Triploidy leads to a mismatch of smoltification biomarkers in the gill and differences in the optimal salinity for post-smolt growth in Atlantic salmon

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ARTICLE INFO

ABSTRACT

Sterile triploid Atlantic salmon (*Salmo salar*) show inconsistent seawater grow-out, but the reason why remains unclear. The purpose of this study was to determine the salinity optima of triploid post-smolts. Diploids and triploids were assessed for smoltification status during an underyearling smolt regime before being transferred to one of four different salinities, 0, 11, 23 and 35 ppt at 12 °C and under 24 h continuous light for 83 days. During this period, fish growth, plasma biochemistry, and production traits (vertebral deformities, ocular cataracts, sexual maturation) were monitored. Molecular biomarkers in the gill (*nkaα1a*, *nkaα1b*, *nkcc1a*) suggested triploids reached peak smolt earlier than diploids and began the desmoltification process before the start of the salinity treatments, however this was not reflected in gill Na$^+$/K$^+$-ATPase enzyme activity. At the initiation of the salinity treatments triploids were significantly larger than diploids (mean weight g $\pm$ SE: 71 $\pm$ 0.7 and 87.2 $\pm$ 0.8 for diploids and triploids, respectively) and there was a ploidy effect on post-smolt growth, with body weight showing a clearer positive trend with salinity in diploids (0 < 11 = 23 = 35 ppt) than in triploids (0 < 11 < 35 = 23 ppt) (final mean weight g $\pm$ SE: 255.2 $\pm$ 7.4, 303.9 $\pm$ 9, 313.9 $\pm$ 9 and 342.4 $\pm$ 12 for diploids and 322.9 $\pm$ 9.7, 361.7 $\pm$ 10.7, 425.9 $\pm$ 12.1, 415.2 $\pm$ 12.2 for triploids at 0, 11, 23, and 35 ppt, respectively). Plasma Na$^+$ and Cl$^-$ increased, but plasma pH decreased, with increasing salinity in both ploidy. However, ploidy only had transient effects on plasma biochemistry depending on the salinity treatment. There was no ploidy effect on vertebral deformities (21% of both ploidy had one or more deformed vertebra). In contrast, triploids had a significantly higher prevalence of ocular cataracts (84 vs 98% in diploids and triploids, respectively) with a higher mean cataract score (mean $\pm$ SE: 1.93 $\pm$ 0.1 and 2.78 $\pm$ 0.1 for diploids and triploids, respectively), but a significantly lower prevalence of pubertal male post-smolts (15 vs 2% in diploids and triploids, respectively). Salinity treatment had no effect on vertebral deformities, cataracts, or post-smolt sexual maturation. In summary, there was a ploidy mismatch for smoltification biomarkers in the gill and salinity had a strong effect on post-smolt growth, but the effects were ploidy dependent.

1. Introduction

Recently, Norway has been trialling the use of sterile triploid Atlantic salmon (*Salmo salar*) in commercial facilities to prevent genetic interactions between farm escapees and wild fish (Stien et al., 2019; Madaro et al., 2021). Triploid salmon are relatively easy to produce using a hydrostatic pressure shock that prevents the removal of the second polar body after fertilisation (Benfey, 2016). The result is an individual that has three complete sets of chromosomes. In salmon, diploidy is the more frequent situation whereby an individual has two

sets of chromosomes. Although the technology and the production methods for large-scale triploid production have been available for several decades, they are rarely used in the commercial production of Atlantic salmon due to concerns regarding their farm performance. Theoretically, considering their sterile nature and energy re-allocation, early predictions were that triploids would outperform diploids, especially during the reproductive seasons. However, large-scale trials have generally provided inconsistent on-farm performance with little evidence that triploids outperform diploids during seawater grow-out (O'Flynn et al., 1997; Madaro et al., 2021). Indeed, triploids will often show lower seawater growth compared to diploids (Fraser et al., 2013). The reasons behind this inconsistency are most likely multiple and probably result from the physiological differences between diploids and triploids, such as differences in temperature optima (Sambraus et al., 2017, 2018) and nutritional requirements (Fjelldal et al., 2016). As such, only Tasmanian (Australia) has used triploids as a fraction of the commercial production as a backup to diploid production that suffers from extreme seasonal highs of pre-harvest sexual maturation (Amoroso et al., 2016).

