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Triploidy effects growth, life history strategies, and bone health in Arctic char (*Salvelinus alpinus*), but does not impact cataract incidence

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ABSTRACT

Skeletal deformities and ocular cataracts have limited the farm performance of sterile triploid salmonids, but have not been assessed in Arctic char (*Salvelinus alpinus*). We repeatedly radiographed mixed-sex diploid and triploid char ($n = 110$/ploidy) of Hammerfest (Norway) origin reared on a natural photoperiod and temperature in freshwater over a 3-year period and assessed cataracts at termination. At the population level, triploids were significantly ($p < 0.001$) heavier at tagging, but lighter at harvest (mass (g) ± SE; tagging, 48 ± 2 vs 67 ± 3; harvest, 2015 ± 83 vs 1639 ± 71, in diploids and triploids, respectively). These growth differences were mainly related to sex and life history, as immature fish were generally smaller than those that matured, and triploids showed lower levels of sexual maturation throughout (mature (%); diploid males, 20 and 65; diploid females, 12 and 83; triploid males, 2 and 40; triploid females 0 and 0, after 2 and 3 years, respectively). In addition, males grew quicker than females, irrespective of ploidy. When comparing fish of the same sex and life history, there was no significant growth disadvantage of triploidy. However, survival was significantly lower in triploids ($p <$ 0.001: 94 vs 86% from tagging to harvest in diploids and triploids, respectively) and they had a significantly higher incidence of fish with one or more deformed vertebra throughout ($p < 0.001$, 99 vs 71% in 3 year-old diploid and triploids, respectively). Cataract prevalence was high (>90% in both ploidy), but severity was low (on average, <10% of the lens shrouded) and not affected by ploidy ($p > 0.1$). Based on the average wet body mass of immature fish and losses due to sexual maturation and mortality, mono-sex stocks of male triploid fish gave the highest return per 100 juveniles stocked, followed by diploid males, diploid females, and triploid females (87.5, 83.0, 80.8, and 69.3 kg, respectively) at the earliest opportunity to harvest (i.e. when the fish first reached 0.7–1.0 kg). Therefore, all-male triploids may provide benefits to char aquaculture although their skeletal health should be addressed.

1. Introduction

Although Arctic char (*Salvelinus alpinus*) has been considered a promising candidate for aquaculture in temperate regions due to its high growth rate at relatively low temperatures (Le François et al., 2002), tolerance of high stocking densities (60–120 kg/m^3, Jørgensen et al., 1993), good flesh quality (Gunnarsson et al., 2012), and niche marketability (Yang et al., 2020), its global production is still low and relatively stagnant (Sæther et al., 2013; Yossa et al., 2019). A major production constraint for Arctic char is preharvest sexual maturation (Yossa et al., 2019), particularly in males, when nutrients are allocated away from somatic growth and into gonad development. This leads to reduced growth (Jobling and Baardvik, 1991) whilst simultaneously reducing flesh quality (McNiven et al., 2012). In addition, sexual maturation elevates cortisol that suppresses the immune system leaving fish more vulnerable to diseases and infections that can be fatal (Yada and Yada and Tort, 2016). Therefore, methods to prevent sexual maturation in Arctic char before they reach the market size of 0.7–1.0 kg are necessary to fully maximize production. The genetic containment of domestic salmonid stocks via sterility would also increase the sustainability of the industry, given the detrimental effects escapees have on wild populations via competition and interbreeding (e.g. Sylvester et al., 2018).

Several alternatives exist to prevent preharvest sexual maturation in char, but to date none are totally effective. For example, it is possible to selectively breed late maturing strains (Nilsson, 1992), produce all-females (Yossa et al., 2019), or delay maturation through photoperiod

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(Liu and Duston, 2019), feed (Liu and Duston, 2019), or water temperature (Gunnarsson et al., 2011) manipulation. Alternatively, one can use chromosome manipulation to produce triploids, fish with three complete chromosome sets compared to the natural diploid state whereby an individual has only two sets. Triploidy is relatively easy to induce in Arctic char using pressure shock to prevent the removal of the second polar body (Gillet et al., 2001). Although triploid male, and to a much lesser extent female, Arctic char may still show some gonadal development, their progeny die early in development and so they are considered to be functionally sterile (Gillet et al., 2001).

Three previous studies have found triploids to have significantly lower rates of sexual maturation than diploids (Gillet et al., 2001; Chiasson et al., 2009; McNiven et al., 2012) and no ploidy effect was found on body mass in immatures males (Chiasson et al., 2009), demonstrating triploid potential for aquaculture. However, other aspects of Arctic char production that may be affected by ploidy have yet to be evaluated. For instance, work in the closely related Atlantic salmon (*Salmo salar*) found triploids to have an increased prevalence of vertebral deformities (Fjelldal and Hansen, 2010) and ocular cataracts that impair vision (Wall and Richards, 1992), both of which reduce growth and are a welfare concern. Subsequently, skeletal deformities in triploid salmon have been alleviated via the use of low incubation temperatures (Fraser et al., 2015) and phosphorus enhanced diets (Fjelldal et al., 2016). Similarly, cataracts in triploids had been alleviated via the use of histidine enriched diets (Taylor et al., 2015). Therefore, from a production and welfare viewpoint, it would seem pertinent to assess skeletal deformities and cataracts in triploid Arctic char reared using current protocols and standard "diploid" feed.

In the current study we assessed vertebral deformities, cataracts, sexual maturation, and growth in diploid and triploid char. Our hypothesis is that triploid char will have lower levels of sexual maturation, but a higher prevalence of vertebral deformities and cataracts than diploids. Therefore, we assessed maturity and radiologically detectable vertebral deformities over time in a mixed sex population of diploid and triploid char up until market size after two years, plus one further breeding season at three years of age when cataracts were also assessed.

2. Material and methods

2.1. Ethics

All experiments were conducted in accordance with the laws and regulations of the Norwegian Regulation on Animal Experimentation 1996, with the Institute of Marine Research (IMR), Matre Research Station, an approved research facility by the Norwegian Food Safety Authority (Mattilsynet, IMR Matre Research Station 110/Virksomhetsnummer 110: Havforskningsinstituttet, Matre havbruksstasjon) for work with salmonids.

