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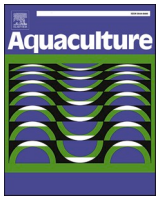

# Triploid Atlantic salmon × brown trout hybrids have similar seawater growth and welfare issues as triploid Atlantic salmon, but both were heavier at harvest than their diploid counterparts

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ABSTRACT

Interspecific hybridisation may improve the farm performance of sterile triploid salmonids via heterosis (i.e. hybrid vigour). We assessed growth over the final 293 days in seawater, and harvest quality, in diploid and triploid Atlantic salmon (*Salmo salar*) × brown trout (*Salmo trutta*) hybrids compared to diploid and triploid Atlantic salmon. We measured vertebral deformities, cataracts, flesh colour, gut mass, and body shape at harvest. In triploids, hybridisation had no effect on harvest size, vertebral deformities, cataracts, or body shape, but did improve fillet colouration (Mean digital SalmoFan™ score [95% CI]: 24.6 [24.4–24.9] and 26.0 [25.7–26.2] for triploid salmon and triploid hybrids, respectively) and lower relative gut size (34% lower). Compared to diploid salmon, triploid salmon were significantly heavier at harvest, triploid hybrids tended to be heavier (Post-hoc, least square means, $p = 0.08$), whereas diploid hybrids were 83% lighter (Mean mass [g] at harvest [95% CI]: 2676 [2470–2898], 3395 [3134–3679], 462 [401–534], and 3086 [2832–3363] for diploid salmon, triploid salmon, diploid hybrids, and triploid hybrids, respectively). However, both triploid groups had a significantly higher incidence of fish with one or more deformed vertebra (Mean % [95% CI]: 23 [14–35], 60 [47–71], 38 [20–60], and 44 [31–57] % in diploid salmon, triploid salmon, diploid hybrids, and triploid hybrids, respectively), more severe cataracts (Mean cataract score [95% CI]: 3.0 [2.7–3.3], 3.5 [3.2–3.8], 2.2 [1.7–2.6], 3.6 [3.3–4.0] for diploid salmon, triploid salmon, diploid hybrids, and triploid hybrids, respectively), and a smaller relative gut size (21% smaller) compared to diploid counterparts. In conclusion, triploid hybrids have no growth advantage over triploid salmon and suffer from similar welfare issues while only benefiting from increased fillet colour.

## 1. Introduction

Atlantic salmon (*Salmo salar*) farming currently relies on broodstock that have been genetically selected for on-farm performance over multiple generations. Although this has led to the development of fast-growing domestic strains, it has become an environmental concern as farm escapees can find their way to spawning grounds resulting in high levels of genetic introgression with wild fish (Karlsson et al., 2016). This has led to feral populations losing their genetic identity and their suitability for a life in the wild (Glover et al., 2017). As such, there is now an urgent need to prevent escapees from reproducing if the industry wants to continue to expand. Although land-based systems would prevent escapees, they require a significant amount of new infrastructure before current net-pen production can be reached. An alternative option is to farm reproductively sterile fish using the current infrastructure.

Numerous methods to produce sterile salmon exist, but to date the most feasible is to use triploids. These are individuals that have three complete chromosome sets, compared to the more natural diploid state whereby an individual has two complete sets. Triploidy is relatively easy to induce in salmonids, results in sterility, and can be done at the high efficiencies required for mass production (reviewed by Benfey, 2016). However, triploidy results in numerous physiological differences when compared to diploids and this can lead to inconsistent farm performance, reduced welfare, and reduced harvest quality. For example, triploidy is a risk factor for vertebral deformities (Fjelldal and Hansen, 2010) and ocular cataracts (Wall and Richards, 1992), both of which can

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limit growth and reduce welfare. In addition, triploidy leads to other morphological differences, such as a reduced mass of pyloric caeca within the gut (Peruzzi et al., 2015), lower fillet colouration (Smedley et al., 2016), and an increased occurrence of gill deformities (Sadler et al., 2001) in Atlantic salmon. Finally, escaped triploid salmon may still return to spawning grounds and interfere with breeding events even though they cannot produce viable offspring (Fjelldal et al., 2014). Therefore, although the technology to produce triploids has been available since the 1970s, their uptake has been limited to Tasmania (Australia) to prevent issues around early sexual maturation (Amoroso et al., 2016) and in North America (Maxwell and Filgueira, 2020; Soga et al., 2020) to prevent genetic interactions between farm escapees and wild fish.

An unexplored method to improve triploid performance is via interspecific hybridisation and heterosis (i.e. hybrid vigour). One of the most viable salmonid hybrids is the cross between female Atlantic salmon and male brown trout (*Salmo trutta*) (Álvarez and Garcia-Vazquez, 2011). When producing these, we found the triploid Atlantic salmon × brown trout hybrid could show superior freshwater (Fraser et al., 2021a) and early seawater growth (Fraser et al., 2021b) compared to diploid and triploid salmon. Galbreath and Thorgaard (1997) also found the triploid hybrid to be 33% larger than diploid salmon after 1 year in seawater. However, the latter study had no triploid Atlantic salmon for comparison. We also found juvenile triploid hybrids did not necessarily have more skeletal deformities than diploid salmon although this depended on year class (Fraser et al., 2021a). Finally, wild diploid hybrids are more frequently observed as parr than returns, suggesting they have a lower potential to interfere with spawning events (Adams et al., 2014). To continue to determine the feasibility of using triploid salmonid hybrids in aquaculture, further information on harvest quality and welfare is required.

In the current study, we continued to follow a group of diploid and triploid Atlantic salmon and Atlantic salmon × brown trout hybrids for which we have previously published growth and vertebral deformity data in parr (Fraser et al., 2021a) and smoltification physiology and early seawater growth (the first 241 days) in post-smolts (Fraser et al., 2021a). Here, we assess seawater growth for the final 293 days prior to harvest and vertebral deformities, cataracts, fillet colour, gut size, and body shape at harvest. We assessed body shape, as diploid hybrids have been found to have longer snouts and thicker caudal peduncle compared to diploid salmon (Solem et al., 2014). We used a cross between an Atlantic salmon female with a brown trout male as this cross is generally considered more viable than the reciprocal cross (Álvarez and Garcia-Vazquez, 2011). Our hypothesis is that triploid hybrids would inherit the characteristics of both triploids and hybrids and would therefore have a higher prevalence of vertebral deformities and cataracts, a longer snout and thicker caudal peduncle, but a smaller gut and lower fillet colouration than diploid counterparts.

## 2. Materials and methods

### 2.1. Ethics

The experimental work was conducted in accordance with the laws and regulations controlling experiments and procedures on live animals in Norway following the Norwegian Regulation on Animal Experimentation 1996. The experiment was approved by the Norwegian Food Safety Authority (FOTS #15240).