Due to being anadromous, it is important that farming protocols ensure Atlantic salmon are adapted for sea migration when moving them from freshwater to seawater. Smoltification is the process in which freshwater parr become smolts, which are adapted to seawater. This developmental process is characterised by changes in behaviour, body colouration, and an increase in hypo-osmoregulatory ability generally epitomised by increases in gill Na^+/K^+-ATPase (NKA) enzyme activity (McCormick, 2012). To date, several studies have suggested triploids complete smoltification earlier than diploids (Leclercq et al., 2011; Taylor et al., 2012). If true, this would mean triploids may require transfer to sea earlier than expected based on the diploid literature, as smoltification is a reversible process if migration to seawater is not accomplished within a short period of time post "peak" smolt. For instance, so called desmoltified salmon, which have reverted to the freshwater phenotype, show poorer osmoregulatory ability and growth in seawater compared to salmon that migrate during the smolt "window" (Stefansson et al., 1998). Indeed, experience from ongoing industry implementation in Norwegian aquaculture has highlighted smolt quality as a significant issue when producing triploid salmon (Stien et al., 2019). Therefore, it is important to determine whether poor smolt quality may explain some of the seawater growth reduction seen in previous triploid studies.

Another aspect of triploid biology poorly studied is salinity tolerance, despite its important implications on seawater growth. Although wild Atlantic salmon generally migrate far out to sea in full-strength seawater (35 ppt), several studies have suggested either freshwater (Saunders and Henderson, 1969) or intermediate salinities (22–28 ppt, Bœuf and Payan, 2001; 15 ppt, Ytrestøyl et al., 2020) may be more optimal for post-smolt growth. Similar trends are found within other members of the Salmonidae family, such as rainbow trout (*Oncorhynchus mykiss*) (McKay and Gjerde, 1985) and Arctic charr (*Salvelinus alpinus*) (Duston et al., 2007), which can tolerate 35 ppt, but grow better at lower salinities. This optimal salinity performance is expected to be related to the energetic cost of osmoregulation. As such, brackish water of around 11 ppt is theorised as being the least costly due to it being isosmotic with the extracellular fluid of salmonids (i.e. approx. 330–340 mOsm kg^{-1}) (Hvas et al., 2018). Here, triploids are expected to have a lower aerobic scope than diploids at relevant rearing temperatures (e.g. 10.5 °C, Riseth et al., 2020) that may limit the energetic resources available for osmoregulation and/or growth. On the other hand, triploid rainbow trout erythrocytes have a higher resistance to osmotic lysis (Maxime and Labbé, 2010). In addition, polyploid brine shrimp (*A. parthenogenetica*) (Zhang and King, 1993) and triploid Chinese shrimp (*Fenneropenaeus chinensis*) (Zhang et al., 2008) have been found to have a wider range of salinity tolerance than diploids for growth and reproduction. Therefore, it would seem beneficial to determine the optimal salinity for triploids to improve our understanding of their seawater performance.

Besides the inconsistent seawater performance, triploids are known to suffer from skeletal deformities, which has restricted their commercial acceptance (Taranger et al., 2010; Madaro et al., 2021). Deformities in triploids can be caused by multiple factors including smoltification regime (Fraser et al., 2014a), elevated incubation temperature (Fraser et al., 2015), and insufficient dietary phosphorus (Fjelldal et al., 2016), an essential mineral for supporting bone health. For instance, when comparing feed containing 9.4 vs 16.3 (Fjelldal et al., 2016) and 10.5 vs 16.3 g/kg (Sambraus et al., 2020) total phosphorus, ploidy effects on bone health were evident at the lower, but not the higher, dietary inclusion. These total phosphorus values are relatively high, and above values found in older commercial diets. For example, Sullivan et al. (2007) reported three commercial salmon feeds to have between 4.1 and 6.4 g/kg total phosphorus, whereas more recently Witten et al. (2019) and Fraser et al. (2019a) used commercial diets that contained 8.4 and 9.1 g/kg total phosphorus, respectively. As such, the phosphorus content of commercial diets has historically not met the expected triploid requirement of approx. 16 g/kg total phosphorus.

