review & editing. IM: Investigation, Writing – review & editing. EP: Investigation, Writing – review & editing. JK: Investigation, Writing – review & editing. MH: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The study was financially supported by the Ministry of Agriculture of the Czech Republic, National Agency for Agricultural Research project QK22020041 *Application of new knowledge on genetics and genomics for production of high-performing triploid populations of fish in order to improve performance traits and market product quality*. The hydrostatic pressure chamber used was funded by the Ministry of Education, Youth and Sports of the Czech Republic, project Biodiversity (CZ.02.1.01/0.0/0.0/16_025/0007370). All funding to support this research was secured by MF.

Acknowledgments

The authors are indebted to Ing. Jiří Lipka and Klatovy Fishery, Czech Republic (Klatovské rybářství a.s.) for providing fish for the study. The technical assistance provided by Ivana Samková, Josef Vobr and Ing. Pavlína Gápová is gratefully acknowledged.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2025.1481117/full#supplementary-material

References

Adams, K. L., Cronn, R., Percifield, R., and Wendel, J. F. (2003). Genes duplicated by polyploidy show unequal contributions to the transcriptome and organ-specific reciprocal silencing. *Proc. Nat. Acad. Sci.* 100, 4649–4654. doi: 10.1073/pnas.0630618100

Aussanasuwannakul, A., Kenney, P. B., Weber, G. M., Yao, J., Slider, S. D., Manor, M. L., et al. (2011). Effect of sexual maturation on growth, fillet composition, and texture of female rainbow trout (*Oncorhynchus mykiss*) on a high nutritional plane. *Aquaculture* 317, 79–88. doi: 10.1016/j.aquaculture.2011.04.015

Babaheydari, S. B., Keyvanshokooh, S., Dorafshan, S., and Johari, S. A. (2016). Proteomic analysis of skeletal deformity in diploid and triploid rainbow trout (*Oncorhynchus mykiss*) larvae. *Comp. Biochem. Physiol. Part D Genomics Proteomics* 19, 1–7. doi: 10.1016/j.cbd.2016.05.001

Beaumont, A. R., Fairbrother, J. E., and Hoare, K. (1995). Multilocus heterozygosity and size: a test of hypotheses using triploid *Mytilus edulis*. *Heredity* 75, 256–266. doi: 10.1038/hdy.1995.133

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

Benfey, T. J. (2016). Effectiveness of triploidy as a management tool for reproductive containment of farmed fish: Atlantic salmon (*Salmo salar*) as a case study. *Rev. Aquac.* 8, 264–282. doi: 10.1111/raq.12092

Benfey, T. J., Dye, H. M., Solar, I. I., and Donaldson, E. M. (1989). The growth and reproductive endocrinology of adult triploid Pacific salmonids. *Fish Physiol. Biochem.* 6, 113–120. doi: 10.1007/BF01875483

Benfey, T. J., and Sutterlin, A. M. (1984). Triploidy induced by heat shock and hydrostatic pressure in landlocked Atlantic salmon (*Salmo salar* L.). *Aquaculture* 36, 359–367. doi: 10.1016/0044-8486(84)90328-4

Blanco, G., Presa, P., Vázquez, E., and Sánchez, J. A. (1998). Allozyme heterozygosity and development in Atlantic salmon, *Salmo salar*. *Fish Physiol. Biochem.* 19, 163–169. doi: 10.1023/A:1007771417270

Blazer, V. S. (2002). Histopathological assessment of gonadal tissue in wild fishes. *Fish Physiol. Biochem.* 26, 85–101. doi: 10.1023/A:1023332216713

Bonnet, S., Haffray, P., Blanc, J. M., Vallée, F., Vauchez, C., Fauré, A., et al. (1999). Genetic variation in growth parameters until commercial size in diploid and triploid freshwater rainbow trout (*Oncorhynchus mykiss*) and seawater brown trout (*Salmo trutta*). *Aquaculture* 173, 359–375. doi: 10.1016/S0044-8486(98)00460-8

Boulanger, Y. (1991). Performance comparison of all-female, diploid and triploid brook trout. *Can. Tech. Rep. Fish. Aquat. Sci.* 1789, 111–121.