### 2.2. Fish stock and rearing conditions

The incubation and early life rearing of the fish stock was previously described in a multiyear class study (Fraser et al., 2021a, see the 2017 year class) whereas smoltification, early seawater growth, and survival is reported in Fraser et al. (2021b). In brief, on the 17th January 2017 (day 0) eggs from one domesticated Mowi strain Atlantic salmon were divided into two equal parts and fertilised with either sperm from one first generation offspring of wild (River Vosso, Norway) Atlantic salmon or one non-anadromous (lake Tunhovd, Eastern Norway) brown trout from a recently domesticated stock. After fertilization, half the eggs from each cross were subject to a hydrostatic pressure shock of 655 bar for 6 min and 15 s (TRC-APV, Aqua Pressure Vessel, TRC Hydraulics inc., Dieppe, Canada) exactly 37.5 mins after fertilization at 8 °C to induce triploidy. Ploidy confirmation ($n$ = 48–50 from each group) was later achieved using blood cell diameter and reported in Fraser et al. (2021a). The mean red blood cell diameter of triploids was approx. 18% larger than diploids, irrespective of genotype (salmon vs hybrid), with no overlap between individual mean values of putative diploids and triploids.

The resulting four groups (diploid salmon, triploid salmon, diploid hybrids, and triploid hybrids) were all reared under the conditions found in Fig. 1 for the production of yearling (i.e. 1+) smolts. Each group was incubated in a single tray before being moved to single fiberglass tanks at first feeding (1 × 1 × 0.43 m). At first feeding on the 27th April 2017, the number of fish was reduced to 800 per tank. Mortality between fertilization and first feeding was 21, 28, 48, and 16% for the diploid salmon, triploid salmon, diploid hybrid, and triploid hybrid, respectively. On the 7th September 2017, 180 fish per group were implanted with a passive integrated transponder (PIT tag), had their fork length and body mass recorded, and were equally distributed between 3 tanks for common garden rearing (1 × 1 × 0.43 m, $n$ = 60/group/tank). On the 2nd February 2018, 72 fish from each group ($n$ = 24/tank) were removed and used to assess the development of smoltification. The water inflow to the tanks was changed to full strength seawater over a 5-day period beginning on the 16th May 2018 (20 ppt on the 16th, 28 ppt on the 18th, and 35 ppt on the 21st May 2018). On the 11th September 2018, all three tanks of fish were transferred into one common garden large (6 m ø) tank (density of 11 kg/m$^3$). These fish remained on 35 ppt up until 11th January 2019 after 241 days in seawater.

The current experimental period began on the 11th January 2019 (day 724 post first feeding). At this point, we had a total of 89 diploid salmon, 85 triploid salmon, 37 diploid hybrids, and 83 triploid hybrids. The difference in numbers was largely due to bimodality during freshwater growth in the hybrids, with the lower fraction not undergoing the parr-smolt transformation and therefore they were not adapted to seawater and removed (Fraser et al., 2021b). The upper mode hybrids all showed the physiological changes expected during the parr-smolt transformation, albeit the upper mode diploid hybrids still displayed poorer early seawater growth and survival (Fraser et al., 2021b). At the initiation of the current sampling, we also noted that 58 fish had mild sores on the belly. Retrospectively, these were all found to be salmon and not hybrids, but there was no effect of ploidy ($n$ = 28, 30, 0, and 0, for diploid salmon, triploid salmon, diploid hybrids, and triploid hybrids, respectively. GLM, Ploidy; $\chi^2 = 0.3$, df = 1, $p = 0.592$). Due to the occurrence of sores, the salinity was lowered to 28 ppt and remained so for the remainder of the study until the 31st October 2019 (day 1017, final tank density of 45 kg/m$^3$). Throughout the experiment all fish that appeared moribund or developed severe external deformities were removed and considered to have died.

### 2.3. Sampling procedures

All fish were anaesthetised in buffered 100 mg/L MS222 (Finquel®) on day 724 and 862 (25th May 2019) and measured for PIT number, body mass (to 1 g), and fork length (to 0.1 cm). On day 1017, all fish were euthanised with an overdose of 200 mg/L MS222, a lateral photograph was taken with a digital camera, and then the PIT number, body mass, and fork length recorded. Sex and sexual maturation were assessed at the final sampling via visual examination of the gonads. Only 1 fish, a diploid male, was deemed sexually mature as it showed some gonad development. The gut (including the liver and adipose tissue) was also removed and weighed (to 1 g).

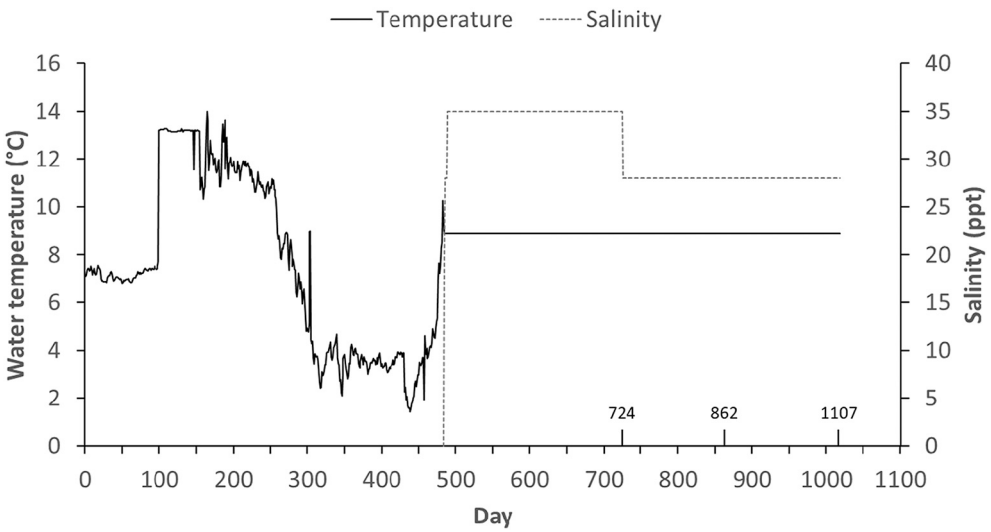


**Fig. 1.** The environmental conditions from fertilization until the end of the study. Day 0 was the 17th January 2017. The rugs on the x axis indicate the sampling days.

Fulton's condition (*K*) factor (body mass (g)/fork length (cm)$^3$ × 100) was used as a measure of body condition. Specific growth rates for individual fish were calculated using the formula ($e^q - 1$) × 100 (Houde and Scheckter, 1981), where $q = [\ln(W_2) - \ln(W_1)]\,(t_2 - t_1)^{-1}$ (Bagenal and Tesch, 1978), and $W_2$ and $W_1$ are average body mass at times $t_1$ and $t_2$, respectively.