2.2. Fish stock and rearing conditions

The Arctic char broodstock were of the anadromous Hammerfest strain (Rikardsen et al., 1997), originally purchased from a commercial supplier, and brought to the IMR Matre Research station as eyed eggs. The family relatedness of the broodstock used was unknown. On the 19th October 2016 (day 0), eggs from 10 females were pooled in a container and mixed with the sperm from 3 males that had been pooled in a separate container. Half of the eggs were subject to hydrostatic pressure shock to induce triploidy (see below). The eggs were incubated in trays in a flow-through system at 6 °C, and the fry were first fed on day 126 at 10 °C in 0.6 m tanks. The fish were transferred to two (1 tank/ploidy) 1 m tanks (total weight = 45 and 160 g for triploids and diploids, respectively, density 0.12–0.44 kg/ m^3) with natural temperature on day 183. Here, we kept additional diploids for use in another study and due to a lack of available free tanks their density was slightly higher prior to the initiation of the current work. The current experiment began on day 387 when the fish ($n = 110$ diploids and 107 triploids) were PIT-tagged and both ploidy were reared in common garden (equal numbers of each ploidy per tank) between three 1 m tanks ($n = 217$, mean weight = 61 g, density = approx. 12.2 kg/ m^3). On day 616, the fish were divided amongst six 1 m tanks ($n = 216$, mean weight = 418 g, density = approx. 42 kg/ m^3). On day 763, all fish were moved into one 5 m ø tank ($n = 206$, mean weight = 983 g, density = 12 kg/ m^3) until the end of the study ($n = 200$, mean weight = 2048 g, density = 24 kg/ m^3). The freshwater was sourced locally and ranged from 1.4 to 15.9 °C depending on the time of year (Fig. 1). The photoperiod was continuous from first feeding up until day 348 and simulated natural thereafter according to civil twilight hours (60°N). Throughout, the fish were fed ad libitum with standard commercial salmonid feed ("Nutra sprint" and "Spirit – Supreme", Skretting, Stavanger, Norway) adjusted for the appropriate pellet size.

2.3. Sampling

Body mass, fork length, and PIT tag were recorded on seven occasions, days 387, 616, 714, 763, 878, 983, and 1106 (Fig. 1). Fish were anaesthetised in 100 mg/L Finquel® (MS 222) prior to handling. Live fish were radiographed on days 387, 616, and 714 whereas freshly slaughtered fish were x-rayed on day 1106.

Fulton's condition (K) factor (body mass (g)/fork length $(cm)^3 \times 100$) was used as a measure of body condition. Specific growth rates for individual fish were calculated using the formula $(e^q - 1) \times 100$ (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. Sexual maturation

We recorded sexual maturation based on external appearance and the occurrence of running milt/ovulating eggs (when the fish were fully sexually mature and showed strong secondary sexual characters) around the expected times of breeding in October at 2 and 3 years of age (days 714 and 1106, respectively). On day 1106, we also visually assessed the gonads as all the fish were euthanised. Based on this data, we identified four different life histories, fish that matured only at 2 years of age, fish that matured only at 3 years of age, fish that matured at 2 and 3 years of age, and fish that remained immature throughout (from here on referred to as early, late, successive, and non-maturing, respectively).

2.5. Radiology and deformity classification

Each individual was radiographed on four separate occasions. On day 387, all fish were radiographed alive whist lightly sedated (Porta 100 HF, Eickemeyer Medizintechnik für Tierärzte KG, Tuttlingen, Germany) using a 35 × 43 cm image plate in a rigid cassette (Dürr Medical, Bietigheim-Bissingen, Germany) with 40 kV and 10 mA at a distance of 70 cm. The image plate was scanned (CR 35 VET, Dürr Medical, Bietigheim-Bissingen, Germany), and the resulting image was converted into a TIFF file (Vet-Exam Plus Software, version 4.14.0). On days 616 and 714, all fish were radiographed alive whilst lightly sedated using 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. On day 1106, the fish were radiographed using the same equipment as on days 616 and 714, but the fish had already been euthanized. Vertebral deformities were evaluated according to the classification of Witten et al. (2009).

2.6. Cataracts

Fish were inspected for cataracts using a slit lamp microscope (HEINE® HSL 150 hand-held slit lamp, HEINE Optotechnik) on day

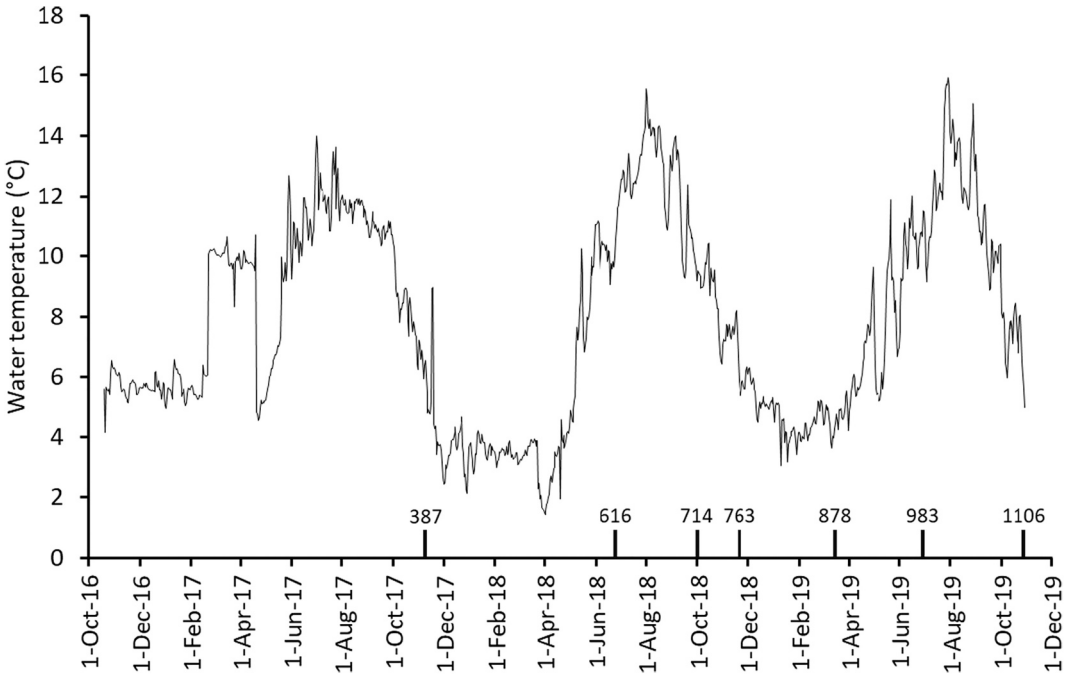

Fig. 1. Water temperature and sampling times (days post fertilization) during the study. The vertical ticks on the x axis represent the different sampling times according to experimental day (0 being the day of fertilization on the 19th October 2016).

1106. 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).

Histidine and N-Acetylhistidine (NAH) concentration was determined in 10 lenses from each ploidy at the termination of the trial, according to the method described by O'Dowd et al. (1990) and slightly modified by Breck et al. (2005).

