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REGULAR ARTICLE

Effect of heterozygosity, ploidy and incubation temperature on post-cranial axial skeletal meristics and deformities in Atlantic salmon (*Salmo salar*)

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Abstract

The teleostean post-cranial axial skeleton is a highly specialized structure for an aquatic mode of life. However, there is limited knowledge regarding parental contributions, early-life environmental impacts on its meristic variation and if reduced heterozygosity challenges its development. To address this, the present study used isogenic homozygous and heterozygous lines of Atlantic salmon (*Salmo salar*) combined with ploidy manipulation (triploidization) to manipulate parental contributions, and incubation temperature (4 vs. 8°C) as an early-life variable, and reared the fish to ~150 g for a detailed radiological examination. Genetically identical fish incubated at 4°C, but not 8°C, segregated into two size modes (upper/lower), which differed in dorsal and tail fin lepidotrich counts as well as anal-fin pterygiophore counts. Incubation temperature did not impact on vertebrae counts, whereas 8°C incubation produced more supraneurals than 4°C incubation. After 8°C incubation, homozygous diploids (100% maternal chromosomes) and heterozygous triploids (67% maternal chromosomes) developed lower total vertebrae and dorsal- and anal-fin pterygiophore counts than heterozygous diploids (50% maternal chromosomes). For tail fin lepidotrichs, the same groups showed the following pattern: diploid heterozygous > triploid heterozygous > diploid homozygous. Homozygous diploids developed a high level of complete fusions in the vertebral column. The result of the present study indicates that the ability to enter different growth modes is dependent on embryo incubation temperature and may be controlled by epigenetic mechanisms. Further, the results show a strong maternal dosage effect on tail fin lepidotrich counts, whereas for other post-cranial skeletal parts, the presence of extra maternal chromosomes seems to overrule the paternal contribution. The findings may reflect

evolutionary adaptations for the shaping of offspring phenotypes. Such mechanisms would impact on important fitness-related traits, such as swimming ability and fecundity, which are relevant for conservation and evolutionary biology and ecological and aquaculture sciences. Vertebral deformities developing in homozygous fish seem to be supported by active repair mechanisms, which may reflect an organism's ability to reduce the cost of inbreeding.

KEYWORDS
Atlantic salmon, deformities, incubation temperature, meristic, ploidy, vertebra

1 | INTRODUCTION

The post-cranial axial skeleton of teleost fish is made up of the vertebral column, supraneurals and median (unpaired) fins (dorsal, anal and tail fins). The first structure to form is the notochord (Witten & Hall, 2022) followed by the development of the skeletal parts (Gwyn, 1940). In Atlantic salmon *Salmo salar* L. 1758, the notochord is present from 110 degree-days (d°) post-fertilization, and the first post-cranial axial skeletal elements to form are the lepidotrichia of the dorsal and anal fins, along with the hypurals of the caudal fin (Kryvi et al., 2017). Thereafter, the remaining skeletal parts are formed in a successive manner, and all parts are present at 750 d° (Kryvi et al., 2017), corresponding with the time point when the alevin leaves the protective gravel and swims up to the surface to fill the swim bladder and start exogenous feeding (Crisp, 1988; Tait, 1960).

There is some variation in the number of elements that form within each post-cranial axial skeletal part in Atlantic salmon (Mottley, 1937; Sanford, 2000), but possible functional implications are unknown. There are, however, studies on other teleosts showing that this type of meristic variation may impact on important fitness-related traits, such as fecundity (*Rutilus rutilus* L. 1758) (Komova, 2023), predator survival (*Gasterosteus aculeatus* L. 1758) (Swain, 1992a, 1992b), body shape (*Galaxias plate* Steindachner, 1898) (Barriga et al., 2013) and escape performance (*Salvelinus alpinus* L. 1758) (Campbell et al., 2021).

Several factors may influence meristic variation in the post-cranial axial skeleton in teleosts. The water temperature during embryonic development has long been recognized as an important factor (Hubbs, 1922; Jordan, 1891). For instance, in salmonids, the number of vertebrae is inversely related to rate of development, so that it is increased at lower egg incubation temperatures (Beacham & Murray, 1986; Garside, 1966; Kwain, 1975; Lindsey et al., 1984). Recently, De Clercq et al. (2018) found no difference in total vertebrae counts between Chinook salmon (*Oncorhynchus tshawytscha*, Walbaum 1792) incubated at either 8 or 12°C, whereas the number of vertebrae in specific vertebral regions was different between the two temperatures. Similarly, temperature-induced regional differences in vertebrae counts have also been reported in *Astyanax mexicanus* De Filippi, 1853 (Reyes Corral & Aguirre, 2019). Fraser et al. (2015) found higher total vertebrae counts in Atlantic salmon incubated at 6 and 8°C compared to those incubated at 10°C, but no difference was observed between 6 and 8°C. That study did not investigate possible regional differences within the vertebral column, and it is unknown if a further lowering of the incubation temperature and development rate to a more natural level would produce even higher vertebrae counts. Currently, Atlantic salmon farmers typically use a stable temperature of 8°C to speed up development, which is far outside the natural thermal range at this life stage. In nature, wild Atlantic salmon spawn in November and December at low temperature, with developing embryos experiencing a mean of 4.5°C (minimum 2.2°C and maximum 8.2°C) before hatching (Jonsson & Jonsson, 2018).

Another factor that may impact on post-cranial axial skeletal meristics is the genetic make-up of the fish. As an example, and in relation to the earlier introduced factor temperature, Ali and Lindsey (1974) studied heritable and temperature-induced post-cranial axial skeletal meristics in medaka (*Oryzias latipes* Temminck & Schlegel, 1846) and found that the heritable variation within different temperatures equalled the phenotypic variation between them. Likewise, between family variation in total vertebrae counts after equal egg incubation temperatures has been reported in several salmonid species, such as masu salmon (*Oncorhynchus masou* Brevoort, 1856) (Ando et al., 2008), chum salmon (*Oncorhynchus keta* Walbaum 1792) (Ando et al., 2011), rainbow trout (*Oncorhynchus mykiss* Walbaum 1792) (Mottley, 1937) and brown trout (*Salmo trutta* L. 1758) (Schmidt, 1919). The induction of triploidy has long been used in salmonid aquaculture to produce functionally sterile fish to mitigate problems associated with reduced meat quality by early sexual maturation or genetic introgression caused by cross-breeding between escaped farmed fish and wild fish (Benfey, 2001; Benfey, 2016; Fraser et al., 2012). The genetic make-up of triploids is altered by the extra maternal chromosome set (Glover et al., 2015), and the total vertebrae number has been reported to be lower in triploid compared to diploid Atlantic salmon (*S. salar* L. 1758) (Fraser et al., 2015).