Sterile triploid production would make the Atlantic salmon industry more sustainable, but their use is challenging due to changes in general physiology. Indeed, concerns over their welfare has now led the Norwegian Food Authorities to ban commercial triploid production as of 2023 without further improvements in production protocols. Therefore, optimal conditions for their growth and welfare need to be determined if they are ever going to have a future in the world's largest salmon producing nation. The purpose of this study was to determine the salinity optima of triploid Atlantic salmon post-smolts reared for 3 months after smoltification at 12 °C. Our hypothesis was that triploids would have either a lower (due to reduced aerobic scope) or broader (based on trends in plants and crustaceans) salinity optimum compared to diploids. In addition, as we used a modern commercial diet with a high phosphorus content (15.7 g/kg total phosphorus) we assessed skeletal deformities as a secondary objective. Our hypothesis being that there will be no ploidy effect on skeletal deformities. We also assessed ocular cataracts as these typically occur at sea transfer, may potentially be related to salinity fluctuations (Breck and Sveier, 2001), and are generally higher in triploids than diploids (Taylor et al., 2015). Therefore, our final hypothesis is that triploidy and salinity exposure will lead to an increase in the occurrence and severity of cataracts.

2. Methodology

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 #20820).

2.2. Fish stock and rearing

Triploid and diploid Atlantic salmon were produced and reared at the Institute of Marine Research, Matre Research Station, Western Norway. On the 16th November 2018, 650 ml of eggs from one Aquagen strain female were mixed with 5 ml of sperm from one Aquagen strain male. Only one family was used to reduce genetic variation. The eggs were then divided into two equal groups (325 ml of fertilised eggs each), with one group receiving a hydrostatic pressure shock of 655 bar (TRC-APV, Aqua Pressure Vessel, TRC Hydraulics Inc., Dieppe, Canada) for 6 min and 15 s at 37 mins and 30 s post-fertilisation at 8 °C to induce triploidy.

The environmental conditions before and during the experiment can be found in Fig. S1. The fish were incubated at ~6 °C and hatched after approximately 510° days. On the 3rd April 2019, the fish were moved from the hatchery into 1 × 1 m tanks (1 tank/ploidy) for first feeding

(approx. body weight of both ploidies, 0.18 g). From the 4th of April 2019 to the 10th of October 2019, the temperature ranged between 5.9 and 16.7 °C. The photoperiod was continuous from first feeding up until the 19th August 2019 when the fish were given a "winter signal" of 12 h light/12 h darkness. On the 29th August 2019, fish were tagged with passive integrated transponder (PIT) tags for individual recognition. On the 9th October 2019, the fish were split between 8 tanks (1×1 m, $n = 4$ tanks/ploidy, $n = 78$–79 fish/tank with a stocking density of 8.4 kg/tank). The following day, the experimental conditions began (Day 1, 10th October 2019) as the photoperiod was set to continuous light to induce smoltification, and the water temperature was maintained at 12 °C.

On day 23, the fish were split into 8 tanks per ploidy ($n = 71$ fish/tank) where they remained for the rest of the experiment. On day 29, the fish received one of four salinity treatments, continuation on 0 ppt or exposure to 11, 23, or 35 ppt. The salinity was changed by altering the inflow water to the tank ($n = 4$ tanks/salinity, $n = 2$ tanks/ploidy/salinity) which was regulated by an automated computer program. The fish remained on these treatments for 83 days until the experiment was terminated on day 112. All fish were initially fed Nutra sprint (Skretting AS, Stavanger, Norway, total phosphorus content of 15.9 and 15.5 g/kg and total histidine of 12.3 and 11.8 g/kg for the 0.8 and 1 mm feed, respectively) starter feed of the appropriate pellet size continuously during daylight hours. From the 4th July 2019, all groups were fed Nutra trio (Skretting AS, 15.7 g/kg and 11.6 g/kg total phosphorus and histidine, respectively) continuously throughout daylight hours with 20% excess based on sampling weights and expected growth rates.