2.7. Triploidisation and ploidy confirmation

Thirty-seven minutes and 30 s after fertilization at 8 °C, those eggs to be triploidised were subjected to a hydrostatic pressure of 655 bar for 6 min and 15 s (TRC-APV, Aqua Pressure Vessel, TRC Hydraulics Inc., Dieppe, Canada) to induce triploidy. Thereafter, the ploidy level was assessed by erythrocyte diameter (Yossa et al., 2018) taken from blood smears collected from the first 120 fish sampled at the end of the study on day 1106 ($n = 63$ and 57 putative diploids and triploids, respectively) using Image J and the and the ObjectJ project 'Elliptical oocytes' as described in Thorsen and Kjesbu (2001). Of these, there was no overlap in mean erythrocyte diameter between putative diploids and triploids (mean ± min, max; diploids, 14.5 ± 13.7, 15.5; triploids, 16.5 ± 15.6, 17.1 μm) indicating 100% successful triploidisation.

2.8. Statistics

R version 3.6.1 was used for statistical analysis. Significance was assumed at $p < 0.05$. Throughout, model diagnostics were assessed via qqplots and standardised versus predicted residual plots. Least-square (LS) means were used as post-hoc tests where significant model effects were identified.

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

To assess sexual maturation, a generalised linear model (GLM) was used with a binomial response using the data for males only, as no triploid females matured. Mature (Yes/No) was the dependent variable, with ploidy (2 levels) and time (2 levels, day 714 and 1106) as independent variables, and the interaction between ploidy and time.

To assess vertebral deformity prevalence, a GLM was used with a binomial response. Deformed (Yes/No) was the dependent variable, with ploidy (2 levels) and time (continuous) as independent variables, and the interaction between ploidy and time. Sex and life history were not included in the models as preliminary plots of the data showed no association with the prevalence of skeletal deformities (see supplementary R script). To assess deformity severity, the number of deformed vertebra 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 time (continuous) as the independent variables, and the 2-way interaction between ploidy and time. To assess vertebral deformities and growth, a GLM was used with body mass as the dependent variable and the number of deformed vertebra (continuous), body mass from the initial time point (continuous), ploidy (2 levels), life history (5 levels), and sex (2 levels) as independent variables. Three separate models were run, one for days 387 to 616, 616 to 714, and 714 to 1106.

To assess cataract prevalence, a GLM was used with a binomial response. Cataracts (Yes/No) was the dependent variable, with ploidy (2 levels) as an independent variable. Following this, a proportional odds linear regression (POLR) was used to assess cataract severity. Cataract score (ordered) was set as the dependent variable and ploidy (2 levels) was set as the independent variable. Sex and life history were not included in the models as preliminary plots of the data showed no association with cataract prevalence or severity (see supplementary R script).

To assess body mass over time, initially a linear mixed effect (LME) model was used with body size (mass, fork length, condition) as the dependent variable, ploidy (2 levels) and time (7 levels) as categorical independent variables, and fish as a random effect. Body mass and length were natural logged transformed, and time was converted from a continuous to categorical variable, to improve model diagnostics (see supplementary R script). Subsequent plots of body mass over time in male and female diploid and triploid fish showed notable time trends due to life history and a negative effect of deformities (see results), but not cataracts (see supplementary R script). As all the triploid females were non-maturing and triploid males only matured at 3 years of age (i.

e. the late life history strategy) a further four LME models were made to investigate ploidy effects when controlling for life history and skeletal deformities. The first assessed body size in only those fish that were recorded as immature at 2 years of age (day 714) with ploidy (2 levels), sex (2 levels), time (continuous, up to day 714 only) and the number of deformed vertebrae on day 714 (continuous) as independent variables. The second assessed body mass in non-maturing fish throughout the whole experiment only, with ploidy (2 levels), sex (2 levels), time (7 levels) and the number of deformed vertebrae on day 1106 (continuous) as independent variables. The third model assessed ploidy, life history (only 2 levels, as there were not enough triploids that displayed either the early [no fish] or successive [1 fish] strategies), time (7 levels) and the number of deformed vertebrae on day 1106 (continuous) in males only. The final model assessed the interaction between sex (2 levels), life history (only 3 levels, as just a single male fish showed the early strategy), time (7 levels), and the number of deformed vertebrae on day 1106 in diploids. In addition, non-maturing fish displayed bimodality in growth from day 714. Therefore, we split all life histories into upper and lower modes based on a specific growth rate of 0.09 between days 714 and 1106 (Fig. 2). It was also noted that triploid females appeared to show bimodality in body mass gain between days 387 and 714 that was clearly observable on day 614 (fish above and below 400 g, see supplementary R script), but this was not modelled further as it was not seen in any other group and was not clearly associated with later life performance (see supplementary R script). When applicable, in all the previously described models, we allowed for all possible 2- and 3-way interactions between ploidy, time, sex, and life history, and individual fish was included as a random effect to account for repeated measures. Where 3-way interactions were included, the model was then compared to another model that included only 2-way interactions using the Akaike Information Criterion with a correction for small sample sizes (AICc). The model with the lowest AICc score was considered the best data fit in relation to model complexity. The same approach was used to assess body length and condition.

3. Results

3.1. Survival

Triploids had a significantly lower survival rate than diploids (COXPH, $p < 0.001$) between tagging (day 387) and termination (survival at termination (%), 95% CI; 94, 92–95 and 86, 84–89 in diploids and triploids, respectively). The most notable drops in survival for triploids occurred between days 616–714 (Jun and Oct 2018) and 878–983 (Mar and Jun 2019). In contrast, the most notable drop in diploid survival occurred between days 714–763 (Oct and Nov 2018). The cause of death for individual fish was not determined.

3.2. Maturation and life history

No triploid females matured during the study, whereas maturity incidence increased between days 724 and 1106 (2 and 3 years of age) in diploid females (Table 1). Triploid males showed a significantly lower maturity incidence compared to diploids (GLM; $\chi^2 = 14$, df $= 1$, $p < 0.001$), although both ploidy showed a significant increase at 3 compared to 2 years of age (GLM; $\chi^2 = 47$, df $= 1$, $p < 0.001$).

In those fish that survived until the end of the study, whereas diploid males demonstrated all four life history strategies (non-maturing, early, late, successive), only three strategies were recorded in triploid males (Table 1). Similarly, diploid females showed three strategies, whereas triploid females only the one. In diploids, the most common strategy was late maturing for both sexes, followed by non-maturing fish. In triploid males, the most common strategy was non-maturing followed by the late maturing strategy.