### 2.4. Final sampling for cataracts, morphology, radiology, and fillet colour

All fish were inspected by one researcher for ocular cataracts using a slit lamp microscope (HEINE® HSL 150 hand-held slit lamp, HEINE Optotechnik). Cataracts were graded according to severity based on a scale of 0 to 4 (0, <10, 10–50, 50–75, and > 75% of the lens shrouded, respectively) for each eye and 0–8 for each fish (Wall and Bjerkås, 1999).

A digital picture of the left lateral side of euthanised fish was taken for later morphometric analysis prior to the whole fish being radiographed with a direct radiology system (Canon CXDI-410C Wireless, Canon Inc., Kawasaki, Japan) using a portable X-ray unit (Portable X-ray Unit Hiray Plus, Model Porta 100 HF, JOB Corporation, Yokohama, Japan) at 88 cm distance with 40 kV and 10 mA. Vertebral deformities were evaluated by one researcher according to the classification of Witten et al. (2009).

Fillets were hand processed and assessed for colouration using a digital DSM SalmoFan™ (Netherlands) following the manufacturer's instructions. Each fillet was scanned five times (3 above and 2 below the lateral line) from the Norwegian Quality Cut.

### 2.5. Quantifying morphology

A total of 22 landmarks (Fig. 2) were manually placed by one researcher along the bodies of the digital images of fish with no deformed vertebrae ($n$ = 59, 15, 32, and 38 for diploid salmon, diploid hybrids, triploid salmon, and triploid hybrids) using ImageJ. We chose not to include fish with any radiologically detectable vertebral deformities or the one sexually mature individual as these may lead to alterations in body shape. The landmark xy coordinates were collected and analysed using the R package Geomorph (Adams and Otarola-Castillo, 2013). The landmarks were projected into tangent space using Generalised Procrustes Analysis (GPA) to remove variation in landmark position due to rotation, translation, and scaling using the *gpagen* function in geomorph. The function also calculates the centroid size (a measure of relative size) for every individual. We also measured pectoral and pelvic fin length, and maxilla length (distance between landmarks 5 and 11, Fig. 1), for a separate analysis.

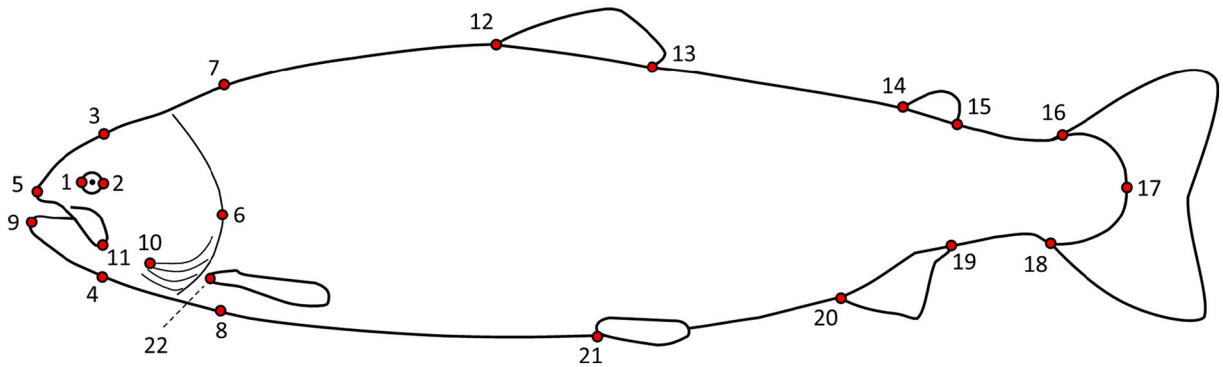


**Fig. 2.** The 22 landmarks used in this study. (1) anterior eye, (2) posterior eye, (3) dorsal depth at posterior eye, (4) ventral depth at posterior eye, (5) anterior tip of the snout, (6) posterior operculum, (7) dorsal body depth at the posterior of the operculum, (8) ventral body depth at the posterior of the operculum, (9) anterior tip of the lower jaw, (10) dorsal anterior point of branchiostegal rays, (11) posterior end of the maxillary, (12) origin of dorsal fin, (13) insertion of the dorsal fin, (14) origin of adipose fin, (15) insertion of the adipose fin, (16) anterior attachment of dorsal membrane of the caudal fin, (17) base of middle caudal rays, (18) anterior attachment of the ventral membrane from the caudal fin, (19) insertion of the anal fin, (20) origin of the anal fin, (21) ventral point corresponding to the origin of the pelvic fin, and (22) origin of the pectoral fin.

### 2.6. Statistics

We used R version 3.6.1. Significance was assumed at $p < 0.05$. Throughout, model diagnostics were assessed via qqplots and standardised versus predicted residual plots. Least-square means (LSM) were used as post-hoc tests where significant model effects were identified. The raw data (Hybrids.xlsx) and the R script used for analysis with all the models described below (Hybrids.R) can be found in the supplementary material.

Due to the occurrence of mild sores in the diploid and triploid Atlantic salmon (see methods), we initially checked whether they impacted on all endpoints using the same approaches described below albeit without the inclusion of genotype as no hybrids had sores. Sore only had a significant effect on the interaction with body mass (linear mixed effect [LME] model, $\chi^2 = 20$, df = 1, $p < 0.001$) and length (LME, $\chi^2 = 6$, df = 2, $p = 0.049$) with time. However, post-hoc analyses found no significant size differences within time point (LSM, $p > 0.07$) and there was no interaction between ploidy and sores on any endpoint.

For survival we used a cox proportional hazards (COXPH) model. Data on the time the individual was within the experiment in days (continuous), group (4 levels), and the outcome when leaving the study (2 levels, dead/alive) were included.

To assess the sex ratio, we used a generalised linear model (GLM) with a binomial response. Sex (Female/Male) was the dependent variable, with ploidy (2 levels) and genotype (2 levels) as the independent variables, and we included the interaction between ploidy and genotype.

To assess vertebral deformity prevalence, we used a GLM with a binomial response. Deformed (Yes/No) was the dependent variable, with ploidy (2 levels) and genotype (2 levels) as the independent variables, and we included the interaction between ploidy and genotype. To assess deformity severity, the number of deformed vertebrae per deformed fish was assessed using a GLM. The model included the number of deformed vertebra (natural log transformed) as the dependent variable, ploidy (2 levels) and genotype (2 levels) as independent variables, and their interaction. For both models, sex was considered for inclusion in the analysis, but preliminary plots and an increase in the models AICc score demonstrated it explained little of the variation within the dataset (see supplementary material). The same approach was used to model cataract severity, only proportional odds linear regression (POLR) models were used and not GLM. If interactions were not significant, it was compared to a model without the interaction using the AICc score. The model with the lowest AICc score was then used in the final analysis (see Table 1).