2.3. Sampling procedures

2.3.1. Sampling

To assess plasma parameters, 16 fish/ploidy ($n = 4$ fish/tank) were sampled on days 1, 12, 19, and 26 ($n = 2$ fish/tank), and 16 fish/ploidy/salinity ($n = 8$/tank) on days 33, 65, and 112. The fish were euthanised with an overdose of anaesthetic (200 mg/l buffered MS-222, Finquel®). On days 1, 12, 19, and 26, the second gill arch on the right side of the body was removed and stored on RNA*later* for analysis of gene expression. At the same time, the second gill arch on the left side of the body was sampled and stored on dry ice before moving to −80°C for analysis of Na^+/K^+-ATPase (NKA). At all-time points, blood was taken from the caudal vein, spun at 13200 rpm (13.2 rfg) for 120 s to separate blood plasma, and the plasma stored at −80 °C for later analysis. In addition, on day 29 (immediately prior to the onset of the different salinity treatments), length and weight and a gill biopsy were taken from 128 ($n = 64$ fish/ploidy, $n = 8$/tank) lightly anesthetised (100 mg/l Finquel) fish and stored at −80 °C to assess NKA activity.

To assess growth, all the fish within a tank were lightly anesthetised in 100 mg/l Finquel and had their PIT tag recorded along with their length (to the nearest 1 mm) and weight (to the nearest 1 g) on days 23, 65, and 112. At the final sampling on day 112, air-dried blood smears were made for ploidy verification ($n = 128$/putative ploidy), all fish were frozen for later radiology, organ weights (heart, liver, and viscera to 0.01 g) from 10 fish/ploidy/salinity were recorded, and 34–42 fish/ploidy/salinity were assessed for ocular cataracts.

2.3.2. Blood cell diameter and ploidy verification

Determination of ploidy was carried out measuring the mean erythrocyte diameter, with triploid erythrocytes expected to be larger than diploids (Benfey et al., 1984). A Scion camera (CFW-1312C) which was mounted on a light microscope DMRE (type 020–525.755) was used to take two images of each blood smear at a resolution of 4.396 pixels/μm at 40× magnification. Mean cell diameter was measured using ImageJ, randomly counting 81–555 cells/picture.

2.3.3. Gill Na^+K^+-ATPase assay (NKA)

The gill NKA enzyme activity was assessed using the method of McCormick (1993). Briefly, the assay measured the hydrolysis of ATP based on the two enzymatic reactions that convert NADH into NAD^+ by pyruvate kinase and lactic dehydrogenase in the presence or absence of Ouabain, an inhibitor and the baseline indicator of NKA. The readings were taken from a Tecan SPARK® (BERGMAN DIAGNOSTIKA) microplate reader at 25 °C and 340 nm wavelength. The protein concentration within homogenized samples was determined by Bicinchoninic acid (BCA) protein analysis (Smith et al., 1985). NKA activity during smoltification was tested using $n = 32$–37/ploidy during the parr-smolt transformation and $n = 11$–17/salinity/ploidy during the different salinity treatments. The sample size was inconsistent due to some practical errors (lost samples).

2.3.4. Gene expression

The mRNA expression levels of *nkaα1a*, *nkaα1b*, and *nkcc1a* were done by Pharmaq Analytiq AS (Bergen, Norway). The primer information and assay procedure are proprietary information. Previously, the expression of *nkaα1b* and *nkcc1a* have been shown to be elevated during smoltification, whereas *nkaα1a* is downregulated (Nilsen et al., 2007).

2.3.5. Blood plasma analysis

Plasma ions (Cl^-, Na^+, K^+, and Ca^{2+}) and biochemical parameters (glucose, lactate and pH) were analysed using an ABL 90 FlEX PlUS ® blood gas analyser. Plasma cortisol was assessed using an ELISA (RE 52061, IBL).

2.3.6. Vertebral deformities

Radiography (40 kV, 10 mAs, 70 cm) was conducted using a Porta 100 HF, X-ray machine (HI- Ray 100, Germany). All fish were thawed before been X-rayed. Vertebral deformities were classified according to Witten et al. (2009) using electronic images. Fish with ≥1deformed vertebra were counted as a deformed whilst fish with ≥5 deformed vertebrae were considered severely deformed.

2.3.7. Cataract identification

Ocular cataracts were assessed using a Kowa SL-15 slit lamp to check the transparency of the fisheye, and the cataract score was graded following the rating scale of Wall and Richards (1992). Eyes were scored based on the degree of opacification (score of 0–4 for a single eye, thus 0–8 for each individual fish).