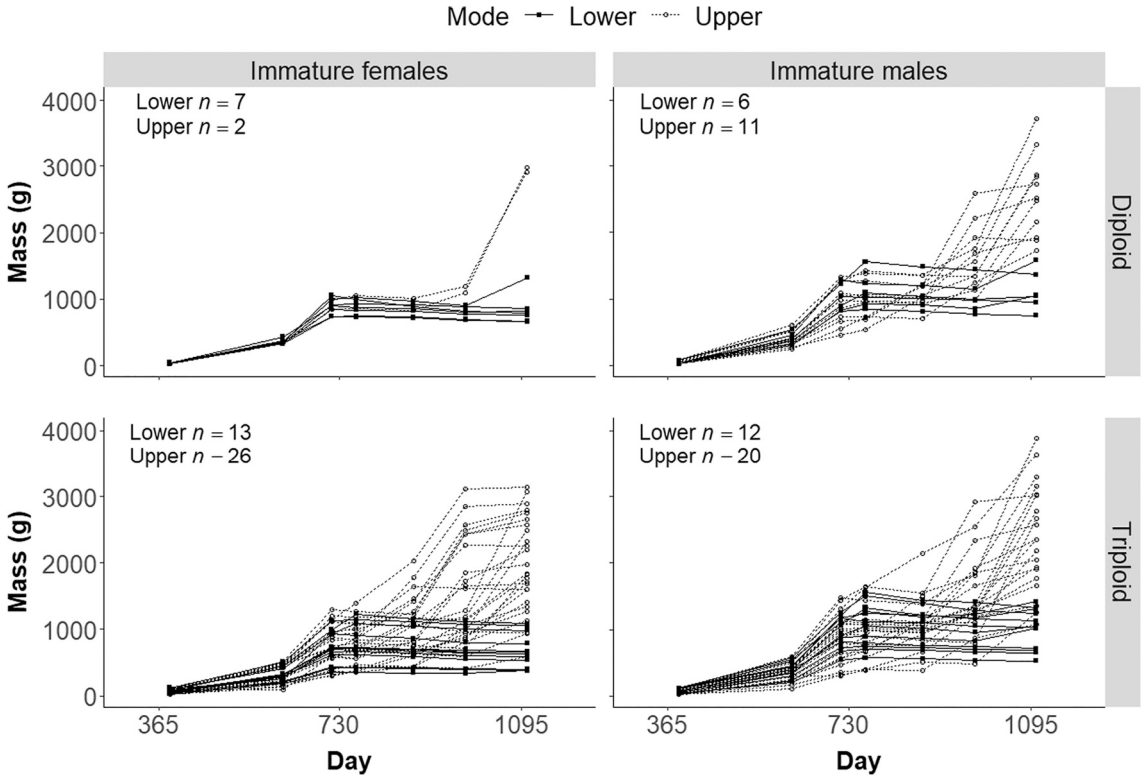

Fig. 2. Bimodality in growth. Line plots for individual fish showing body mass over time in upper and lower mode non-maturing fish. The distinction between upper and lower mode fish was based on a specific growth rate of above (upper mode) or below (lower mode) 0.09%/day between days 714 and 1106. On day 1106, the coefficient of variation (Standard deviation/ Mean × 100) at the final sampling for non-maturing fish (pooled for sex) was 53% for both diploids and triploids.

Table 1
The prevalence of sexual maturation and life history strategy within diploid and triploid Arctic char reared over 3 years.

Sex	Ploidy	*n*	Mature (%)		Life history (%)			
			Age 2	Age 3	Non-maturing (lower/upper)*	Early	Late	Successive
Female	Diploid	52	11.5	82.7	17.3 (13.5/3.8)	0.0	71.2	11.5
	Triploid	40	0	0	100 (33.3/66.7)	0.0	0.0	0.0
Male	Diploid	51	19.6	64.7	33.3 (11.8/21.6)	3.0	47.1	17.6
	Triploid	53	1.9	39.6	60.4 (22.6/37.7)	0.0	37.7	1.9

* Non-maturing fish were split into an upper and lower mode based on a specific growth rate of 0.09% day^{-1} between days 714 and 1106.

3.3. Deformities

There was a significant interaction between ploidy and day for the incidence of fish with ≥1 deformed vertebra (LME, Ploidy × Day; $\chi^2 = 189$, df = 1, $p = 0.017$). The prevalence of deformed fish increased over time and triploid char had a significantly higher prevalence of fish with vertebral deformities than diploids at all time points, although the ploidy effect decreased over time as triploid values began to plateau after surpassing 85% from day 616 onwards whereas diploid values continued to increase until the end of the study (Fig. 3A). There were significant ploidy (LME; $\chi^2 = 40$, df = 1, $p \leq 0.001$) and day (LME; $\chi^2 = 475$, df = 3, $p \leq 0.001$) effects, but no interaction, on the number of deformed vertebrae per deformed fish. The number of deformed vertebrae increased over time in both ploidy, with triploid values being significantly higher than diploids (Fig. 3B).

The majority of deformities were related to compression and/or fusion and their relative frequency were similar between the two ploidy (Fig. S1). In diploids, deformed vertebra were mostly located between vertebra 1 and 18 at the initiation of the study (Fig. S2) with newer deformities mainly detected between vertebra 1 and 38 and 49 and 54 on day 616 and between vertebra 1 and 58 on day 714. At the termination of the study, there was a prominent peak in deformity rate around vertebra no. 36 that was not seen at earlier timepoints. The results in triploids mirrored those in diploids, with deformities occurring progressively further along the vertebral column from the head to the tail between days 387 and 714, with a prominent peak in deformity rate around vertebra no. 33 on day 1106. Radiographs of representative deformities can be found in Fig. S3.