To assess body mass, length, and condition over time, we began with a LME model with body size (mass, fork length, condition) as the dependent variable, ploidy (2 levels) and genotype (2 levels) as categorical independent variables, time as a categorical independent variable (3 levels), and fish ID as a random effect to account for repeated measures. We also included a correction for correlation in the repeated measures (correlation = corAR1()) and body mass and length were natural logged transformed to improve model diagnostics (see supplementary material). Ploidy, genotype, and time were included as a 3-way interaction, and we also included sex and its interaction with ploidy, the number of deformed vertebrae, and cataract score in the initial model. Subsequently, the ploidy × sex interaction was removed, although sex was left in the model, and the cataract score was removed, as neither explained much of the variation in the dataset when comparing model AICc scores (see supplementary material).

To assess relative gut size, we took the residuals from linear models (LM) of gut mass vs gutted mass and logged gut mass vs logged length to create an index of relative gut size and included these in two GLMs with relative gut size as the dependent variable, ploidy (2 levels), genotype (2 levels), and sex (2 levels) as categorical independent variables, and cataract score, and the number of deformed vertebrae as continuous independent variables. Ploidy, genotype, and body mass minus gut mass or body length were included as a 3-way interaction. Subsequently we used the AICc score to arrive at the most parsimonious model as the interactions were not significant. We created a third GLM model that included total gut mass as the dependent variable to compare absolute gut size.

For body shape we performed a Procrustes regression using the function *procD.lm* with the GPA scores as the dependent variable, log centroid size as a continuous independent variable, and ploidy (2 levels), genotype (2 levels), and sex (2 levels) as categorical independent variables. Initially, ploidy, genotype, and sex were allowed to interact (3-way) before non-significant interactions were removed until we achieved the most parsimonious model. Post hoc analyses were done using morphological disparity with the function *morphol.disparity*. We also specifically compared pelvic and pectoral fin length, and maxilla length, as the latter two, but not the former, were previously found to be longer in diploid hybrids compared to diploid salmon (Solem et al., 2014). To assess relative fin and maxilla length, we took the residuals from LMs of fin/maxilla length vs fork length to create an index of relative size and included these in three GLMs with relative size as the dependent

**Table 1**
Survival, sex ratio, vertebral deformities, cataracts, fillet pigmentation, and body dimension data from diploid and triploid Atlantic salmon and Atlantic salmon × brown trout hybrids at termination of the study. The data are predicted means (95% CI) and include the statistics. Different lowercase letters within a row signify a significant difference between groups (Post hoc, LSM, $p < 0.05$).

| Parameter | Salmon | | Hybrid | | Statistics | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Diploid | Triploid | Diploid | Triploid | Model | Final model | $\chi^2$ | df | *p* |
| Survival (%) | 93 (88, 99) | 98 (94, 100) | 89 (78, 100) | 94 (89, 100) | COXPH | Group | 3.4 | 3 | 0.337 |
| Males (%) | 52 (42, 62) | 44 (34, 54) | 55 (41, 68) | 47 (37, 58) | GLM | Ploidy × Genotype | 5.9 | 1 | 0.015 |
| Deformed (%) | 23 (14, 35)[b] | 60 (47, 71)[a] | 38 (20, 60)[ab] | 44 (31, 57)[a] | GLM | Ploidy × Genotype | 4.9 | 1 | 0.027 |
| Deformed vertebrae | 4.1 (2.9, 5.8) | 5.1 (4.0, 6.5) | 4.8 (3.2, 7.2) | 6.0 (4.5, 8.0) | GLM | Ploidy + Genotype | 1.2/0.8 | 1/1 | 0.266/0.373 |
| Cataracts (%)* | 100[a] | 100[a] | 91[b] | 100[a] | – | – | – | – | – |
| Cataract score | 3.0 (2.7, 3.3)[b] | 3.5 (3.2, 3.8)[ab] | 2.2 (1.7, 2.6)[c] | 3.6 (3.3, 4.0)[a] | POLR | Ploidy × Genotype | 7.0 | 1 | 0.008 |
| Fillet colour (SalmoFan™) | 25.1 (26.8, 25.3)[b] | 24.6 (24.4, 24.9)[b] | 23.6 (22.9, 24.3)[c] | 26.0 (25.7, 26.2)[a] | POLR | Ploidy × Genotype | 57.5 | 1 | <0.001 |
| Pectoral fin length index (×10²) | -9 (−16, −1)[b] | 13 (3, 24)[a] | 5 (−10,20)[ab] | 0 (−9, 10)[ab] | GLM | Ploidy × Genotype | 5.5 | 1 | 0.019 |
| Pelvic fin length index (×10²) | −2.2 (−10.9, 6.6) | 0.3 (−11.5, 12.0) | 2.7 (−14.5, 19.9) | 2.0 (8.8, 12.8) | GLM | Genotype | 0.4 | 1 | 0.549 |
| Maxilla length index (×10²) | −10.7 (−17.6, −3.6)[b] | −8.9 (−18.3, 0.6)[b] | 17.0 (3.3, 30.7)[a] | 18.3 (97, 27.0)[a] | GLM | Genotype + Sex | 36/21 | 1/1 | <0.001/<0.001 |

* There was no statistical analysis.

variable, ploidy (2 levels), genotype (2 levels), and sex (2 levels) as categorical independent variables. Ploidy and genotype were initially allowed to interact, and each model was simplified using backwards model building until we arrived at the most parsimonious model with the lowest AICc.

## 3. Results

### 3.1. Survival and sex ratio

Survival ranged from 89% in diploid hybrids to 98% in triploid salmon, but there was no significant effect of group (Table 1). There was no effect of ploidy, genotype, or the interaction on the frequency of males (Table 1).

### 3.2. Growth

The was a significant 3-way interaction between ploidy, genotype, and time for body mass (LME, $\chi^2 = 27$, df = 2, $p < 0.001$), length (LME, $\chi^2 = 119$, df = 1, $p < 0.001$), and body condition (LME, $\chi^2 = 9$, df = 2, $p = 0.014$). Triploids were always heavier and longer than diploids counterparts, irrespective of genotype (Figure 3AB). There was no genotype effect in triploids, whereas in diploids the hybrids were always significantly lighter than salmon, and more so at the end (83% lighter) compared to the start of the experiment (77% lighter). In both ploidies, salmon initially had a higher condition factor than hybrids on day 724, then equal at day 862, before being higher again on day 1017 (Fig. 3C). There was also a general tendency for diploids to have higher condition than triploids in salmon, but the opposite in hybrids.

Cataract score and sex had no effect on mass or length, although females had significantly higher body condition than males (LSM, 95% CI: Female 1.30, 1.28–1.32; Male, 1.27, 1.25–1.28. Estimate (female vs male) = 0.03, df = 242, $t = 2.9$, $p = 0.004$). The number of deformed vertebrae had a significant negative relationship on body mass (LME, $\beta = -0.02$, df = 243, $t = -5.1$, $p < 0.001$) and length (LME, $\beta = -0.41$, df = 243, $t = -8.2$, $p < 0.001$), but a significant positive association with body condition (LME, $\beta = -0.01$, df = 242, $t = 8.7$, $p < 0.001$).