Brake, J., Davidson, J., and Davis, J. (2004). Field observations on growth, gametogenesis, and sex ratio of triploid and diploid *Mytilus edulis*. *Aquaculture* 236, 179–191. doi: 10.1016/j.aquaculture.2003.09.016

Budy, P., Thiede, G. P., Dean, A., Olsen, D., and Rowley, G. (2012). A comparative and experimental evaluation of performance of stocked diploid and triploid Brook Trout. *N. Am. J. Fish. Manage.* 32, 1211–1224. doi: 10.1080/02755947.2012.728172

Comai, L. (2005). The advantages and disadvantages of being polyploid. *Nat. Rev. Genet.* 6, 836–846. doi: 10.1038/nrg1711

Coulson, T. N., Pemberton, J. M., Albon, S. D., Beaumont, M., Marshall, T. C., Guinness, F. E., et al. (1998). Microsatellites reveal heterosis in red deer. *Proc. R. Soc Lond. B Biol. Sci.* 265, 489–495. doi: 10.1098/rspb.1998.0321

Danzmann, R. G., Ferguson, M. M., and Allendorf, F. W. (1987). Heterozygosity and oxygen-consumption rate as predictors of growth and developmental rate in rainbow trout. *Physiol. Zool.* 60, 211–220. doi: 10.1086/physzool.60.2.30158645

Danzmann, R. G., Ferguson, M. M., and Allendorf, F. W. (1988). Heterozygosity and components of fitness in a strain of rainbow trout. *Biol. J. Linn. Soc* 33, 285–304. doi: 10.1111/j.1095-8312.1988.tb00813.x

Deschamps, M. H., Poirier Stewart, N., Demanche, A., and Vandenberg, G. W. (2014). Preliminary study for phenotypic description of vertebral abnormalities in

triploid trout subjected to prolonged deficiency in phosphorus. *J. Appl. Ichthyol.* 30, 833–839. doi: 10.1111/jai.12518

Everson, J. L., Weber, G. M., Manor, M. L., Tou, J. C., and Kenney, P. B. (2021). Polyploidy affects fillet yield, composition, and fatty acid profile in two-year old, female rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 531, 735873. doi: 10.1016/j.aquaculture.2020.735873

Ferguson, M. M. (1990). Enzyme heterozygosity and growth of rainbow trout reared at two rations. *Biol. J. Linn. Soc* 40, 215–227. doi: 10.1111/j.1095-8312.1990.tb00536.x

Ferguson, M. M. (1992). Enzyme heterozygosity and growth in rainbow trout: genetic and physiological explanations. *Heredity* 68, 115–122. doi: 10.1038/hdy.1992.18

Fjelldal, P. A., Hansen, T. J., Lock, E. J., Wargelius, A., Fraser, T. W. K., Sambraus, F., et al. (2016). Increased dietary phosphorous prevents vertebral deformities in triploid Atlantic salmon (*Salmo salar* L.). *Aquac. Nutr.* 22, 72–90. doi: 10.1111/anu.12238

Flajšhans, M., Havelka, M., Lebeda, I., Rodina, M., Gela, D., and Hubálek, M. (2020). Application of hydrostatic pressure shock for retention of the second polar body in sterlet (*Acipenser ruthenus*). *Aquaculture* 520, 734947. doi: 10.1016/j.aquaculture.2020.734947

Flajšhans, M., Kašpar, V., and Hubálek, M. (2023). "Optimalizace šoku hydrostatickým tlakem u lososovitých ryb," in *Methods*, vol. 199. (FFPW USB, Vodňany), 39 p. In Czech.

Flajšhans, M., Ráb, P., and Linhart, O. (2013). "Polyploidie a genomové manipulace u ryb," in *Genetika a šlechtění ryb*. Ed. M. Flajšhans (FFPW USB, Vodňany), 151–195.

Fraser, T. W., Fjelldal, P. G., Hansen, T., and Mayer, I. (2012). Welfare considerations of triploid fish. *Rev. Fish. Sci.* 20, 192–211. doi: 10.1080/10641262.2012.704598

Fraser, T. W., Hansen, T. J., Remø, S. C., Olsen, R. E., and Fjelldal, P. G. (2022). Triploid Atlantic salmon× brown trout hybrids have similar seawater growth and welfare issues as triploid Atlantic salmon, but both were heavier at harvest than their diploid counterparts. *Aquaculture* 552, 737975. doi: 10.1016/j.aquaculture.2022.737975