By eliminating the genetic component of phenotypic variation, clonal fish lines can be used to refine studies on possible temperature, inheritance and ploidy-induced meristic variation in the post-cranial axial skeleton (Nakajima et al., 1996; Taniguchi et al., 1996). Recently, protocols have been established to produce both homozygous double haploid female (Hansen et al., 2020) and male (Fjelldal et al., 2020) lines of Atlantic salmon, allowing for the production of isogenic heterozygous hybrid lines. Comparing diploid homozygous clones with

their diploid and triploid heterozygous half-siblings is an unexplored approach to widen the knowledge about the parental contributions to meristic variation, and how the additional maternal chromosome set of triploids may interact.

Ploidy and incubation temperature may also affect vertebral deformity development in fish, not only meristic variation. Deformity assessment is also relevant for studies involving clonal fish, as deformity rates increase following inbreeding (Kerniske et al., 2021; Winemiller & Taylor, 1982). In triploid Atlantic salmon, the predominant location for deformities is at vertebra number 24 in the posterior part of the abdominal region (Fjelldal & Hansen, 2010; Sankar, Kryvi, et al., 2024), but the location of possible inbreeding/clonal specific vertebral deformities is, however, unexplored. With regard to incubation temperature, the industry standard temperature of 8°C for diploid Atlantic salmon does not support the normal development of the vertebral column in triploids, but lowering the incubation temperature to 6°C helps alleviate issues (Fraser et al., 2015). Increasing the amount of inorganic phosphorus to the diet also reduces the risk of deformity development further (Fjelldal et al., 2016), with first feeding period being most critical (Sambraus et al., 2020).

Different methods have been used for region-specific vertebrae counts in teleosts. In salmonids, regional-specific vertebral counts have been determined either by steaming and removal of the vertebral column (masu salmon, Ando et al., 2008) or histology (Chinook salmon, De Clercq et al., 2017). These methods are time-consuming, reducing their practicality. However, Sankar, Kryvi, et al. (2024) recently developed a new regionalization method for the vertebral column in salmonids based on specific radiographic hallmarks. This method allows accurate vertebrae counts of the post-cranial, abdominal, transitional, caudal and ural regions of the vertebral column in individual fish.

The aim of the present study was to examine the (i) impact of 4°C (natural range) versus 8°C (industry standard) incubation temperature on meristic variation, (ii) maternal versus paternal impact and the effect of doubling the maternal chromosome contribution by triploidy on meristic variation, (iii) possible differences in vertebral deformity phenotype between triploid and diploid clonal Atlantic salmon. Homozygous and heterozygous clonal lines of Atlantic salmon were used. This study is important for understanding the shaping of offspring phenotypes in the concept of evolutionary adaptations and local adaptions, as well as the organism's ability to counteract possible skeletal defects caused by inbreeding.

2 | MATERIALS AND METHODS

Figure 1 summarizes the experimental set-up. In brief, this study used eggs from one double haploid homozygous XX female that were either fertilized with ultraviolet (UV)-irradiated sperm (to produce an isogenic homozygous line) or cryopreserved sperm from a double haploid homozygous YY super male (to produce an isogenic heterozygous line). Thereafter, all the eggs fertilized with UV-irradiated sperm and a half of the eggs fertilized with YY sperm were subjected to hydrostatic pressure to produce either all-female diploid homozygous clones (UV-irradiated sperm) or all-male triploid heterozygous clones (YY sperm). The remaining half of the eggs that were fertilized with YY sperm were not subjected to pressure shock and produced all-male diploid heterozygous clones. During egg incubation, all groups were incubated at 8°C, but some of the diploid heterozygous fish were also incubated at 4°C to make additional groups. Triploids were incubated at 8°C and first fed with a normal diploid diet to promote deformities to enable a comparison with possible clonal line-specific deformities. After this, the fish were reared to ~150 g to allow a detailed radiological examination.

Remark on confounding factors: There is no triploid homozygous group, and homozygous fish are all-female, whereas heterozygous fish are all-male. These were the only options with the currently available homozygous XX and YY founders.

2.1 | Experimental set-up

Eggs from a doubled haploid Atlantic salmon female were divided into four batches of approximately 2000 eggs in each batch. One egg batch was fertilized with diluted (1:40) and UV-irradiated sperm and subject to a pressure shock for 5 min at 655 bar at 300-min degrees (min°C) post-fertilization (second meiotic division), resulting in the production of a diploid homozygous all-female group (XX) (Hansen et al., 2020). This group was incubated at 8°C (DHo 8°C). Another three batches of eggs were fertilized with cryopreserved milt from a doubled haploid super male (YY) (Fjelldal et al., 2020), resulting in the production of heterozygous all-male hybrids (XY), which were split into three separate groups: Two groups were incubated at either 4°C (DHt 4°C) or 8°C (DHt 8°C) as diploid heterozygous groups. The remaining one group was subject to a pressure shock for 5 min at 655 bar at 300 min°C (second meiotic division) post-fertilization for the production of a triploid heterozygous all-male (XXY) group. This group was incubated at 8°C (THt 8°C). All groups were fed with commercial diets (Skretting AS).

During freshwater growth, it was obvious that the DHt 4°C group developed a distinct bimodal size distribution, which is normal for outbred Atlantic salmon reared at a low intensity (Thorpe, 1977). Therefore, the treatment group was further subdivided into a large (DHtL 4°C) and small (DHtS 4°C) group to test for possible differences between phenotypes (Figure 2). The fish were first fed under continuous light and stable 12°C (heated water), where time of first feeding was delayed by 3 months in the fish incubated at 4°C. When the natural freshwater temperature reached 12°C in late June, natural temperature was maintained. After this, the fish were reared under continuous light and natural temperature in fresh water until ~150 g body weight, which allowed a detailed radiological examination for post-cranial axial skeletal meristics and vertebral deformities.

2.2 | Sampling

All the five groups (DHo 8°C, DHt 8°C, THt 8°C, DHtL 4°C and DHtS 4°C) were sampled for 16 fish and measured for fork length and body