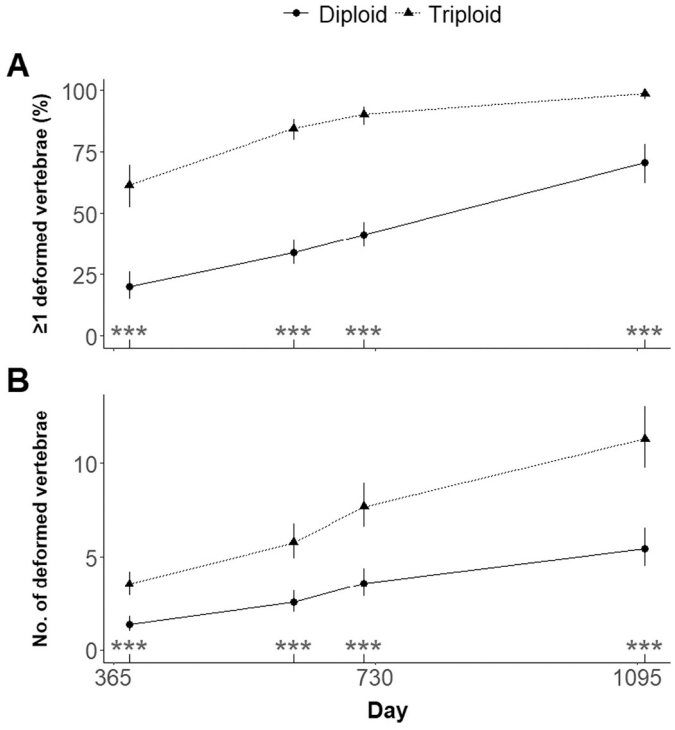

Fig. 3. Deformity incidence in diploid and triploid Arctic char over time. (A) The incidence of fish with one or more deformed vertebra. (B) The number of deformed vertebrae per deformed fish over time (N = 19–92 fish/ploidy/day). Day 0 was the 19th October 2016. Data are means ±95% CI. In (A), asterisks represent significant ploidy effects within sampling day (LS means, *** $p < 0.001$).

3.4. Cataracts and lens histidine NAH concentration

There was no ploidy effect on the prevalence of cataracts (GLM; $\chi^2 = 1.2$, df = 1, $p = 0.269$. LS means probability (%) ± SE; 98.4 ± 0.02 and 94.9 ± 0.03, in diploids and triploids, respectively) or on the cataract score (POLR; $\chi^2 = 2.3$, df = 1, $p = 0.131$. LS means ± SE; 2.0 ± 0.1 and 2.3 ± 0.1 in diploids and triploids, respectively). There was no ploidy effect on the concentrations of lens histidine (GLM; $\chi^2 = 0.001$, df = 1, $p = 0.970$. LS means [μmol/ g] ± SE; 1.48 ± 0.1 and 1.47 ± 0.1 in diploids and triploids, respectively) or NAH (GLM; $\chi^2 = 0.1$, df = 1, $p = 0.757$. LS means [μmol/ g] ± SE; 10.3 ± 0.8 and 10.6 ± 0.7 in diploids and triploids, respectively).

3.5. Ploidy, life history, and growth

At the population level, triploids were significantly heavier than diploids on day 387, but were significantly lighter from day 616 onwards (LME, Ploidy × day interaction; $\chi^2 = 174$, df = 6, $p \leq 0.001$. Fig. 4A). This result held true when assessing body mass over time in fish that were immature on day 714, even when accounting for sex, for which males were larger than females, and the number of deformed vertebrae (see supplementary R script). However, when accounting for life history over the entire 3-year period, triploids were always significantly heavier on day 387, but there was no ploidy effect thereafter in males (LME, Ploidy × day interaction; $\chi^2 = 159$, df = 6, $p \leq 0.001$. Fig. 4B) or females (LME, Ploidy × day interaction; $\chi^2 = 82$, df = 6, $p \leq 0.001$. Fig. 4C). Fish length followed the same trends as for body mass (see supplementary R script).

In diploids only, those fish that matured at 2 and 3 years of age (i.e. a successive life history) were significantly larger than late and non-maturing fish from tagging until day 714 (Table 2). Growth in successive, late, and the upper mode fraction of non-maturing fish was similar between days 714 and 1016, but the lower mode fraction of non-maturing fish became significantly smaller (the comparison of upper and lower mode can be seen in Fig. 2). Females were significantly lighter than males from day 878 onwards irrespective of reproductive strategy (LME, Sex × Day; $\chi^2 = 20$, df = 6, $p = 0.003$). Fish length followed the same trends (see supplementary R script).

At the population level, there was a significant interaction between ploidy and day (LME, Ploidy × Day; $\chi^2 = 43$, df = 6, $p < 0.001$). Body condition was significantly higher (Post-hoc, LS means, $p < 0.05$) in triploids than diploids on day 387, but significantly lower on days 616 and 983. In diploids only, those fish that would mature generally had higher body condition going into the reproductive season than those fish that remained immature (LME, Life history × Day; $\chi^2 = 280$, df = 18, $p < 0.001$, Table 2). In non-maturing fish, those from the lower mode showed a general reduction in condition between days 714 and 1016,