Fraser, T. W., Hansen, T., Skjæraasen, J. E., Mayer, I., Sambraus, F., and Fjelldal, P. G. (2013). The effect of triploidy on the culture performance, deformity prevalence, and heart morphology in Atlantic salmon. *Aquaculture* 416, 255–264. doi: 10.1016/j.aquaculture.2013.09.034

Fraser, T. W., Lerøy, H., Hansen, T. J., Skjæraasen, J. E., Tronci, V., Pedrosa,, et al. (2021). Triploid Atlantic salmon and triploid Atlantic salmon× brown trout hybrids have better freshwater and early seawater growth than diploid counterparts. *Aquaculture* 540, 736698. doi: 10.1016/j.aquaculture.2021.736698

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

Garnier-Géré, P. H., Naciri-Graven, Y., Bougrier, S., Magoulas, A., Héral, M., Kotoulas, G., et al. (2002). Influences of triploidy, parentage and genetic diversity on growth of the Pacific oyster *Crassostrea gigas* reared in contrasting natural environments. *Mol. Ecol.* 11, 1499–1514. doi: 10.1046/j.1365-294X.2002.01531.x

Gillet, C., Vauchez, C., and Haffray, P. (2001). Triploidy induced by pressure shock in Arctic charr (*Salvelinus alpinus*): growth, survival and maturation until the third year. *Aquat. Living Resour.* 14, 327–334. doi: 10.1016/S0990-7440(01)01129-9

Glover, K. A., Harvey, A. C., Hansen, T. J., Fjelldal, P. G., Besnier, F. N., Bos, J. B., et al. (2020). Chromosome aberrations in pressure-induced triploid Atlantic salmon. *BMC Genet.* 21, 1–11. doi: 10.1186/s12863-020-00864-0

Goldstein, D. B., Ruiz Linares, A., Cavalli-Sforza, L. L., and Feldman, M. W. (1995). An evaluation of genetic distances for use with microsatellite loci. *Genetics* 139, 463–471. doi: 10.1093/genetics/139.1.463

Gregory, T. R. (2001). Coincidence, coevolution, or causation? DNA content, cell size, and the C-value enigma. *Biol. Rev. Camb. Philos. Soc* 76, 65–101. doi: 10.1017/S1464793100005595

Hawkins, A. J. S., Magoulas, A., Heral, M., Bougrier, S., Naciri-Graven, Y., Day, A. J., et al. (2000). Separate effects of triploidy, parentage and genomic diversity upon feeding behaviour, metabolic efficiency and net energy balance in the Pacific oyster *Crassostrea gigas*. *Genet. Res.* 76, 273–284. doi: 10.1017/S0016672300004766

Hubálek, M., and Flajšhans, M. (2021). Simple field storage of fish samples for measurement of DNA content by flow cytometry. *Cytometry A* 99, 743–752. doi: 10.1002/cyto.a.24271

Jagiełło, K., Polonis, M., Panasiak, L., Dobosz, S., and Ocalewicz, K. (2021). Production of triploid, doubled haploid (DH) and meiogynogenetic brook trout (*Salvelinus fontinalis*)–efficiency and development of body deformities. *Ann. Anim. Sci.* 21, 141–157. doi: 10.2478/aoas-2020-0051

Janhunen, M., Vehviläinen, H., Koskela, J., Forsman, A., and Kankainen, M. (2019). Added value from an added chromosome: Potential of producing large fillet fish from autumn to spring with triploid rainbow trout, *Oncorhynchus mykiss*. *Aquac. Res.* 50, 818–825. doi: 10.1111/are.13952

Kartavtsev, Y. P. (1992). Allozyme heterozygosity and morphological homeostasis in pink salmon, *Oncorhynchus gorbuscha* (Walbaum): evidences from family analysis. *J. Fish Biol.* 40, 17–24. doi: 10.1111/j.1095-8649.1992.tb02549.x

Kartavtsev, Y. F. (1998). Analysis of association between allozyme variability and fitness characteristics in the fry of pink salmon (*Oncorhynchus gorbuscha*) in mass crosses. *Russ. J. Mar. Biol.* 24, 32–35.