2.3.8. Statistical analysis

R studio was used to perform the statistical analysis. Graphical illustrations were drawn using ggplot 2 or Prism 8. Throughout, model residuals were checked for linearity and normality following visual examination of plots (i.e. histograms/q-q plots/ standardised residuals vs fitted values). Post-hoc tests were done using least square means with a tukey adjustment from the "lsmeans" library. We also used the Akaike information criterion with a correction for small sample sizes (i.e. AICc) to determine the best data fit weighted against the number of variables when constructing models. A spontaneous triploid found within the diploids (see results 3.1) was removed from all analyses described below.

We used linear mixed effect (LME) models to assess smolt parameters over time. In the initial model, ploidy and day (factor, 4–5 levels) were allowed to interact and this model was compared to a model without an interaction using AICc. Tank was included as a random effect. LME models were also used to assess plasma and gill endpoints during the different salinity treatments. We used a model that allowed ploidy (2 levels), salinity (4 levels), and day (3 levels) to interact (i.e. a 3-way interaction model) and tank was included as a random effect. All pubertal fish were removed from the analysis. If the initial model residuals were either non-linear or lacked normality, data was either transformed or the different days were analysed separately (see Table 1). We also used LME models to assess the relationships between *nkaα1a* and *nkaα1b* with NKA enzyme activity. In the initial models we allowed ploidy (2

Table 1

Statistical models and results for the physiology data presented in Table 1.

No mature Parameter	Model		R²m	R²c	χ²	df	p	
NKA	LME (log)	Salinity × Day	0.52	0.52	16.6	6	0.011	*
Chloride	LME	Ploidy × Salinity × Day	0.67	0.67	48.2	6	<0.001	***
Sodium	LME	Ploidy × Salinity × Day	0.74	0.75	41.3	6	<0.001	***
Calcium	LME	Ploidy × Salinity × Day	0.55	0.55	22.0	6	0.001	**
Potassium	LME (log, day 33 only)	Salinity	0.43	0.56	8.1	3	0.043	*
	LME (day 65 only)	Salinity	0.35	0.49	14.4	3	0.002	**
	LME (day 112 only)	Ploidy	0.06	0.07	3.0	1	0.082	
Cortisol	LME (log)	Ploidy × Salinity × Day	0.36	0.40	17.2	6	0.008	**
Glucose	LME	Day	0.48	0.50	34.9	2	<0.001	***
		Ploidy × Salinity			10.9	3	0.012	*
		Ploidy × Salinity × Day			12.1	6	0.060	
Lactate	LME	Ploidy × Salinity × Day	0.49	0.50	36.2	6	<0.001	***
pH	LME (reflected + log)	Ploidy × Salinity × Day	0.50	0.51	19.3	6	0.004	**

levels) to interact with gene expression (either $nka\alpha1a$ or $nka\alpha1b$) and included tank as a random effect. If the interaction was not significant, it was removed and the AICc score for each model compared.

We used LME models to assess body size and condition over time. We assessed the inclusion of various factors in the final model including sex, maturity status (immature vs pubertal), and deformities (yes vs no) based on AICc values. Of these, only sex was included in the final model. We included fish within tank as a random effect to account for repeated measures of the same individuals. Body weight was natural log transformed. For the biopsy fish, we used an LME model to investigate whether NKA enzyme activity on day 29 explained the variation in body weight on day 65 when accounting for ploidy, salinity, and body mass on day 29, with sex and tank included as random effects.

For the incidence of deformed fish, cataracts, and male puberty we used generalised linear models (GLM) or generalised linear mixed-effect (GLMER) models with a binomial distribution to compare the prevalence of fish with one or more deformed vertebra, fish with or without cataracts, or immature vs pubertal fish between ploidy, respectively. Initially, we included sex or salinity within these models, however, these variables increased the AICc score (i.e. the best data fit weighted against the number of variables) and so they were not included in the final models. For the number of deformed vertebra per deformed fish, we used a Kruskal Wallis test and compared group (8 levels, one for each ploidy and salinity combination), ploidy (2 levels), or salinity (4 levels) independently. For cataract severity, we used a proportional odds linear regression (POLR). Based on the AICc score, salinity, but not sex, was included in the final model. Tank effects were checked and found to not change the conclusion of any model.