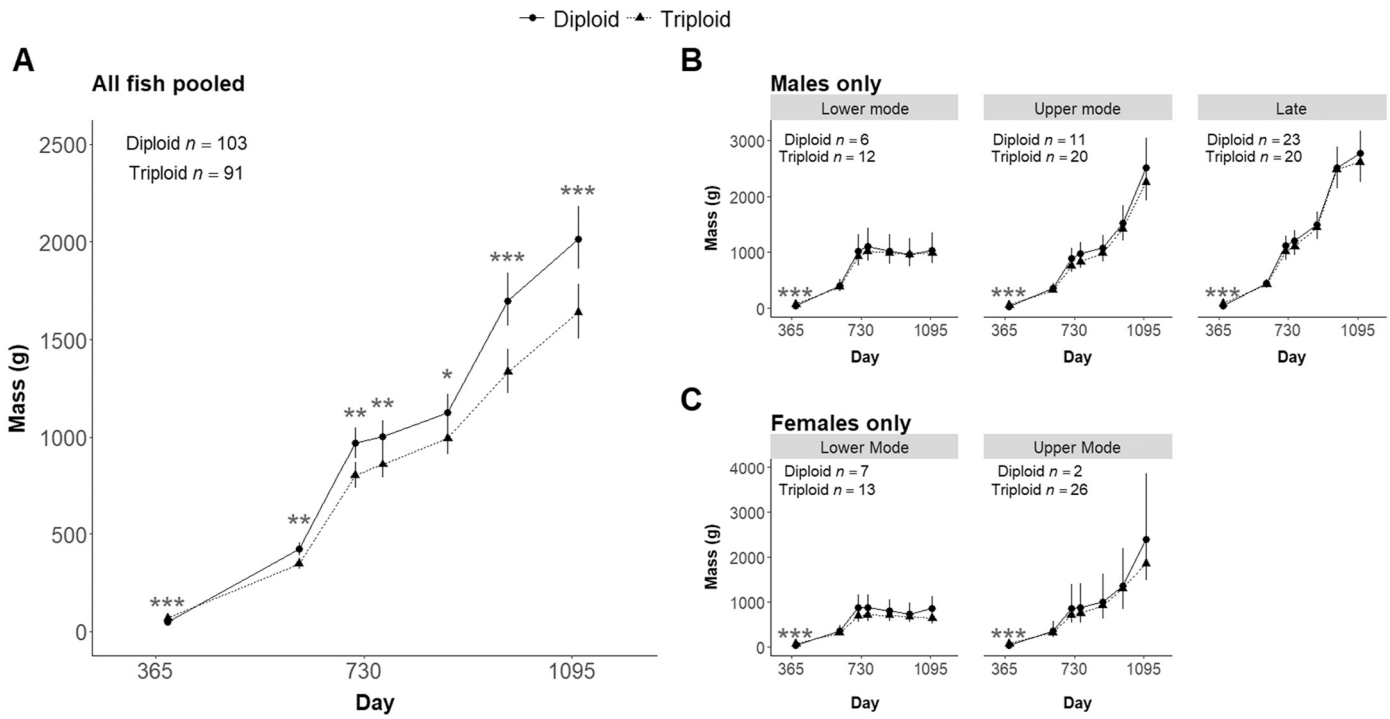

Fig. 4. Body mass over time. (A) Diploid and triploid Arctic char over the entire period of study. Data does not consider sex, life history, or vertebral deformities. There was a significant interaction between ploidy and day. (B) Body mass in diploid and triploid male char separated by life history. (C) Body mass in diploid and triploid female char separated by life history. Day 0 was the 19th October 2016. (A-C) Data are back transformed means ±95% CI and asterisks represent significant ploidy effects within sampling day (LS means, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

Table 2
Body weight and condition factor in diploid Arctic char with different life history strategies over time. The four life histories are immature fish with low growth after day 714 (Lower mode), immature fish with high growth after day 714 (Upper mode), fish that matured at 3 years of age (Late) and fish that matured at both 2 and 3 years of age (Successive). Data are means ±95% CI. Different lowercase letters indicate life history effects within time point (those life histories that do not share a lowercase symbol are significantly different from one another, post-hoc LS means, $p < 0.05$).

Parameter	Day	Lower mode ($n = 13$)	Upper mode ($n = 13$)	Late ($n = 59$)	Successive ($n = 15$)
Weight (g)	387	38 (32–43)^{b}	39 (32–46)^{b}	49 (46–53)^{ab}	69 (60–79)^{a}
	616	373 (322–432)^{b}	378 (316–451)^{b}	425 (397–456)^{b}	537 (468–616)^{a}
	714	926 (800–1073)	881 (737–1052)	1028 (959–1102)	932 (813–1068)
	763	962 (830–1114)^{ab}	917 (768–1096)^{ab}	1091 (1018–1170)^{a}	868 (757–995)^{b}
	878	928 (801–1074)^{b}	1013 (848–1211)^{ab}	1278 (1192–1370)^{a}	995 (867–1140)^{b}
	983	885 (764–1025)^{c}	1400 (1172–1673)^{b}	2101 (1959–2253)^{a}	1829 (1595–2097)^{ab}
	1106	931 (804–1078)^{b}	2462 (2061–2941)^{a}	2351 (2193–2521)^{a}	2194 (1913–2516)^{a}
Body condition (K)	387	1.19 (1.08–1.29)	1.15 (1.03–1.27)	1.16 (1.11–1.21)	1.22 (1.12–1.32)
	616	1.65 (1.55–1.76)	1.67 (1.55–1.79)	1.62 (1.57–1.67)	1.73 (1.63–1.83)
	714	1.80 (1.69–1.90)^{a}	1.74 (1.62–1.86)^{ab}	1.79 (1.74–1.84)^{ab}	1.62 (1.53–1.72)^{b}
	763	1.64 (1.53–1.74)^{ab}	1.60 (1.48–1.72)^{ab}	1.66 (1.61–1.71)^{a}	1.49 (1.39–1.59)^{b}
	878	1.46 (1.35–1.56)^{b}	1.53 (1.41–1.64)^{ab}	1.67 (1.62–1.72)^{a}	1.53 (1.43–1.63)^{ab}
	983	1.28 (1.17–1.38)^{c}	1.73 (1.61–1.85)^{b}	1.97 (1.92–2.02)^{a}	1.92 (1.82–2.01)^{ab}
	1106	1.25 (1.15–1.35)^{c}	1.94 (1.82–2.06)^{a}	1.73 (1.68–1.78)^{b}	1.76 (1.66–1.86)^{ab}

whereas the upper mode showed an increase (Table 2).

3.6. Vertebral deformities, cataracts, and growth

Although the number of deformed vertebrae had no significant effect on final body mass (GLM; $\chi^2 = 0.4$, df = 1, $p = 0.504$), there were some significant negative associations between the number of deformed vertebrae at the beginning and body mass at the next time period (between days 387 to 616. GLM; $\chi^2 = 4$, df = 1, $p = 0.045$) and a strong tendency in the final time period (between days 714 to 1106. GLM; $\chi^2 = 4$, df = 1, $p = 0.054$). There was no association between cataract score and body mass at termination (GLM; $\chi^2 = 5$, df = 6, $p = 0.521$), although fish with higher cataract scores were generally heavier throughout the study (see supplementary material).

3.7. Harvest mass

Taking the average mass of male and female immature fish on day 714 (i.e. when the fish reached the market size of 0.7–1.0 kg) for each ploidy and subtracting losses due to the prevalence of sexual maturation and mortalities, we calculate that per 100 juveniles stocked, returns would be highest for triploid males, diploid males, diploid females, and then triploid females, respectively (Table 3).

4. Discussion

Triploidy had no impact on body weight in Artic char at 2 or 3 years of age when accounting for sex and life history. Furthermore, although triploids had lower survival and more skeletal deformities, these issues

Table 3
Predicted sum of harvest mass at 2 years of age from immature fish based on stocking 100 juveniles of each sex and ploidy.

Parameter	Female		Male	
	Diploid	Triploid	Diploid	Triploid
Mean harvest mass (g)*	945	745	1072	961
Losses due to maturation (%)	11.5	0	19.6	1.9
Losses due to mortality (%)	3	7	3	7
Harvest mass (kg) 100^{-1} juveniles	80.8	69.3	83.0	87.5

* Mean mass of immature fish within ploidy/sex on day 714.

were "offset" in terms of harvest yield by reduced maturation. Subsequently, using all-male triploids would theoretically have produced the heaviest yield of market size immature fish at the earliest opportunity using our genetic stock. Future work should address the nutritional and environmental requirements for triploid Arctic char to improve their welfare.

At the population level, although our triploids were 40% heavier at tagging, they were 18% lighter after 2 to 3 years. However, the perceived triploid growth disadvantage was lost when we took sex and life history into account. Similarly, although mixed sex triploid char were found to be 13% lighter than diploid counterparts, there was no ploidy effect when comparing males only in a Labrador (Canada) strain (Chiasson et al., 2009). Nevertheless, diploids still had a relatively higher proportion of fish demonstrating the quickest growing strategies and fewer vertebral deformities that resulted in their better population level growth. As such, irrespective of ploidy, the most efficient production strategy may be to rear males that mature at 3 years of age as they were the largest immature fraction at market size (0.7–1.0 kg). Age of puberty is heritable in char (Nilsson, 1992), and triploid families show similar rankings in terms of growth performance as diploids (Chiasson et al., 2009), but whether it is possible to genetically select for age of puberty in triploid males is unclear.