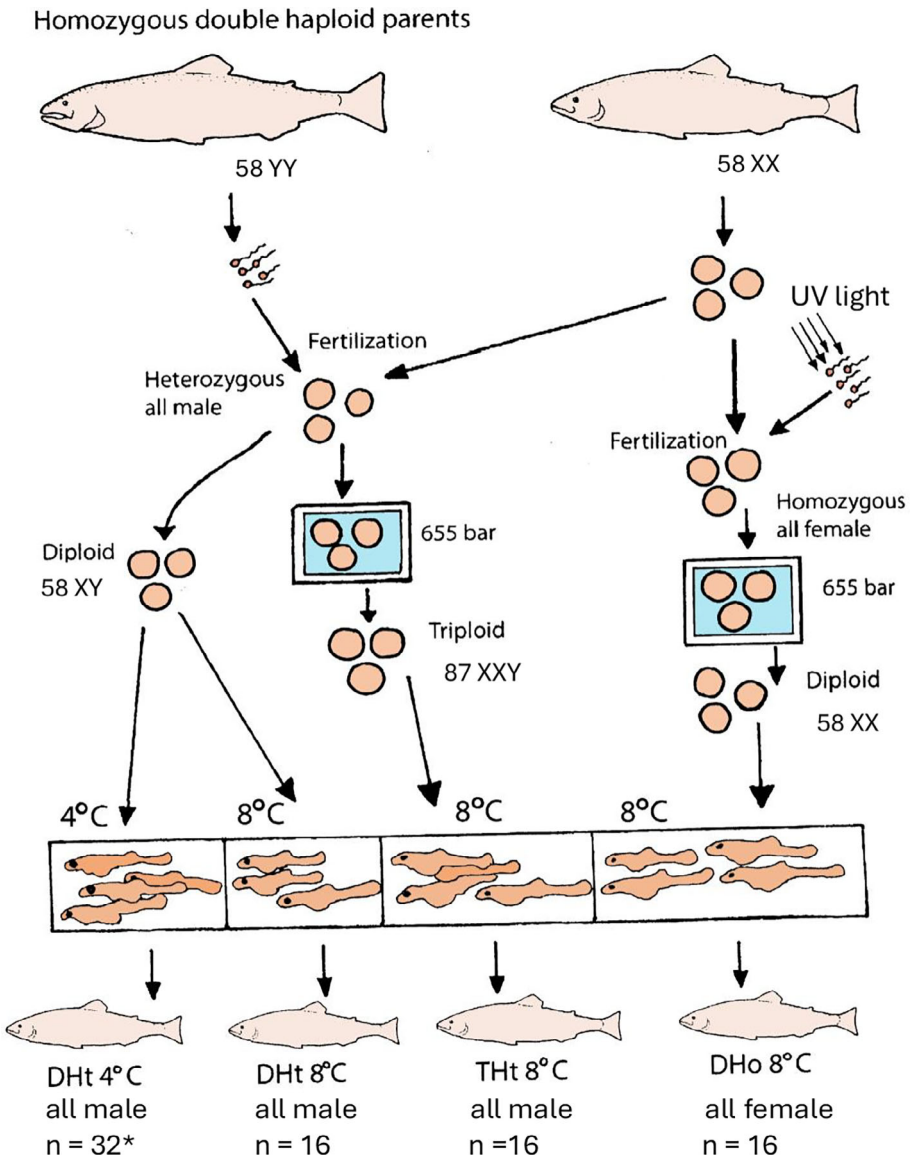

FIGURE 1 Schematic drawing of the experimental set-up. Homozygous and heterozygous offspring were created by fertilizing eggs from a double haploid homozygous female with either ultraviolet (UV)-irradiated sperm (all-female homozygous offspring) or fertile sperm from a double haploid homozygous YY supermale (all-male heterozygous offspring). Hydrostatic pressure (655 bar) was applied to produce heterozygous triploids and homozygous diploids. Different incubation temperatures (4 vs. 8°C) were applied to produce the following groups: diploid heterozygous 4°C (DHt 4°C), diploid heterozygous 8°C (DHt 8°C), triploid heterozygous 8°C (THt 8°C) and diploid homozygous 8°C (DHo 8°C). * The DHt 4°C group showed a bimodal growth pattern, resulting in two subgroups: diploid heterozygous small 4°C (DHtS 4°C, $n = 16$) and diploid heterozygous large 4°C (DHtL 4°C, $n = 16$). Diploid Atlantic salmon have 58 chromosomes (Grammeltvedt, 1975), which is indicated in parents and offspring.

weight and radiographed to analyse post-cranial axial skeletal meristic counts and vertebral deformities. Additionally, adipose fins were clipped for DNA microsatellite analyses to confirm clonal, ploidy and zygosity status.

2.3 | Dissection, radiology, meristic counts and vertebral deformities

The vertebral column and fins from each specimen were dissected and radiographed. First the complete – lepidotrich and pterygiophores – dorsal and anal fins were dissected free from the vertebral column and radiographed (Amos et al., 1963). Then the vertebral column was radiographed with tail fin lepidotrichs. Radiographs were taken using a direct radiology system (resolution: 4.0 lp/mm, CANON CXDI410C Wireless, CANON INC, Kawasaki, Japan) and portable X-ray unit (portable X-ray unit Hiray Plus, Model Porta 100 HF, Job Corporation, Japan) with 40 kV and 4 mA input at 88-cm distance. The digital TIF images were analysed for region-specific vertebrae counts, and supraneural, fin lepidotrich and pterygiophores counts. Vertebral column regions were identified based on radiographic hallmarks described by Sankar, Kryvi, et al. (2024), which allowed for the region-specific vertebrae counts within the post-cranial, abdominal, transitional, caudal and ural regions of the vertebral column. The vertebral deformities observed in each vertebral region were classified based on the phenotypes described in Witten et al. (2009).

2.4 | Confirmation of isogenic, zygosity and ploidy verification

Microsatellite DNA analyses were performed on sampled adipose fins to confirm isogenic, zygosity and ploidy status. Total DNA was extracted from fin-clips using a commercially available extraction kit (Qiagen DNeasy 96 Blood & Tissue Kit). Nineteen microsatellite DNA markers were genotyped using standard isolation and amplification

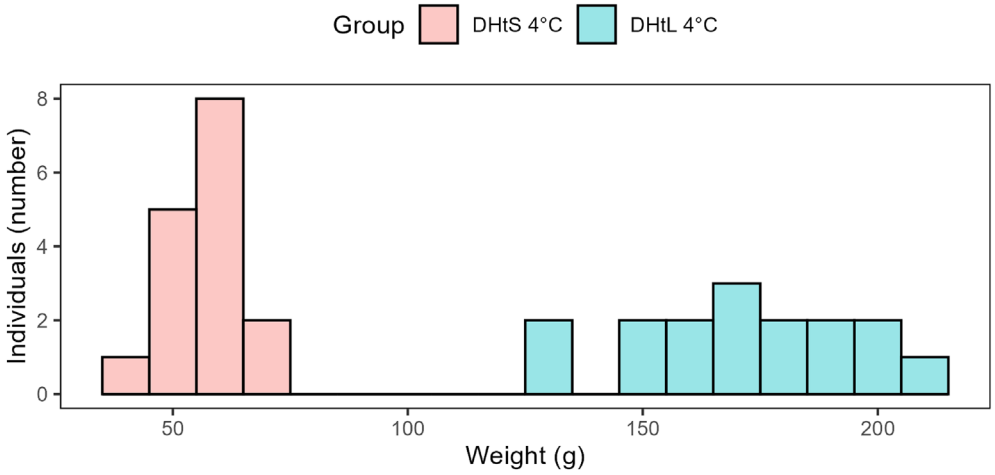

FIGURE 2 Histogram showing the weight distributions in the DHtS 4°C and DHtL 4°C groups.

protocols previously described in detail (Glover et al., 2015; Wennevik et al., 2019).

Individuals were classified as heterozygous or homozygous based on the presence or absence of two alleles for the various microsatellites assessed, respectively. Triploidy could be inferred from a higher amplification in those alleles that were doubled (Delaval et al., 2024).