We used LME models to assess relative organ size. Organ weight (heart, liver, or viscera) was set as the dependent variable, ploidy (2 levels), salinity (4 levels), and sex (2 levels) were included as categorical independent variables, body size (weight or length) was included as a continuous independent variable, and tank was included as a random effect. Ploidy, salinity, and body size (length or weight) were initially allowed to interact in all possible combinations. We ran separate models with either body weight or length set as the continuous independent variable. As we had no prior expectations, we computed the AICc score for all possible model complications (including the null model) and ran the model with the lowest AICc score as the final model. All pubertal fish were removed from the analysis ($n = 5$ diploids, one from 0 ppt, and two from 23 and 35 ppt).

3. Results

3.1. Ploidy verification

Based on erythrocyte dimensions, we found one spontaneous triploid amongst the putative diploid individuals whereas no diploids were found within those fish that had received the pressure-shock confirming a 100% triploidisation success rate (Fig. S2). The mean (range) values for diploids and triploids were 13.0 (12.3–13.8) and 15.2 (14.4–16.1) μm, respectively.

3.2. Survival

All groups finished with >95% survival following the different salinity treatments. In total 8 fish died, 5 diploids from the 35 ppt treatment (2 had received biopsies) and 3 triploids, one from each of the 0, 11, and 35 (biopsy fish) ppt treatments.

3.3. Smoltification parameters

Both ploidy showed the expected trends in smoltification markers. There was an increase in gill NKA enzyme activity and mRNA abundance of the seawater subunit ($nka\alpha1b$) and the $nkcc1a$ transporter over time, and a decrease in the mRNA abundance of the freshwater subunit $nka\alpha1a$, the $nka\alpha1a/nka\alpha1b$ ratio, and body condition (Fig. 1A-F). For both ploidy, peak smoltification based on the highest NKA enzyme activity and mRNA abundance was on day 19. In addition, NKA enzyme activity was positively associated with $nka\alpha1b$ (LME, $\chi^2 = 52$, df $= 1$, $p < 0.001$), but negatively associated with $nka\alpha1a$ (LME, $\chi^2 = 75$, df $= 1$, $p < 0.001$) with no interaction effect with ploidy. However, although there were no significant ploidy effects on NKA enzyme activity, mRNA abundance suggested triploids had started to desmoltify by day 26 due to a significant decrease in $nka\alpha1b$ and $nkcc1a$ and a significant increase in $nka\alpha1a$ and the $nka\alpha1a/nka\alpha1b$ ratio, which was not seen in diploids (Fig. 1B-E). Although triploids generally had a significantly lower body condition than diploids, the response overtime during smoltification showed no effect of ploidy (Fig. 1F).

Plasma ions (Na⁺, Cl⁻, K⁺, and Ca²⁺), cortisol, and glucose all showed transient trends during smoltification (Fig. 2A-F). Plasma Cl⁻ and lactate both showed a significant ploidy × day interaction with triploids having significantly lower Cl⁻ values on day 26, whereas the opposite occurred for lactate (Fig. 2G). Plasma cortisol showed a tendency to increase on day 19, but triploids had significantly higher values than diploids irrespective of time (Fig. 2E). Plasma pH showed no significant time or ploidy effects (Fig. 2H).