### 3.3. Vertebral deformities at harvest

There was a significant interaction between ploidy and genotype on the prevalence of deformed fish, with diploid salmon having fewer deformed fish than both triploid salmon and triploid hybrids (Table 1). However, there was no significant effect of ploidy, genotype, or the interaction, on the number of deformed vertebrae per deformed fish (Table 1). Most deformed vertebrae were found in the cranial and caudal trunk (between vertebrae 1–30, Fig. S1). In triploid salmon there was a clear deformity peak around vertebra no. 29 that was not observed in the other groups.

### 3.4. Cataracts

The diploid hybrids were the only group not to have 100% cataract prevalence (Table 1). Triploid hybrids, but not triploid salmon, had a significantly higher cataract score than diploid salmon (Table 1), although the latter was close to being significant (LSM; estimate = −0.55, z ratio = −2.5, $p = 0.056$). The diploid hybrid had a significantly lower cataract score than all other groups.

### 3.5. Gut index

There was no interaction between ploidy and genotype on relative gut mass, but triploids and hybrids had relatively lighter guts than diploids and salmon, respectively (Fig. 4A). The results when using body length were identical to those when using gutted mass (see supplementary R script). For absolute gut mass, there was a significant interaction between ploidy and genotype (Fig. 4B). Triploid hybrids had lighter, but triploid salmon had a heavier, absolute gut mass compared to diploid salmon. Hybrids had lighter guts than salmon in both ploidies, only the effect was much greater in diploids.

### 3.6. Fillet colouration

There was a significant interaction between ploidy and genotype on fillet colour, with triploid hybrids having significantly higher values than diploid and triploid salmon, whereas diploid hybrids had

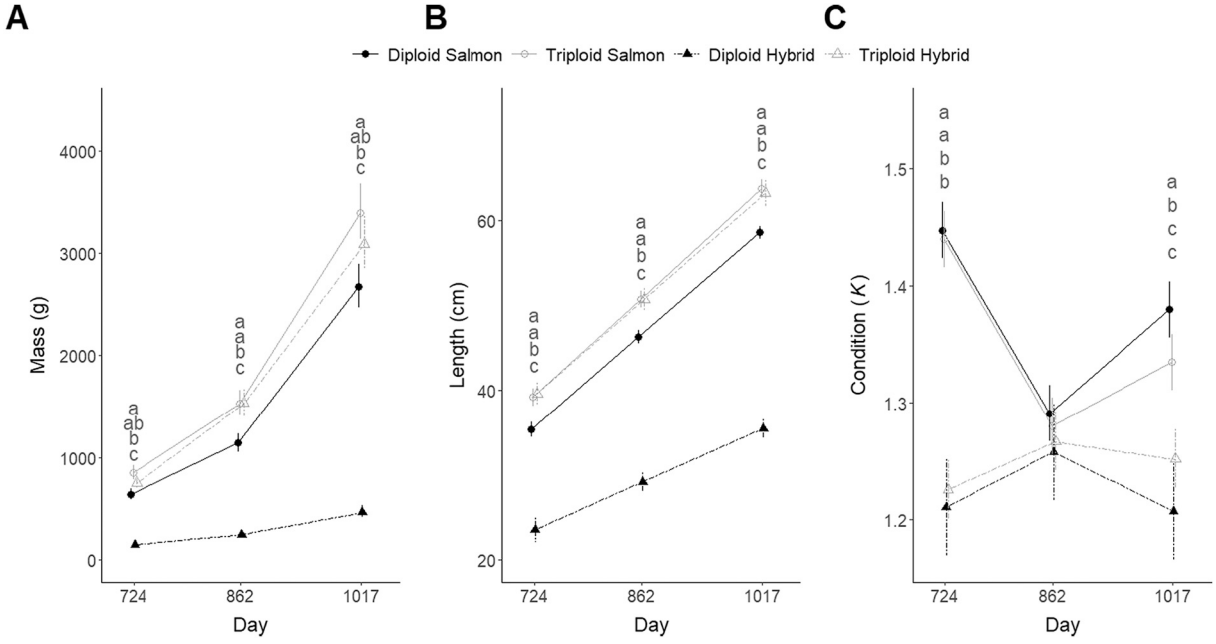


**Fig. 3.** Predicted body size and condition in diploid and triploid Atlantic salmon and Atlantic salmon × brown trout hybrids. (A) Body mass, (B) fork length, and (C) body condition. The data are predicted (LME models) means ±95% CI. Different lowercase letters within a timepoint signify significant differences between groups (LSM post hoc, $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

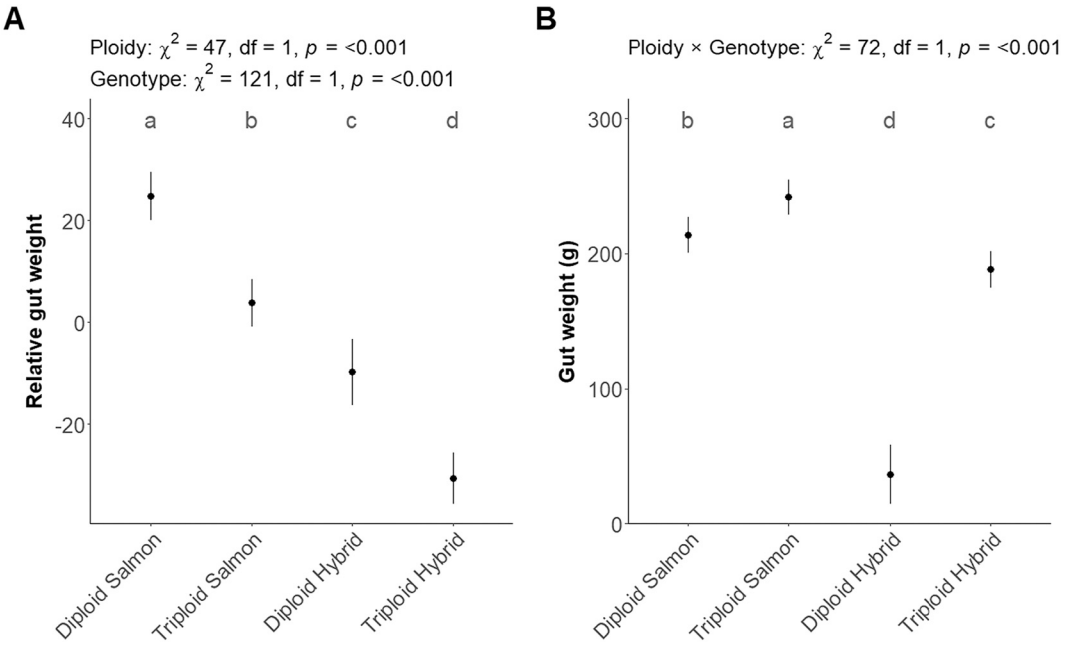


**Fig. 4.** Gut size in diploid and triploid Atlantic salmon and Atlantic salmon × brown trout hybrids. (A) Relative gut weight with respect to gutted mass. (B) Total gut weight without any correction for body size. The data are predicted (GLM) means ±95% CI. The statistics are from GLMs. Different lowercase letters indicate a significant effect between groups (LSM post hoc, $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

significantly lower values than all other groups (Table 1).