2.5 | Calculations and statistical analysis

The condition factor (CF) was calculated as $CF = 100 \times BW/L^3$, where BW was the body weight (g), and L was the fork length (cm).

The data were transferred to the R Statistical software (version 4.0.4, R Core Team, 2021) for all analyses. The packages 'MASS' (Venables & Ripley, 2002), 'emmeans' (Lenth, 2021) and 'ggplot2' (Wickham, 2016) were used for analysis and graphical presentation. Throughout, model diagnostics were assessed via q-q plots and standardized versus predicted residual plots. The raw data (Sankar JFB.RData) and the R script used for the analysis (Sanker JFB.R) are available in the supplementary material.

To assess for genetic effects, either a proportional odds logistic regression (POLR) model or a generalized linear model (GLM) with a binomial distribution (when the endpoint only had two outcomes) was fitted to the total vertebrae count and fin lepidotrichs and pterygiophores data from the DHt 8°C, DH 8°C and THt 8°C groups (see Table 1). Group differences on length, weight and condition were assessed by GLMs, the prevalence of deformed fish was assessed by a GLM within a binomial distribution (deformed; yes/no) and the number of deformed vertebrae per deformed fish was assessed by GLM. To assess for incubation temperature and size phenotype effects, the same approach was used for the data from the DHt 8°C, DHtL 4°C and DHtS 4°C groups albeit the Kruskal–Wallis test was used to assess the number of deformed vertebrae per deformed fish due to the data being heavily right skewed. To assess for differences in the prevalence of deformity phenotypes, we used POLR models. To aid the analysis, the vertebral deformity phenotypes were grouped into compressions (phenotypes 2 and 5), unstable fusions (phenotypes 6 and 8) and stable fusions (phenotype 7), resulting in three levels.

3 | RESULTS

The microsatellite analyses confirmed that all sampled fish were isogenic and had the correct ploidy and zygosity in accordance with their group (Hvas et al., 2025 under review). Specifically, all individuals within groups had the same alleles (DNA repetitions), and heterozygous fish had two different alleles, whereas homozygous had only one allele for each marker. Meanwhile triploidy was inferred from higher amplification of the doubled maternal allele (Delaval et al., 2024).

3.1 | Incubation at 4 versus 8°C

There was a significant difference in the number of dorsal and tail fin lepidotrichs and anal-fin pterygiophores between the DHtL 4°C and DHtS 4°C groups (Figure 3; Table 1). As such, these were considered as separate groups in the comparison for the incubation temperature effect on meristic characters (DHtS 4°C vs. DHtL 4°C vs. DHt 8°C). Mean length, weight and condition factor are shown in Table 1. The DHtS 4°C group had significantly lower weight and length than the DHtL 4°C and DHt 8°C groups, and significantly higher condition factor than the DHtL 4°C group (Table 1). Figure 2 is a histogram showing the weight distributions of the DHtS 4°C and DHtL 4°C groups.

The DHt 8°C group had significantly more supraneurals and tail fin lepidotrichs compared to the DHtS 4°C, but not the DHtL 4°C group, whereas for the anal fin, the DHt 8°C group had significantly less pterygiophores than the DHtL 4°C, but not the DHtS 4°C group (Figure 3; Table 1). The DHt 8°C group was not different from the 4°C groups for dorsal-fin lepidotrich counts, whereas the DHtS 4°C group had significantly higher counts than the DHtL 4°C group (Figure 3; Table 1). The frequency and severity of vertebral deformities were not significantly different between the groups (Table 1;

TABLE 1 Meristic counts, deformity levels and body size metrics from diploid (D) and triploid (T), homozygous (Ho) or heterozygous (Ht) Atlantic salmon reared at either 4 or 8°C.

Region	Model	*p*-Value	Range	DHt 8°C	DHo 8°C	THt 8°C	Model	*p*-Value	Range	DHt 8°C	DHtL 4°C	DHtS 4°C
Total	polr	**<0.001**	57–61	60.6 (60.3–60.9)^{a}	59.8 (59.5–60.2)^{b}	59.8 (59.4–60.2)^{b}	polr	0.408	59–61	60.6 (60.3–60.9)	60.6 (60.3–60.9)	60.4 (60.1–60.6)
Abdomen	polr	0.532	24–27	25.0 (24.7–25.3)	25.0 (24.7–25.4)	25.3 (24.9–25.7)	polr	0.551	24–26	24.9 (24.7–25.2)	25.1 (24.8–25.3)	24.9 (24.6–25.1)
Transitional	polr	0.150	6–11	9.3 (9.0–9.7)	9.4 (9.0–9.7)	8.8 (8.4–9.3)	polr	0.116	8–11	9.4 (9.1–9.7)	9.5 (9.2–9.7)	9.8 (9.5–10.1)
Caudal	polr	**0.023**	16–19	17.9 (17.6–18.3)^{a}	17.3 (17.0–17.7)^{ab}	17.2 (16.9–17.6)^{b}	polr	0.088	16–19	17.9 (17.6–18.3)	17.7 (17.4–18.1)	17.4 (17.1–17.7)
Ural	polr	0.407	5–7	6.3 (6.1–6.6)	6.2 (6.0–6.4)	6.4 (6.2–6.7)	glm	0.747	6–7	6.3 (6.1–6.5)	6.4 (6.1–6.6)	6.3 (6.0–6.5)
Dorsal-fin lepidotrichs	glm	**<0.001**	13–14	13.4 (13.1–13.7)^{b}	13.2 (13.0–13.4)^{b}	13.9 (13.814.0)^{a}	glm	**0.004**	13–14	13.4 (13.1–13.7)^{ab}	13.1 (13.3–13.9)^{b}	13.6 (13.3–13.9)^{a}
Dorsal-fin pterygiophores	polr	**<0.001**	11–13	12.6 (12.3–12.9)^{a}	11.9 (11.5–12.2)^{b}	11.7 (11.4–12.0)^{b}	polr	0.128	11–13	12.6 (12.3–12.9)	12.3 (12.0–12.5)	12.6 (12.4–12.9)
Supraneurals	polr	0.063	11–15	13.5 (13.1–13.9)	13.0 (12.6–13.4)	12.9 (12.5–13.2)	polr	**0.014**	11–15	13.5 (13.1–13.9)^{a}	12.7 (12.3–13.2)^{ab}	12.6 (12.2–13.0)^{b}
Anal-fin lepidotrichs	polr	0.218	7–11	10.0 (9.8–10.3)	9.6 (9.3–10.0)	9.9 (9.6–10.2)	polr	0.133	9–11	10.1 (9.9–10.2)	10.2 (10.0–10.4)	9.9 (9.8–10.1)
Anal-fin pterygiophores	polr	**<0.001**	6–10	8.9 (8.7–9.2)^{a}	7.9 (7.5–8.4)^{b}	8.3 (8.0–8.7)^{b}	polr	**<0.001**	8–10	8.9 (8.8–9.1)^{b}	9.4 (9.2–9.7)^{a}	8.9 (8.8–9.1)^{b}
Tail fin lepidotrichs	polr	**0.018**	36–42	41.6 (41.3–42.0)^{a}	40.0 (40.0–41.4)^{b}	40.8 (40.0–41.5)^{ab}	polr	**<0.001**	36–42	41.7 (41.3–42.0)^{a}	41.5 (41.0–41.9)^{a}	39.1 (38.5–39.6)^{b}
Deformed	glm	**<0.001**		12.5 (3.1–38.6)^{b}	62.5 (37.7–82.1)^{a}	75.0 (49.2–90.3)^{a}	glm	0.855		12.5 (3.1–38.6)	18.8 (6.2–44.7)	18.8 (6.2–44.7)
Deformed vertebra per deformed fish	glm	0.834		7.5 (0.3–14.7)	9.3 (6.1–12.5)	8.3 (5.3–11.2)	KW	0.899		7.5 (5.8–9.3)	4.0 (0.8–7.3)	7.0 (5.0–9.0)
Deformity phenotypes	polr	**<0.001**	3*	0.0 (−1.0–1.0)^{b}	1.8 (1.3–2.3)^{a}	−0.3 (−0.7–0.0)^{b}	polr	**0.001**	3*	0.0 (−0.9–0.9)^{b}	1.6 (0.8–2.5)^{a}	−0.5 (−1.4–0.3)^{b}
Weight	glm	0.951		170 (158–181)	169 (158–181)	167 (156–179)	glm	**<0.001**		170 (159-181)^{a}	171 (160-182)^{a}	57 (53-61)^{b}
Length	glm	0.719		24.2 (23.6–24.8)	24.1 (23.5–24.6)	23.9 (23.3–24.5)	glm	**<0.001**		24.2 (23.6–24.8)^{b}	25.2 (24.6–25.8)^{a}	17.0 (16.6–17.4)^{c}
Condition	glm	0.220		1.20 (1.17–1.22)	1.22 (1.19–1.24)	1.23 (1.20–1.26)	glm	**<0.001**		1.20 (1.17–1.22)^{a}	1.07 (1.04–1.09)^{b}	1.16 (1.14–1.19)^{a}