Our growth analysis was complicated by life history and bimodality within non-maturing fish. For example, fish that matured at 3 years of age were significantly larger than non-maturing fish more than 1 year earlier. However, this pattern was lost when non-maturing fish were further split into upper and lower modes based on their growth between 2 and 3 years of age. Here, it would have been interesting to attain physiological data to determine whether these differences could be explained by "failed" attempts to mature or "dummy" runs, especially given the upper mode non-maturing fish grew at a similar pace to late maturing fish. A "dummy" run is when the brain-pituitary-gonad axis is activated one year prior to maturation, although no gonad development occurs (Okuzawa, 2002). Similarly, both female (Andersson et al., 2013) and male (Fraser et al., 2019b) Atlantic salmon are capable of initiating, but then stopping gonad development prior to reaching full sexual maturity. These "failed" attempts were linked to hormonal surges and growth spurts and were associated with an increased likelihood of completing maturation during the next breeding season (Fraser et al., 2019b). However, triploid females also showed bimodality in growth even though they showed no maturation and do not show surges in sex steroids or significant gonad maturation (Lincoln and Scott, 1984; Benfey et al., 1989). As such, it appears unlikely the anabolic effects of reproductive hormones could explain this result. Nevertheless, triploid female brook char were found to show seasonal fluctuations in vitellogenin production, albeit at much lower levels than diploids (Schafhauser-Smith and Benfey, 2001), and 2 and 3 year-old female triploid rainbow trout showed seasonal elevations of plasma gonadotropin that corresponded to minor elevations of plasma testosterone (Kobayashi et al., 1998), suggesting they do have some capacity to respond to seasonal environmental signals.

One of the most common reasons for using immature/sterile triploid fish is that they should have a growth and survival advantage over mature fish around the reproductive window (Sumpter et al., 1991; Trippel et al., 2014). We found some evidence of catch-up growth in non-maturing fish around the breeding seasons, but we also observed a lower mode that showed very limited growth between 2 and 3 years of age. Nevertheless, at no time point were the non-maturing fish heavier or longer than those that matured, demonstrating little long-term "cost" to maturation within our culture environment. A previous laboratory experiment in Atlantic cod (*G. morhua*) also found little evidence of a long-term cost of reproduction on growth over 4 years, although some transient differences were observed, and survival was lower in maturing fish (Trippel et al., 2014). In contrast, Sumpter et al. (1991) found cultured immature rainbow trout males (all the females matured), although initially lighter, were significantly heavier by the end of the reproductive season compared to mature males. Here, it is noted that Sumpter et al. (1991) used an elevated winter temperature (10 °C) in contrast to the current study and that of Trippel et al. (2014) that used natural temperatures. Consequently, our temperatures were around 4 °C following the breeding season, which is considerably lower than the optimum temperature for growth in char (e.g. 11 °C, Eriksson et al., 2010). These low temperatures would have limited the effect of reduced appetite on growth in mature fish and allowed them to recondition prior to the next "growing" season (Boyer and Van Toever, 1993).

The occurrence of the lower mode fraction in 12–33% of the non-maturing fish is puzzling and difficult to explain. Boyer and Van Toever (1993) also experienced that 30–50% of previously mature Canadian char never re-initiate feeding after the spawning season. As our non-maturing fish were not significantly smaller than any other life history strategy at the beginning of year 2, we feel it is unlikely this result is explained by the formation of social hierarchies. Alternatively, "loser" fish or "stunts", fish that stop growing and become emaciated, are found in Atlantic salmon farming. The reasons behind their occurrence have yet to be elucidated, although they do occur more frequently in response to challenging environmental conditions of high temperature and hypoxia during seawater adaption (Fraser et al., 2020). However, we do not consider our culture environment to be particularly challenging with maximum temperatures of 15.9 °C. The bimodality in growth led to extremely high variation in individual growth that was not related to gonad development. A greater understanding of the genetic and environmental factors that regulate this variation is therefore required to improve the sustainability of the char industry.

We found males outgrew females, even in non-maturing fish. Sexual dimorphism in immature farmed fish has previously been reported in char (Árnason et al., 2014) and in other salmonids, such as Atlantic salmon (Gjerde and Gjedrem, 1984), rainbow trout (*Oncorhynchus mykiss*) (Gjerde, 1989), and brown trout (*Salmo trutta*) (Bonnet et al., 1999), although the mechanism has not been studied. As the sex difference in non-maturing fish occurred between 2 and 3 years of age, it is unclear whether this is due to life history (the age in which the fish will mature) or dummy runs. For instance, if we had kept the fish until they all matured, we would have been able to back-track their growth for a more complete comparison.

We found triploid males to have significantly lower levels of sexual maturation than diploids, and no sexual maturation in triploid females. Similarly, Chiasson et al. (2009) reported 13 and 37% of male triploid and diploid Arctic char, respectively, matured at age 2, although female maturation was not reported. Using the same population as Chiasson et al. (2009), McNiven et al. (2012) reported no sexual maturity in triploids of either sex at approx. 1 kg, although gonad development occurred in both diploid sexes. In contrast, in a Lake Geneva (France) strain grown relatively slowly (approx. 600–700 g at 3 years of age), Gillet et al. (2001) reported sexually mature triploid females and no ploidy effect on the prevalence of maturation in males. Although female triploid salmonids are capable of producing mature oocytes, they number just a fraction of what is observed in diploids and generally do not result in increases in the gonadosomatic index during the reproductive season (e.g. Kobayashi et al., 1998; Schafhauser-Smith and Benfey, 2001). Therefore, our results align more closely with work in

other triploid salmonids, rather than the work of Gillet et al. (2001) in char. However, an equal incidence of sexual maturation in male diploid and triploid males is not uncommon and has been reported in several salmonid species, including Atlantic salmon (Oppedal et al., 2003), pink salmon (*Oncorhynchus gorbuscha*) (Benfey et al., 1989), and rainbow trout (Benfey et al., 1986). Nevertheless, using our genetic stock and rearing protocol, triploidy provided an effective method to reduce sexual maturation. Indeed, around market size, sexual maturation was 0 and 2% in female and male triploids, respectively, which is similar to low maturation strains in Sweden that have <5% maturation in fish <800 g (Eriksson et al., 2010).