### 3.7. Morphology

An example of the external appearance of each group can be seen in Fig. S2. The two most significant axes explaining shape variation explained 49.5% of the variation in the landmark data and the diploid hybrids were notably clustered apart from all other groups (Fig. 5A). This group separation was confirmed by a significant interaction between ploidy and genotype on body shape, with diploid hybrids being significantly different to all other groups (Procrustes regression, df = 1, SS = 0.002, $F = 5.8$, $p < 0.001$). Diploid hybrids had a longer snout and greater body depth in the caudal peduncle (Fig. 5B). There was also a significant interaction between ploidy and sex (Procrustes regression, df = 1, SS = 0.001, $F = 2.2$, $p = 0.029$) although the groups were not clearly distinguished with Principle Component Analysis (PCA) (Fig. 6A). Diploid males were significantly different to triploid salmon and triploid hybrids, the major difference being the distance between landmarks 12 and 13 (the origin and insertion of the dorsal fin) being shorter in triploids (Fig. 6B). There was also a general effect of sex, with females having a shorter snout than males (Fig. 6B).

There were general genotype and sex effects on relative maxilla

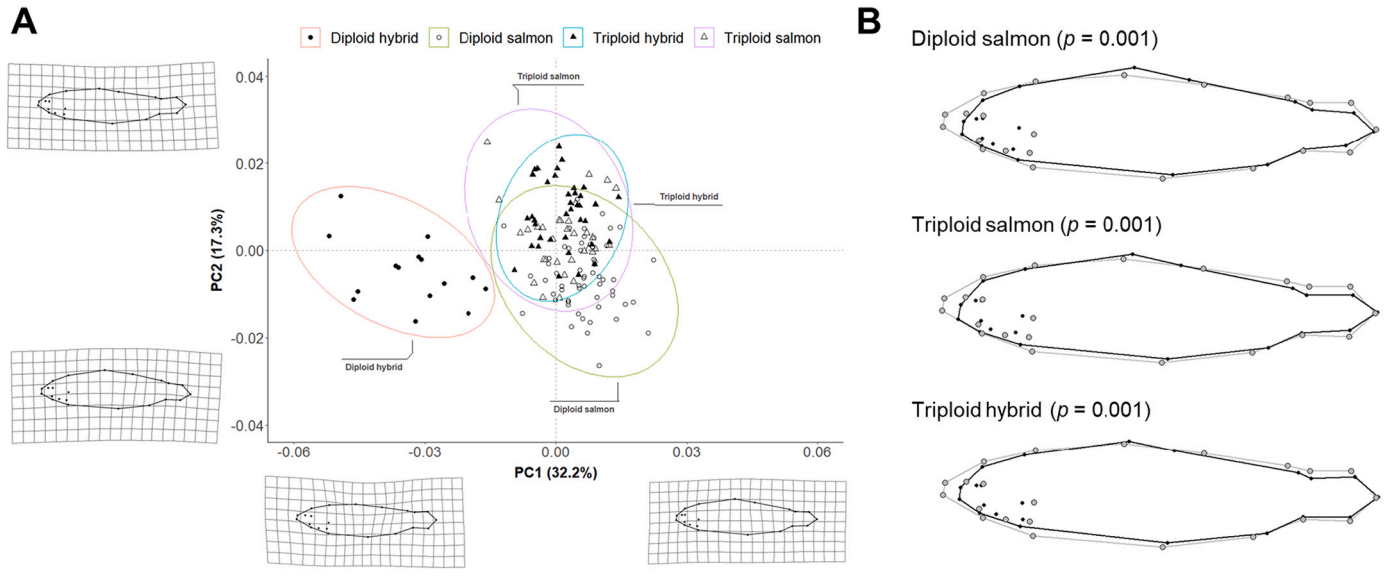


**Fig. 5.** Body shape analyses in diploid and triploid Atlantic salmon and Atlantic salmon × brown trout hybrids. (A) Principal components (PC) one and two with examples of the min and max thin plate splines for each PC. (B) Comparison of mean body shape for each group (black dots and lines) compared to diploid hybrids (grey dots and lines). There was a significant interaction between ploidy and genotype with diploid hybrids being significantly different to all other groups (statistics are from morphological disparity). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

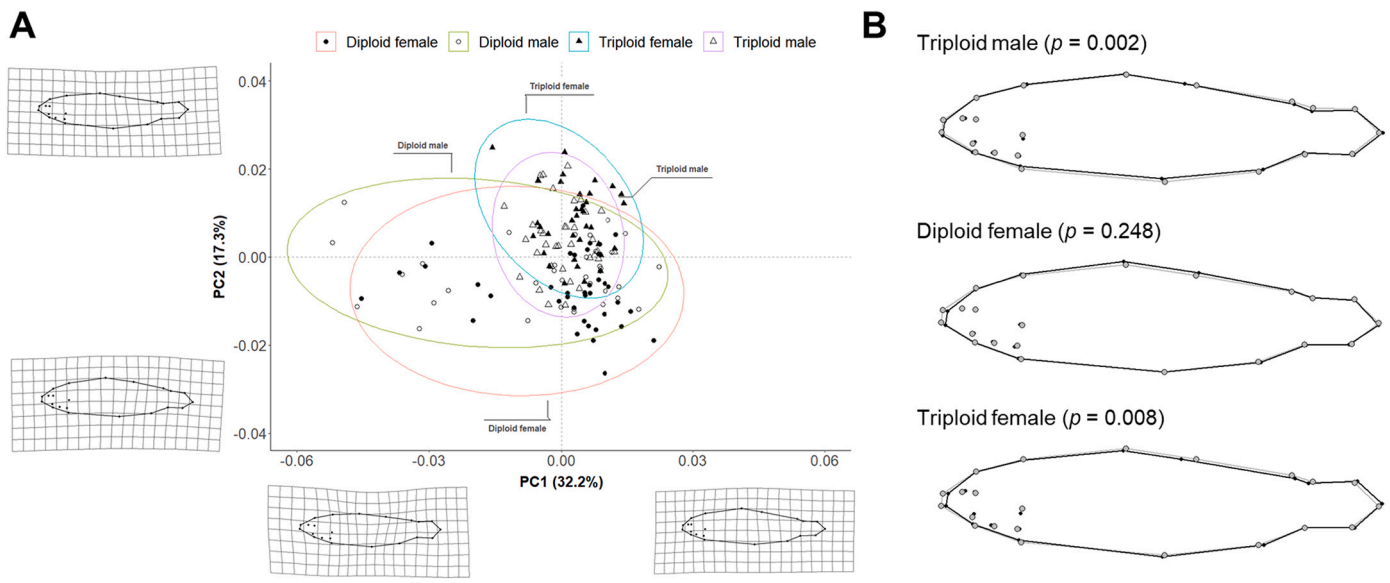


**Fig. 6.** Body shape analyses in male and female diploid and triploid Atlantic salmon and Atlantic salmon × brown trout hybrids. (A) Principal components (PC) one and two with examples of the min and max thin plate splines for each PC. (B) Comparison of mean body shape for each group (black dots and lines) compared to diploid males (grey dots and lines). There was a significant interaction between ploidy and sex with diploid males being significantly different to triploid salmon and triploid hybrids (statistics are from morphological disparity). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