Note: The table includes the models used (polr = ordered logistical regression, glm = generalized linear model), the model output, the range of the raw data (meristic counts only) and the least-square means (with 95% confidence intervals) for each endpoint. Different superscript letters indicate significant differences between groups within a row. The analysis consists of two parts, only comparing the 8°C groups to one another (left-hand side) and only comparing the diploid heterozygous groups to one another (righthand side). Values in bold indicate significant differences ($p < 0.05$).

*3 levels; 1 = compression (phenotypes 2 and 5); 2 = unstable fusion (phenotypes 6 and 8); 3 = stable fusion (phenotype 7).

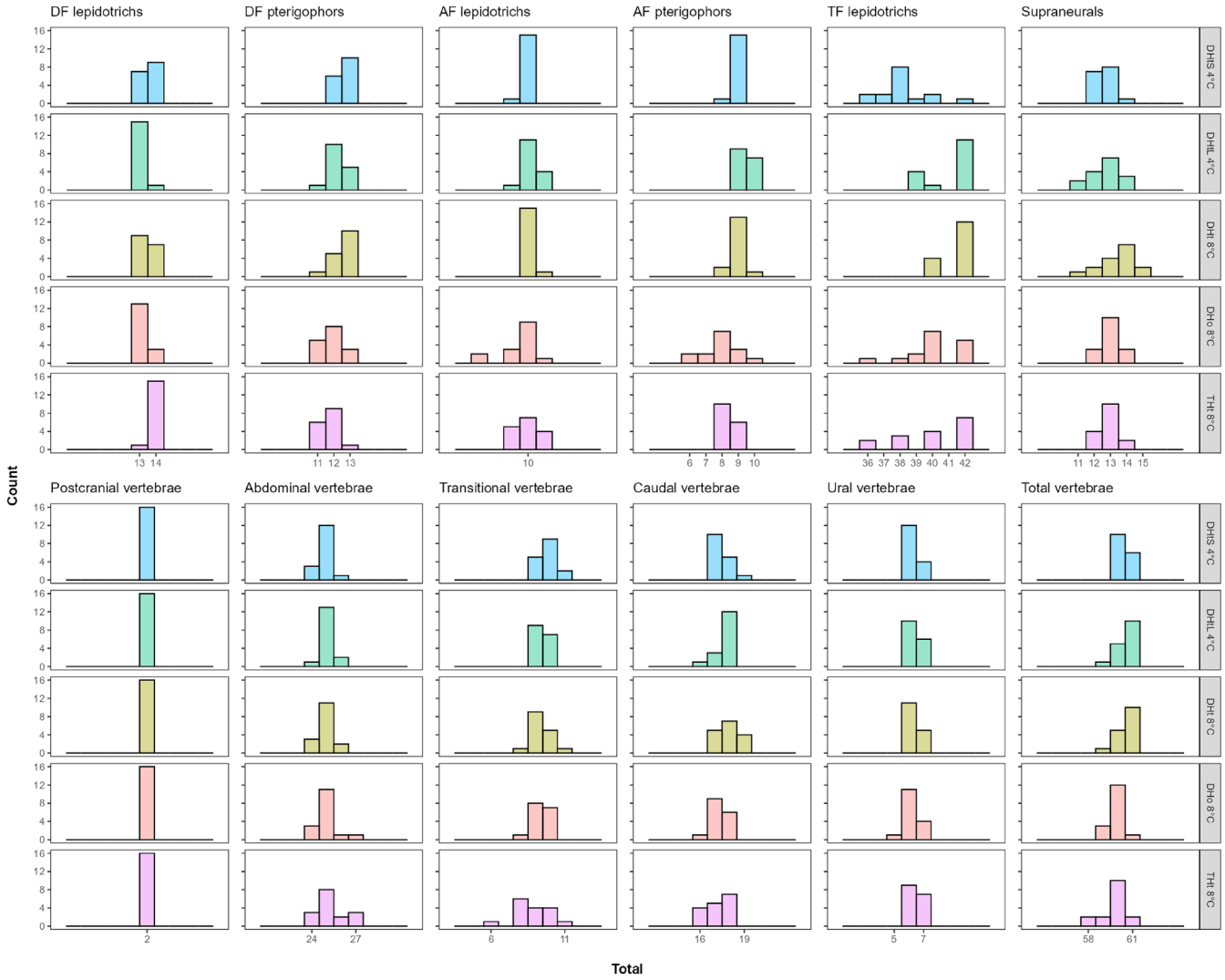

FIGURE 3 Histograms of fin ray (dorsal, anal and tail fins), supraneural and regional and total vertebrae meristic counts. AF, anal fin; DF, dorsal fin; TF, tail fin.