Kazyak, D. C., Hilderbrand, R. H., and Holloway, A. E. (2013). Rapid visual assessment to determine sex in brook trout. *N. Am. J. Fish. Manage.* 33, 665–668. doi: 10.1080/02755947.2013.785998

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., et al. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28, 1647–1649. doi: 10.1093/bioinformatics/bts199

Koljonen, M. L. (1986). The enzyme gene variation of ten Finnish rainbow trout strains and the relation between growth rate and mean heterozygosity. *Aquaculture* 57, 253–260. doi: 10.1016/0044-8486(86)90203-6

Körner, O., Vermeirssen, E. L., and Burkhardt-Holm, P. (2007). Reproductive health of brown trout inhabiting Swiss rivers with declining fish catch. *Aquat. Sci.* 69, 26–40. doi: 10.1007/s00027-006-0842-5

Kozfkay, J. R., Wagner, E. J., and Aplanalp, D. (2005). Production of triploid lake trout by means of pressure treatment. *N. Am. J. Aquac.* 67, 93–97. doi: 10.1577/A04-049.1

Leary, R. F., Allendorf, F. W., and Knudsen, K. L. (1984). Superior developmental stability of heterozygotes at enzyme loci in salmonid fishes. *Am. Nat.* 124, 540–551. doi: 10.1086/284293

Leary, R. F., Allendorf, F. W., Knudsen, K. L., and Thorgaard, G. H. (1985). Heterozygosity and developmental stability in gynogenetic diploid and triploid rainbow trout. *Heredity* 54, 219–225. doi: 10.1038/hdy.1985.29

Li, F., Xiang, J., Zhang, X., Zhou, L., Zhang, C., and Wu, C. (2003). Gonad development characteristics and sex ratio in triploid Chinese shrimp (*Fenneropenaeus chinensis*). *Mar. Biotechnol.* 5, 528–535. doi: 10.1007/s10126-002-0103-0

Lindstrom, E. W. (1936). Genetics of polyploidy. *Bot. Rev.* 2, 197–215. doi: 10.1007/BF02872444

Madsen, L., Arnbjerg, J., and Dalsgaard, I. (2000). Spinal deformities in triploid all-female rainbow trout (*Oncorhynchus mykiss*). *B. Eur. Assoc. Fish Path.* 20, 206–208.

Maxime, V. (2008). The physiology of triploid fish: current knowledge and comparisons with diploid fish. *Fish Fish.* 9, 67–78. doi: 10.1111/j.1467-2979.2007.00269.x

McCarthy, I. D., Sanchez, J. A., and Blanco, G. (2003). Allozyme heterozygosity, date of first feeding and life history strategy in Atlantic salmon. *J. Fish Biol.* 62, 341–357. doi: 10.1046/j.1095-8649.2003.00026.x

O'Flynn, F. M., McGeachy, S. A., Friars, G. W., Benfey, T. J., and Bailey, J. K. (1997). Comparisons of cultured triploid and diploid Atlantic salmon (*Salmo salar* L.). *ICES J. Mar. Sci.* 54, 1160–1165. doi: 10.1016/S1054-3139(97)80022-7

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

Okomoda, V. T., Pradeep, J. P., Oladimeji, A. S., Abol-Munafi, A. B., Alabi, K. I., Ikhwanuddin, M., et al. (2020). Effect of electric induced triploidization on sex ratio, growth and gonad histology of red hybrid tilapia. *Aquaculture* 520, 734991. doi: 10.1016/j.aquaculture.2020.734991

Otto, F. J. (1994). High-resolution analysis of nuclear DNA employing the fluorochrome DAPI. *Method. Cell Biol.* 41, 211–217. doi: 10.1016/S0091-679X(08)61719-7

Otto, S. P., and Whitton, J. (2000). Polyploid incidence and evolution. *Annu. Rev. Genet.* 34, 401–437. doi: 10.1146/annurev.genet.34.1.401

Piferrer, F., Beaumont, A., Falguière, J. C., Flajšhans, M., Haffray, P., and Colombo, L. (2009). Polyploid fish and shellfish: production, biology and applications to aquaculture for performance improvement and genetic containment. *Aquaculture* 293, 125–156. doi: 10.1016/j.aquaculture.2009.04.036

Pongtippatee, P., Laburee, K., Thaweethamsewee, P., Hiranphan, R., Asuvapongpatana, S., Weerachatyanukul, W., et al. (2012). Triploid *Penaeus monodon*: sex ratio and growth rate. *Aquaculture* 356, 7–13. doi: 10.1016/j.aquaculture.2012.06.004

Poontawee, K., Werner, C., Müller-Belecke, A., Hörstgen-Schwark, G., and Wicke, M. (2007). Flesh qualities and muscle fiber characteristics in triploid and diploid rainbow trout. *J. Appl. Ichthyol.* 23, 273–275. doi: 10.1111/j.1439-0426.2007.00843.x