length, with it been 28% and 21% longer in hybrids compared to salmon, and females compared to males, respectively (Table 1). There was an interaction between ploidy and genotype for pectoral fin length, with triploid salmon having relatively longer fins than diploid salmon (Table 1). There was no effect of ploidy, genotype, or sex on relative pelvic fin length (Table 1).

## 4. Discussion

Our objective was to determine whether hybridisation would have any beneficial effects on triploid performance in terms of seawater growth and production characteristics and welfare at harvest. Compared to triploid salmon, only fillet colour was enhanced in the triploid hybrid. Otherwise, triploid hybrids suffered from similar production issues as triploid salmon with impaired bone health and more severe cataracts compared to diploid salmon. These results are discussed in relation to the current knowledge on triploid salmonids.

Hybridisation had no effect on triploid body size, but significantly reduced diploid performance. As triploid hybrids were initially larger as parr and pre-smolts (Fraser et al., 2021a, 2021b), but became progressively smaller than triploid salmon (this study and Fraser et al., 2021b), we find they lose their growth advantage over triploid salmon as they become bigger/develop. Whether this is due to a limited size potential or poorer long-term seawater tolerance is unknown. Against the general trend in the literature, triploid salmon out-grew diploid salmon with these differences already established at the beginning of the study period. For example, triploid salmon were 34% heavier than diploid salmon at the start of the study period. We presume the poor performance of the diploid hybrid is due to the genetic makeup of the fish. For example, Atlantic salmon are considered more seawater tolerant than brown trout (Tanguy et al., 1994; Urke et al., 2010), therefore one may expect hybrids would be less tolerant of prolonged periods in seawater. As triploids were two-thirds' salmon (we doubled the maternal contribution), one may expect they are more akin to salmon than the diploid hybrids, which were equal parts salmon and trout. We saw some evidence of reduced seawater adaptiveness in the physiology data around smoltification as diploid hybrids showed a lower surge in cortisol and smaller changes in the gill indicative of smoltification (e.g. the mRNA abundance of the *nkaα1a/nkaα1b* ratio) during the spring, whereas triploid hybrids had an equal surge, compared to diploid salmon (Fraser et al., 2021b). This poor seawater performance may explain why diploid hybrids are rarely observed post parr stages in the wild (Adams et al., 2014). Alternatively, if they are sterile, they may not undergo return migrations.

The growth of triploid salmon in seawater is relatively inconsistent compared to diploids with reports of lower (Fraser et al., 2013), equal (Smedley et al., 2016), or greater growth (O'Flynn et al., 1997). The underlying cause of the inconsistency remains unknown, but may be explained by differences in temperature optima (Sambraus et al., 2018), nutritional requirements (Taylor et al., 2015; Fjelldal et al., 2016), or the optimal timing of sea transfer (Taylor et al., 2011). Recently, we have also observed ploidy effects on salinity optima for post-smolt growth (Fonseka et al., 2022), with triploids performing relatively better at 23 compared to 35 ppt. In addition, ploidy effects on growth in Arctic char were explained by life history, with diploids having a higher proportion of the quickest growing strategies (Fraser et al., 2022). Therefore, numerous hypotheses exist to explain inconsistent triploid performance. In the current work we used 9 °C seawater that is likely to favour triploid salmon (Sambraus et al., 2017b, 2018), we ensured the optimal timing of sea transfer (Fraser et al., 2021b), and we used 28 ppt during the final phase in seawater that may also favour triploid salmon (Fonseka et al., 2022). Therefore, triploid salmon may be a viable alternative to diploids when favourable environmental conditions can be guaranteed, and triploid specific diets are available.

Hybridisation did not significantly reduce the incidence of triploids with deformed vertebra. Triploidy is a well-known risk factor for skeletal deformities in Atlantic salmon (Fjelldal and Hansen, 2010), rainbow trout (*Oncorhynchus mykiss*) (Weber et al., 2014), brown trout (Preston et al., 2017), and Arctic char (*Salvelinus alpinus*) (Fraser et al., 2022). Bone health in triploid Atlantic salmon is improved by increasing the dietary phosphorus level (Fjelldal et al., 2016), particularly during the very early life stages when dietary phosphorus requirements are generally at their highest (Sambraus et al., 2020). This ploidy effect may be explained by larger genomes having a higher per cell nucleic acid content for which phosphorus is a key component (Neiman et al., 2012). Previously, we found no consistent effect on the prevalence of vertebral

deformities in three year-classes of triploid Atlantic salmon × brown trout hybrid parr, as they either had equal or more deformities than diploid salmon (Fraser et al., 2021a). The triploid hybrids used in the current study had an equal prevalence of deformities as diploid when parr (the 2017 year class in Fraser et al., 2021a), but we now find a higher occurrence at harvest. However, it is not uncommon to find the ploidy effect becoming more apparent as fish grow (Smedley et al., 2016). As such, we suggest triploid hybrids should be given a diet with a high total phosphorus content (i.e. ≥ 16 g/kg Fjelldal et al., 2016; Sambraus et al., 2020) to prevent vertebral deformities, with further studies required to determine whether such diets are required throughout the entire production cycle or can be used only during critical periods.

In diploid salmon, the prevalence of radiologically detectable deformed vertebrae was towards the low end of the scale for harvest size fish. For example, values can range between 10 and 70% in market size fish (Fjelldal et al., 2007, 2009) whereas values of 35 (Fraser et al., 2014) to 43% (Sambraus et al., 2014) have been reported in wild migrating adults. As such, our value in triploid hybrids is not particularly high compared to other studies, although our triploid salmon were towards the higher end of commonly reported values. Our triploid Atlantic salmon had a deformity peak around vertebra 27 to 29, which is commonly reported in both juveniles (Fjelldal and Hansen, 2010) and harvest size fish (Fraser et al., 2013). The triploid hybrids had a similar peak as juveniles (6–8% of vertebrae no. 24–26 deformed, Fraser et al., 2021a), but we did not observe it now in harvest size fish. This appears to be due to a relatively greater increase in the number of deformities observed at other locations, rather than a reduction in the prevalence of deformities in vertebrae no. 24 to 26. These vertebrae lie just below the dorsal fin and are the first to form in Atlantic salmon (Grotmol et al., 2003), although why they are generally most affected by triploidy remains unknown.