Figure 4), although there was a significant group effect on the prevalence of deformity phenotypes with the DHtL 4°C having more stable fusions (phenotype 7), whereas the DHtS 4°C and DHt 8°C groups had more compressions (phenotypes 2 and 5) and unstable fusions (phenotypes 6 and 8).

3.2 | Different groups incubated at 8°C

The DHo 8°C, DHt 8°C and THt 8°C groups all developed a uniform distribution in size, and there were no differences in length, weight or condition factor between them (Table 1). There was a significant group effect on total and caudal region vertebrae counts, and the DHo 8°C and THt 8°C groups had significantly lower total vertebrae counts compared to the DHt 8°C group (Table 1), which was mainly attributed to more caudal vertebrae in the latter (Figure 3). Similarly, for dorsal- and anal-fin pterygiophores, there was a significant group effect, as the DHo 8°C and THt 8°C groups had significantly lower counts compared to the DHt 8°C group (Table 1; Figure 3). There was also a significant group effect on dorsal and tail fin lepidotrich counts, and the THt 8°C group had significantly higher counts than the DHt 8°C and DHo 8°C groups for the dorsal fin, whereas the DHt 8°C group had significantly higher count than the DHo 8°C group for the tail fin, with the THt 8°C group displaying intermediate counts (Table 1; Figure 3).

The frequency of fish with vertebral deformities was, however, significantly higher in the DHo and THt groups compared to the DHt group (Table 1; Figure 4). The deformity severity was equal between the groups, and the number of deformed vertebrae among affected fish ranged between 7.5 and 9.3 (Table 1; Figure 4). However, the deformity location and phenotype prevalence were different between the DHo and THt groups. The predominant location for deformities was in the mid part of the abdominal region, with vertebra number 16 most often affected in the DHo group, whereas it was at the border between the abdominal and transitional regions, with vertebra number 27 most often affected in the THt group (Figure 4). In

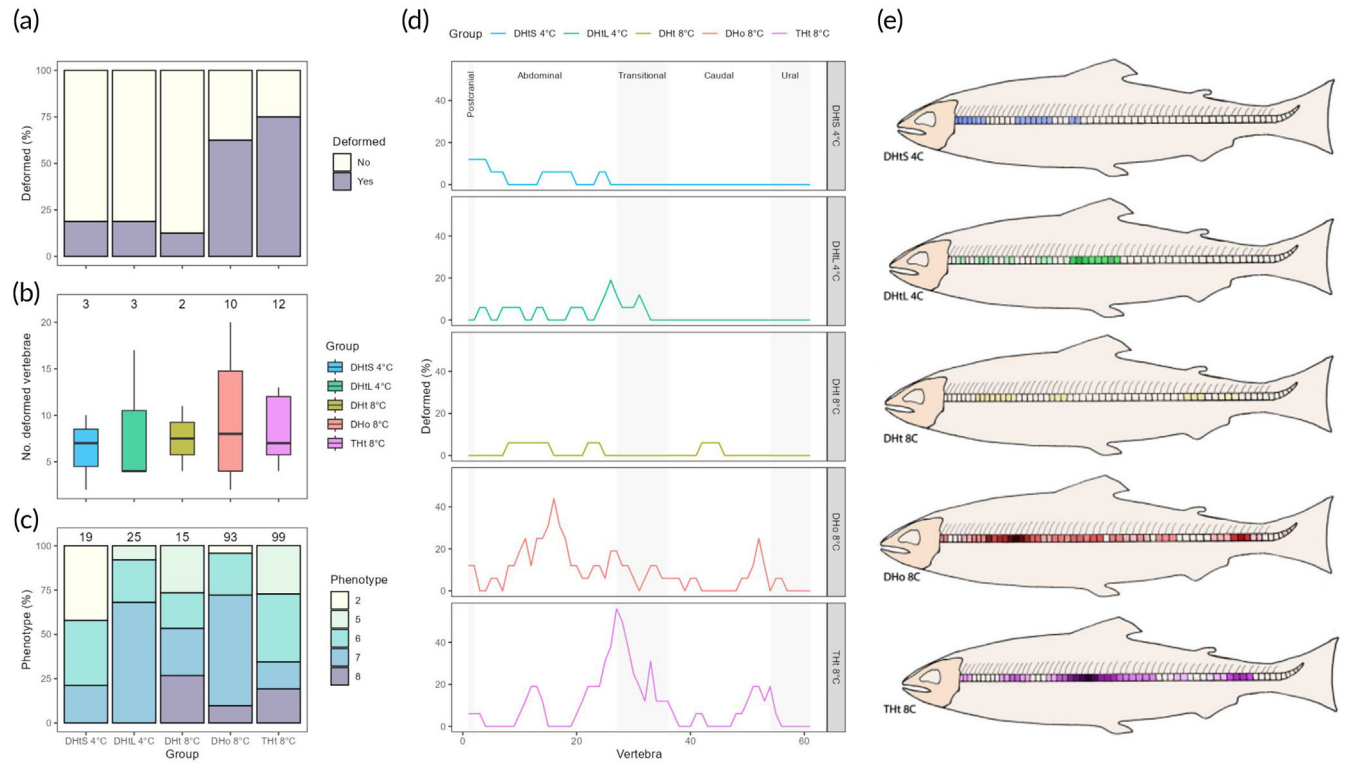

FIGURE 4 (a) Occurrence, (b) severity, (c) type and (d and e) location of vertebral deformities. 'e' shows schematic drawings illustrating vertebral deformity location and level of occurrence. White vertebrae illustrate no deformities; increasing colour intensity illustrates increasing deformity rate. Vertebral deformity types: 2 = homogenous compression, 5 = one-sided compression, 6 = compression and fusion, 7 = complete fusion, 8 = fusion centre.