Triploidy increased the prevalence and severity of vertebral deformities as reported in other salmonids, such as Atlantic salmon (Fjelldal and Hansen, 2010), brown trout (Preston et al., 2017), and rainbow trout (Weber et al., 2014), when reared on diploid protocols. In contrast, lower incubations temperatures (Fraser et al., 2015) and increased dietary phosphorus (Fjelldal et al., 2016), an important mineral component of the vertebra, alleviate vertebral deformities in triploid Atlantic salmon suggesting they have different temperature optima and nutritional requirements. The former is generally associated with cell size, as larger cell sizes/genomes are either better adapted or not a disadvantage in colder environments that conforms with work in Atlantic salmon (Sambraus et al., 2017a, 2018; Riseth et al., 2020). To date, no study has determined temperature optima in triploid char, although it is suggested to be lower in triploids in the closely related brook trout (*Salvelinus fontinalis*) (Atkins and Benfey, 2008). The higher dietary phosphorus requirement of triploids is most noticeable during early development (Sambraus et al., 2020), the life stage with the highest requirement in salmonids (Shearer, 1995), and is possibly due to larger genomes having higher per cell nucleic acid content for which phosphorus is an important component (Neiman et al., 2012). The commercial salmonid diet we used is designed for diploids, and we assume there is scope to reduce deformities in triploid char via tailored diets as in Atlantic salmon.

Although no radiological studies of deformities in Arctic char exist, the location and type share similarities with work in Atlantic salmon. For example, deformities were mainly related to compression and fusion, as commonly found in both diploid and triploid Atlantic salmon (Fjelldal and Hansen, 2010). Vertebral deformities were most apparent towards the head, with newer cases being found further along the spine towards the tail as the fish grew. Similarly, in Atlantic salmon, vertebral deformities in the tail are generally a greater issue later in life (Fjelldal et al., 2009), but may occur during early development if inappropriate incubation temperatures are used (Fraser et al., 2015). Of note, both ploidy showed a peak in deformities at vertebra 33 and 36 for diploids and triploids, respectively, during the final year. In triploid Atlantic salmon, and to a lesser extent in diploids, deformity peaks around vertebrae 24–29 are common (Fjelldal and Hansen, 2010), especially when using higher (≥8 °C) incubation temperatures (Fraser et al., 2015). Intriguingly, in diploid Atlantic salmon × Arctic char (Skogseidvatnet strain) hybrids we found vertebral deformity peaks at vertebrae 28 (similar to salmon) and a second peak between vertebrae 34–37 (as in the current work on Arctic char), albeit in smaller fish of around 45 g (Fraser et al., 2021). This may suggest these peaks are species specific, but more work is required into defining the distinct anatomical characteristics of the vertebral column in Arctic char and Atlantic salmon before reliable cross-species comparisons can be made.

The prevalence of diploids with radiologically detectable deformed vertebra was around 20 and 40% in juvenile and market size fish, respectively. Our juvenile levels are comparable to similar sized diploid salmon (approx. 20%, Fraser et al., 2021), and it is typical to see more deformities in harvest size fish compared to juveniles (Fjelldal et al., 2007). At market size, the occurrence of Atlantic salmon with radiologically detectable deformed vertebra can range between approx. 10–70% (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. Therefore, our data suggests cultured char do not have additional welfare concerns regarding skeletal health compared to Atlantic salmon. Nevertheless, in Atlantic salmon, growth is impaired in fish with ≥10 deformed vertebra (Hansen et al., 2010). We also found some negative associations between the number of deformed vertebrae and growth during the subsequent period, although the number of deformed vertebrae at termination was not associated with mass at the final sampling. However, the latter may simply indicate high growth rate is causing the vertebral deformities, as it is a risk factor in other salmonids (Fjelldal et al., 2006).

The prevalence of cataracts in the present study was high, but it matches a previous report of >90% prevalence of cataracts in two populations of 3-year-old farmed Arctic char from Finland (Peuhkuri et al., 2009). There was no ploidy effect on the prevalence or severity of cataracts, which was somewhat unexpected given triploidy is a risk factor for their development in Atlantic salmon (Wall and Richards, 1992) due to a higher dietary requirement of histidine (Taylor et al., 2015; Sambraus et al., 2017b). However, we found both ploidy to have lens NAH concentrations above those considered safe in Atlantic salmon (Remø et al., 2014). Therefore, Arctic char maybe less susceptible to developing cataracts that may negate any ploidy effect on dietary histidine requirement. Similarly, rainbow trout have higher lens NAH concentrations and they are less susceptible to developing cataracts when reared on the same conditions and diet as Atlantic salmon (Remø et al., 2017). Alternatively, as the requirement for histidine to maintain a sufficient NAH pool in the lens increases after seawater transfer in Atlantic salmon (Breck et al., 2005; Remø et al., 2014), keeping our char in freshwater throughout may have lowered the general risk of cataract development. The severity of the cataracts was generally low with mean scores around 2 (<10% of the lens shrouded). This is unlikely to impact welfare and performance, given we previously found a score of >4 (>50% of the lens shrouded) was required before we saw an impairment in growth of Atlantic salmon (Fraser et al., 2019a). The high prevalence in both diploids and triploids may be related to either fluctuations in the ambient rearing temperature (Bjerkås et al., 2001), and/or periods with temperatures above the optimum for growth (Waagbo et al., 2010; Sambraus et al., 2017b).

Using our current production protocol and genetic stock, the optimum strategy to maximize harvest mass at the earliest opportunity (i.e. when the fish first reached market size) would have been to stock all-male triploid char. This is due to the higher growth rate in males and the lower losses due to sexual maturation of triploids outweighing the losses from their higher mortality. In contrast, the worst strategy would have been to produce all-female triploid stocks. This is somewhat ironic, given all-female triploids are generally advised for aquaculture as their lower maturation is expected to provide an advantage around harvest. However, we found late maturing fish had enhanced growth over non-maturing fish even 1 year prior to spawning, and this strategy was more prevalent in triploid males as the females showed no signs of maturation. To date, no one has made all-male char although sex is genetically determined in this species (Woram et al., 2003) and all female production is currently in use (Yossa et al., 2019). Therefore, it should be possible to produce all-male populations via sex reversal as in rainbow trout (Chevassus et al., 1988), brook trout (Schill et al., 2016), and Atlantic salmon (Fjelldal et al., 2020). However, if triploids are to be used, we recommend their optimal rearing conditions are determined to help alleviate skeletal deformities.

In conclusion, triploidy does not impair growth when accounting for life history in Arctic char, but does come at a cost of increased mortality and impaired bone health. Future studies should determine whether triploid skeletal health can be improved via nutritional intervention and determine protocols for all male-production.

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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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