Hybridisation did not reduce the incidence of cataracts in triploids although the prevalence was generally high with all groups having an occurrence >90%. We are unaware of any published results on cataracts in brown trout, but triploid Atlantic salmon (Wall and Richards, 1992; Taylor et al., 2015), although not arctic char (Fraser et al., 2022), are more susceptible to developing cataracts than diploids. Here, brown trout are more closely related to Atlantic salmon, but have a more similar life history to char (e.g. short sea migrations), and we are unsure as to which is most relative on the likelihood of developing cataracts in our experimental setup. Although not significant, triploid salmon also had a strong tendency ($p = 0.06$) to have more severe cataracts than diploid salmon. The population mean scores in the current work were all <4, with an average of <10–50% of each lens shrouded, which is not expected to impede growth (Fraser et al., 2019). Indeed, we found no negative correlation between cataract score and final body mass. The ploidy effect on the cataract score is likely due to nutritional requirements as we used "diploid" diets and triploids have a higher dietary histidine requirement to prevent cataracts (Taylor et al., 2015), particularly during challenging environmental and developmental stages (Sambraus et al., 2017a). Therefore, future trials with triploid hybrids should use histidine-enforced diets to improve welfare. Diploid hybrids had the lowest cataract scores of all, which may be explained by their low growth. Growth rate is a risk factor for cataracts in Atlantic salmon (Bjerkås et al., 2001), and we found the largest groups at termination generally had the highest cataract scores (i.e. the triploid groups).

Hybridisation had an additive effect with triploidy on gut size. The smaller guts in the triploid hybrid are expected to increase their slaughter yield above triploid salmon, although we did not have adequate facilities to process fillets to a commercial standard to assess this. A greater slaughter yield would be an advantage of hybridisation if the feed conversion ratio (FCR) is the same. However, we did not measure FCR. We also found a general effect of ploidy, with triploids having a 21% reduction in relative gut size compared to diploids. This matches previous reports on gut length and the number and mass of pyloric caeca in triploid salmon (Peruzzi et al., 2015) and Atlantic cod (*Gadus morhua*) (Peruzzi et al., 2013). However, triploid salmon still had an absolute gut mass heavier than diploid salmon as they were larger fish. As such, it is unclear whether the relatively smaller gut effects triploid salmon growth. Although FCR was not measured in the current study, it has previously been found to be higher (Fraser et al., 2013) or equal (Sambraus et al., 2018) in 2–3 kg triploid salmon. Regarding the biological significance, although we did not specifically weigh or count caeca, the most detailed work on gut morphology use these criteria and have found mixed results. For example, a positive association between caeca number and feed conversion has been reported in rainbow trout, although there was no consistent effect of caeca number on growth (Bergot et al., 1981). Similarly, the number and length of caeca explained little variation in protein or fat digestibility in rainbow trout (Ulla and Gjedrem, 1985). Therefore, other aspects of gut morphology and physiology may be of more importance in explaining triploid and hybrid performance than caeca number and/or length.

The triploid hybrids had a redder fillet colouration than triploid salmon, which is favoured by consumers (Alfnes et al., 2006). In contrast, triploid salmon had a lower colouration than diploid salmon. Previously, triploid Atlantic salmon have been found to have equal or higher (Bjørenvik et al., 2004) or lower red colouration (Smedley et al., 2016) than diploids. Neither study identified the cause of the ploidy effect on colouration, although season (Bjørenvik et al., 2004) and sexual maturation (Choubert and Blanc, 1989) have significant influences on pigmentation in general. We only found one sexually mature individual, therefore this would not explain our general group effect. Work in rainbow trout has found little to no ploidy effect on fillet colouration (Choubert and Blanc, 1985), or the ability of triploids to fix the carotenoid canthaxanthin that is important for fillet colouration (Choubert and Blanc, 1989). The diploid hybrid had the lowest colouration score, which is likely to reflect their general poor growth in seawater. Future work should focus on other measures of fillet quality that may explain the ploidy effects on colouration.

Hybridisation had no effect on triploid body shape. Triploid salmon and triploid hybrids were very similar to diploid salmon in the morphometric analysis, although they did show some differences related to fin and maxilla length. Therefore, it is unlikely triploids will affect marketability based on body shape. The shorter length of the dorsal fin and the longer pelvic fin in triploids is difficult to explain, but it may be interesting to investigate whether it is due to interactions between loading capacity and bone mineralisation, the latter of which can differ between the ploidies (Sambraus et al., 2020). Hybridisation did result in alterations in head shape in diploids that could have been expected based on previous work in salmonids. For example, wild trout were found to have longer snouts and maxilla, and a thicker caudal peduncle, than wild salmon, with the wild hybrid intermediate (Solem et al., 2014). Similar results were found by Wilkins et al. (1994), with hybrids (female salmon × male trout) having a relatively longer maxilla and thicker caudal peduncle than salmon, in a mixture of wild and hatchery-bred populations. In addition, in a comparison of land-locked purebred strains, trout had longer snouts than salmon, but there was no difference in head and body depth (Pakkasmaa et al., 1998). We also found diploid hybrids had a longer snout and thicker peduncle than diploid salmon demonstrating the consistency of these findings across studies. As the triploid hybrid was more akin to diploid salmon than the diploid hybrid, as also observed in Wilkins et al. (1994), this could be explained by it being two-thirds' salmon and only one-third trout. Sex had some effects on maxilla length and snout length, being shorter in females. Sexual dimorphism in the jaw does occur in mature fish, with males developing a kype whereas females do not, although our analysis was done on fish that were immature during the natural breeding season.

In conclusion, triploid hybrids show similar performance to triploid salmon in a farm environment, the only benefit being an increase in fillet colour. We found no evidence of hybrid vigour on triploid size at harvest. Compared to diploids, triploid hybrids had the same issues with

vertebral deformities and cataracts as seen with triploid salmon and would most likely require unique diets to prevent these issues.

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## CRediT authorship contribution statement

**Thomas W.K. Fraser:** Investigation, Data curation, Formal analysis, Writing – original draft. **Tom J. Hansen:** Funding acquisition, Conceptualization, Resources, Investigation, Writing – review & editing. **Sofie C. Remø:** Investigation, Formal analysis, Writing – review & editing. **Rolf Erik Olsen:** Resources, Writing – review & editing. **Per Gunnar Fjelldal:** Funding acquisition, Conceptualization, Resources, Investigation, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

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