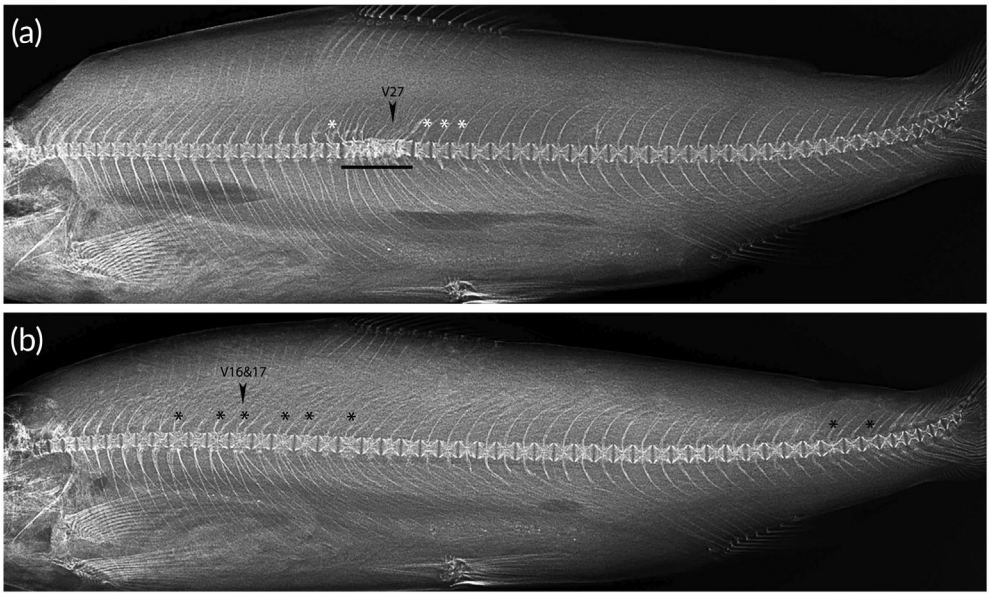

FIGURE 5 Lateral radiography of vertebral deformity type and location. (a) Specimen from the THt 8°C group. (b) Specimen from the DHo 8°C group. Vertebra numbers are indicated in the figure. White asterisk indicates one-sided compressions (type 5). Black bare indicates a fusion centre (type 8) containing eight vertebrae (numbers 21–28). Black asterisk indicates complete fusion (type 7).

addition, the THt group had a smaller deformity peak in the same area as the main peak in the DHo group, and both groups had a smaller deformity peak in the posterior part of the caudal region (Figure 4). Within the centre of the main deformity peaks (DHo V15–17 vs. THt V26–28), the distribution of deformity types (T) were as follows: DHo (T2 < T8 < T6 < T7) (4.3%, 9.7%, 23.7%, 62.3%) and THt (T7 < T8 < T5 < T6) (15.2%, 19.2%, 27.2%, 38.4%). Overall, compressions (phenotypes 2 and 5) and unstable fusions (phenotypes 6 and 8)

dominated both the DHt and THt groups, whereas stable fusions (phenotype 7) were the dominant deformity type in the DHo group (Table 1; Figures 4 and 5).

4 | DISCUSSION

The results of the present study show several effects on meristic counts within the post-cranial axial skeleton, both between phenotypes within family and between incubation temperatures and ploidies. In addition, the vertebral column deformity phenotypes were different in the triploid heterozygous compared to diploid homozygous lines, both with regard to location and type of deformity.

4.1 | Both phenotype and incubation temperature affected meristic counts

Diploid isogenic hybrids (DHt) incubated at 4°C developed a distinct bimodal distribution in size, which was not observed following 8°C incubation. This could be seen as surprising, as the modes contained genetically identical isogenic hybrids. Development of bimodality is the norm in wild Atlantic salmon (Thorpe, 1977), enabling a diversity in smolting and maturity age (Simpson & Thorpe, 1976; Thorpe et al., 1982), and may indeed represent a safeguard for the reproductive success of wild populations. The genetic make-up of the fish and environmental factors, such as proportion of available cover, water velocity (reviewed in Thorpe et al., 1982) and photoperiod (Skilbrei, 1991), may influence the level of segregation into size modes in Atlantic salmon. The development of size modes appears at the parr stage, at a 'threshold' length of 7.5–8 cm (Skilbrei, 1988), below which the 'light-pituitary' axis (Komourdjian et al., 1976) is not functional (Nordgarden et al., 2007). The current study also shows that incubation temperature and developmental speed impact on the likelihood of segregation into size modes in Atlantic salmon.

It is also possible that the temperature during the embryonic development may regulate epigenetic mechanisms that affect the later-developing life-history traits in fish (Jonsson & Jonsson, 2014, 2019). The 4°C incubation is within the natural thermal range for Atlantic salmon embryos, whereas 8°C is higher, and Macqueen et al. (2008) showed that the temperature in the period from fertilization to eyed-egg stage affected the phenotype of the musculature throughout life in Atlantic salmon. How such structural changes are linked to the ability of the fish to enter different growth phenotypes, ultimately allowing variability in smolting and maturity age in nature, is currently unknown. As such, these biological pathways should be further explored to assess the impact of climate change.

Another surprising observation was the difference in dorsal and tail fin lepidotrichs, and anal-fin pterygiophore counts observed between the size modes at 4°C. This is probably not due to the genetic make-up of the fish, as they were all genetically identical. Therefore, the current observation supports the notion of Jonsson and Jonsson (2014, 2019) that epigenetic mechanisms during the embryonic period are involved in the formation of later-life phenotypes. One plausible explanation for the current observations may be that the lower and upper mode fish had different rates of development as embryos, which in turn affected the formation of specific parts of the post-cranial axial skeleton. The dorsal- and caudal-fin lepidotrichs start to form at 370 d° after fertilization, which is after the eyed-egg stage (Kryvi et al., 2017). The direction of formation is bidirectional in both fins and starts at hypural 1 in the caudal fin and in the cranial end in the dorsal fin (Sankar, Fraser, et al., 2025). The anal-fin pterygiophores start to form at 400 d° near the cranial end of the fin followed by a bidirectional sequence of development towards the head and tail (Kryvi et al., 2017; Sankar, Fraser, et al., 2025). One way to explore possible relationships between developmental speed and phenotype is to examine if the time of hatching affects later growth by separating early- and late-hatch siblings.

Embryos incubated at 8°C generally had more supraneurals than those incubated at 4°C. The higher size mode at 4°C was intermediate between the 8°C and 4°C lower mode, but 8°C had on average 13.5 supraneurals, whereas 4°C upper and lower modes had 12.7 and 12.6, respectively. The same trend was observed by Beacham and Murray (1986) who found decreasing dorsal-fin ray counts with decreasing incubation temperature in chum salmon (*O. keta* Walbaum 1792). This is the opposite trend that is believed to exist for the determination of number of vertebrae where decreasing embryo temperature should increase vertebrae number (Beacham & Murray, 1986; Garside, 1966; Kwain, 1975; Lindsey et al., 1984). Indeed, the current study showed no effect of temperature on total vertebrae counts between 4 and 8°C, similar to the observation by Fraser et al. (2015) comparing 6 and 8°C. Further, there was no effect of temperature on region-specific vertebrae counts in the current study, unlike the results of earlier studies showing different vertebrae counts within specific vertebral regions between incubation temperatures in Chinook salmon (*O. tshawytscha*, Walbaum 1792) (De Clercq et al., 2018) and *A. mexicanus* De Filippi, 1853 (Reyes Corral & Aguirre, 2019). The only part of the post-cranial axial skeleton where a low incubation temperature increased meristic counts was the anal-fin pterygiophores, where the upper mode at 4°C produced the highest counts.

4.2 | Triploidy induced a maternal effect on vertebrae number

At 8°C incubation, the present study showed that diploid and triploid isogenic all-male heterozygous fish developed different total vertebrae counts and dorsal- and anal-fin pterygiophore counts, whereas isogenic all-female diploid homozygous fish developed equal counts as the latter. The current result of triploidization most probably reflects a genetic dosage effect by addition of more maternal chromosomes, especially because the triploids developed equally to the all-female homozygous group possessing only maternal chromosomes. Indeed, different vertebrae counts between diploid and triploid Atlantic salmon (Fraser et al., 2015) and rainbow trout (*O. mykiss* Walbaum 1792) (Kacem et al., 2004) have been reported in previous studies.