Preston, A. C., Taylor, J. F., Craig, B., Bozzolla, P., Penman, D. J., and Migaud, H. (2013). Optimisation of triploidy induction in brown trout (*Salmo trutta* L.). *Aquaculture* 414, 160–166. doi: 10.1016/j.aquaculture.2013.07.034

Quillet, E., Chevassus, B., Blanc, J. M., Krieg, F., and Chourrout, D. (1988). Performances of auto and allotriploids in salmonids I. Survival and growth in fresh water farming. *Aquat. Living Resour.* 1, 29–43. doi: 10.1051/alr:1988004

Sadler, J., Pankhurst, P. M., and King, H. R. (2001). High prevalence of skeletal deformity and reduced gill surface area in triploid Atlantic salmon (*Salmo salar* L.). *Aquaculture* 198, 369–386. doi: 10.1016/S0044-8486(01)00508-7

Schafhauser-Smith, D., and Benfey, T. J. (2001). The reproductive physiology of three age classes of adult female diploid and triploid brook trout (*Salvelinus fontinalis*). *Fish Physiol. Biochem.* 25, 319–333. doi: 10.1023/A:1023285008072

Sellars, M. J., Wood, A. T., Dixon, T. J., Dierens, L. M., and Coman, G. J. (2009). A comparison of heterozygosity, sex ratio and production traits in two classes of triploid *Penaeus* (*Marsupenaeus*) *japonicus* (Kuruma shrimp): Polar Body I vs II triploids. *Aquaculture* 296, 207–212. doi: 10.1016/j.aquaculture.2009.08.030

Sheehan, R. J., Shasteen, S. P., Suresh, A. V., Kapuscinski, A. R., and Seeb, J. E. (1999). Better growth in all-female diploid and triploid rainbow trout. *Trans. Am. Fish. Soc* 128, 491–498. doi: 10.1577/1548-8659(1999)128<0491:BGIAFD>2.0.CO;2

Solar, I. I., Donaldson, E. M., and Hunter, G. A. (1984). Induction of triploidy in rainbow trout (*Salmo gairdneri* Richardson) by heat shock, and investigation of early growth. *Aquaculture* 42, 57–67. doi: 10.1016/0044-8486(84)90313-2

Thorgaard, G. H. (1986). Ploidy manipulation and performance. *Aquaculture* 57, 57–64. doi: 10.1016/0044-8486(86)90180-8

Uzunova, E. (2004). Survival, growth and maturation of triploid brook trout (*Salvelinus fontinalis*) induced by heat shock. *Biotechnol. Biotechnol. Equip.* 18, 82–90. doi: 10.1080/13102818.2004.10819235

Vindelov, L. L., Christensen, I. J., Keiding, N., Spang-Thomsen, M., and Nissen, N. I. (1983). Long-term storage of samples for flow cytometric DNA analysis. *Cytometry A* 3, 317–322. doi: 10.1002/cyto.990030502

Wang, Z., Guo, X., Allen, S. K., and Wang, R. (2002). Heterozygosity and body size in triploid Pacific oysters, *Crassostrea gigas* Thunberg, produced from meiosis II inhibition and tetraploids. *Aquaculture* 204, 337–348. doi: 10.1016/S0044-8486(01)00845-6

Wang, H., Li, F., Xiang, J., Zhang, C., and Yu, K. (2008). Microsatellite-centromere distances and microsatellite diversity in different ploidy classes of Chinese shrimp (*Fenneropenaeus Chinensis*). *Genetica* 132, 43–50. doi: 10.1007/s10709-007-9147-4

Werner, C., Poontawee, K., Mueller-Belecke, A., Hoerstgen-Schwark, G., and Wicke, M. (2008). Flesh characteristics of pan-size triploid and diploid rainbow trout (*Oncorhynchus mykiss*) reared in a commercial fish farm. *Arch. Anim. Breed.* 51, 71–83. doi: 10.5194/aab-51-71-2008

Withler, R. E., Beacham, T. D., Solar, I. I., and Donaldson, E. M. (1995). Freshwater growth, smolting, and marine survival and growth of diploid and triploid coho salmon (*Oncorhynchus kisutch*). *Aquaculture* 136, 91–107. doi: 10.1016/0044-8486(95)01036-X