The present study is the first to show that the all-male triploids develop the same number of vertebrae as the all-female diploids that possess only the maternal chromosomes. Unlike total vertebrae number and dorsal- and anal-fin pterygiophore counts, where diploid homozygous and triploid heterozygous developed equal counts, tail fin lepidotrich counts showed the closest association to maternal chromosome dosage: triploid heterozygous (67% maternal dosage) have on average 40.8 lepidotrichs, intermediate between diploid heterozygous (50% maternal dosage) with 40.0 and diploid homozygous (100% maternal dosage) with 41.6. Thus, it may seem as for some parts of the post-cranial skeleton any presence of additional maternal chromosome overrules the paternal contribution, whereas for others, a clearer dosage effect appears. Nonetheless, the currently used model with isogenic homozygous and heterozygous fish seems to be well suited to study the effect of additional maternal chromosomes to their phenotype.

The currently observed reaction norm to ploidy has also been observed when studying the external phenotype of diploid and triploid hybrids created by crossing female Atlantic salmon and male brown trout (*S. trutta* L. 1758), where the triploid hybrid was equal to the dam, and the diploid hybrid is intermediate between the parental species (Fraser et al., 2021, 2022). However, this was not observed by Fleming et al. (2014) who compared total vertebrae counts, number of scales along the lateral line and the number of fin rays in the dorsal fin between diploid and triploid hybrids of female Atlantic salmon and male Arctic char (*S. alpinus* L. 1758). In their study, total vertebrae and dorsal-fin ray counts of the triploid hybrid were similar to diploid Arctic char, whereas the number of scales was similar to diploid Atlantic salmon. The authors suggested a mosaic genetic contribution from the parental species as a plausible explanation for these observations, agreeing with other studies on hybrid organisms (Cassani et al., 1984; Kierzkowski et al., 2011; Vörös et al., 2007) and the present study. In addition to implying a genetic dosage effect on total vertebrae counts by triploidy, the result of the current study indicates a strong paternal effect on total vertebrae counts when comparing isogenic homozygous and heterozygous individuals from the same mother.

The pioneering work by Schmidt (1919) indicates that the maternal vertebrae count may also impact heritability; if the dam had a high vertebrae number, the maternal effect was strong, whereas it was not if the dam had a low vertebrae number. If a similar pattern exists in Atlantic salmon, our results may imply a higher paternal than maternal vertebrae number in the current study. In the present study, we did not assess the maternal and paternal vertebrae number. Furthermore, temperature during early development may impact on the maternal effect; in the three-spined stickleback (*G. aculeatus* L. 1758), Lindsey (1962) found a positive correlation between maternal and offspring vertebrae counts below 21°C, whereas the correlation was negative at higher temperatures.

The present study suggests a paternal contribution to increased vertebrae number in the caudal region in male offspring. Notably, Lindsey (1962) found a maternal effect on abdominal vertebrae number in female offspring in the three-spined stickleback. Looking at the differences between the diploid and triploid heterozygous fish in the present study, it may suggest a maternal contribution to reduced number of caudal and transitional vertebrae and increased number of abdominal vertebrae, resulting in relatively more abdominal vertebrae. As such, this may affect the size of the abdominal cavity and the ability to produce gametes, which is especially important for female fecundity. The current triploids were all male, but the results may still reflect a possible female contribution towards increased egg production capacity, which is also visible in the male offspring. It is difficult to conclude on these aspects, as it is not possible to make triploid homozygous all-female fish with the current approach.

In a wider view, it has, for instance, been showFFn that anadromous populations have higher vertebrae number than non-anadromous populations within the same species, which has been suggested to be a beneficial adaptation to the marine environment (Goin et al., 2008; McDowall, 2003). Information about regional identity, which is currently unknown, could reveal if the trait is most likely related to reproduction or swimming abilities. Indeed, McDowall (2003) suggested a link to water viscosity, as seawater has higher viscosity than fresh water, where more vertebrae may favour higher flexibility and swimming ability in seawater.

4.3 | Vertebral deformity level, location, severity and type affected by heterozygosity and ploidy

The present triploids were challenged with a suboptimal incubation temperature at 8°C (Fraser et al., 2015) and diploid-adapted diets (Fjelldal et al., 2016) to provoke vertebral deformities beneath the dorsal fin (Fjelldal & Hansen, 2010). The approach worked, and the triploid heterozygous fish developed a high level of vertebral deformities, with compression and fusion (type 6, Witten et al., 2009) as the dominant deformity type and vertebra number 27 as the most often affected vertebra. The diploid homozygous fish developed an equal deformity occurrence and severity as the triploids, but the dominant deformity type was complete fusions (type 7), and vertebra number 16 was most often affected. Thus, the deformities in diploid homozygous fish were milder compared to triploids, as these complete fusions had remodelled into normal amphicoelous elongated vertebrae with numerous neural spines and ribs/haemal spines (Witten et al., 2006). Complete fusions are indeed the dominant deformity type in wild Atlantic salmon, which rely on an anatomically functional vertebral column for long-distance migration and river entrance for spawning (Sambraus et al., 2014). In line with the present study, Shikano et al. (2005) found a decreased level of fused vertebrae with two neural and haemal spines with increasing heterozygosity in guppy (*Poecilia reticulata* W. Peters, 1859). Fused and remodelled vertebrae have also been reported to be dominant in humpback zebrafish (*Danio rerio* F. Hamilton, 1822) mutants (König et al., 1999). That the diploid homozygous fish developed a distinct deformity peak at vertebra number 16 is an interesting observation. Further studies should examine if this deformity phenotype links to heterozygosity and/or can be used as a marker for unwanted genotypes.

5 | CONCLUSION

The result of the present study indicates that the ability to enter the lower or upper growth mode as parr is dependent on embryo incubation temperature and is fixed before first feeding. That different growth modes appear in isogenic fish suggests an involvement of epigenetic mechanisms. Further, the results show a strong maternal dosage effect on tail fin lepidotrich counts, whereas for total vertebrae and dorsal- and anal-fin pterygiophore counts, the presence of extra maternal chromosomes seems to overrule the paternal contribution. Vertebral deformity development in homozygous fish seems to be supported by active repair mechanisms.

AUTHOR CONTRIBUTIONS

Murugesan Sankar: methodology, validation, investigation and writing – original draft. Thomas W. K. Fraser: validation, writing – review and editing, visualization, data curation and formal analysis. Harald Kryvi: conceptualization, methodology, validation, investigation, writing – review and editing and visualization. Malthe Hvas: methodology, resources, writing – review and editing. Tom J. Hansen: methodology, resources, writing – review and editing. Per Gunnar Fjelldal: conceptualization, methodology, validation, investigation, writing – original draft and visualization.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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