3.4. Post-smolt growth under different salinities

There was a significant 3-way interaction between ploidy, salinity, and time on body weight. Irrespective of time, triploids were always significantly heavier than diploids independent of salinity (Fig. 3AB) whereas salinity effects were only apparent at the final timepoint and were dependent on ploidy. In diploids, fish maintained on 23 ppt were intermediate between 35 and 11 ppt, and not statistically different from
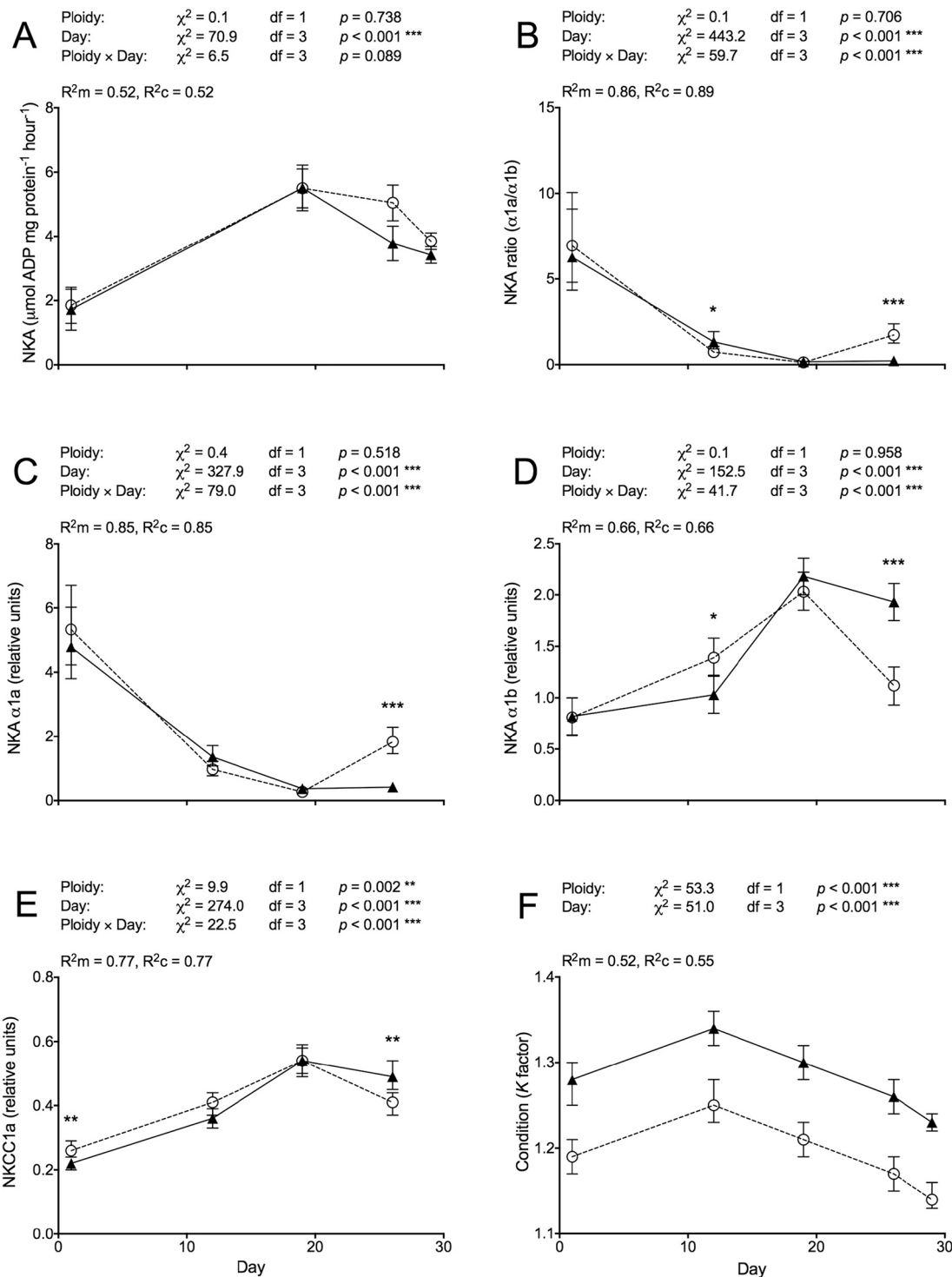
Fig. 1. Smoltification indicators in diploid (triangles, solid line) and triploid (circles, dashed line) Atlantic salmon. Data and statistics are from linear mixed effect models (LME) with mean ± 95% CI. An asterisk indicates a significant ploidy effect within time point (post hoc, least square means, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

either (although there was a strong trend for 35 ppt to be heavier than 23 ppt. LS means post-hoc, $p = 0.052$). In contrast, in triploids, fish on 23 ppt were marginally heavier than those reared on 35 ppt, and both were significantly heavier than those fish reared on 11 ppt. In both ploidy, fish maintained on freshwater were the smallest at the end of the study. Although triploids were heavier throughout, the size difference was unaltered throughout the study in freshwater, marginally reduced following 3 months on 11 and 35 ppt (a 5 and 3% reduction in the ploidy

effect, respectively), but increased when maintained on 23 ppt (a 12% increase in the ploidy effect) (see Fig. S3).

There was also a 3-way interaction on body condition that showed the same trends as in the body weight data, with triploids having lower values in general and there being a ploidy effect dependent on salinity at the end of the study (Fig. 3CD). In diploids, fish reared on 35 ppt had the highest body condition, whereas in triploids it was those fish reared on 23 ppt. For both ploidy, those kept on freshwater had the lowest body
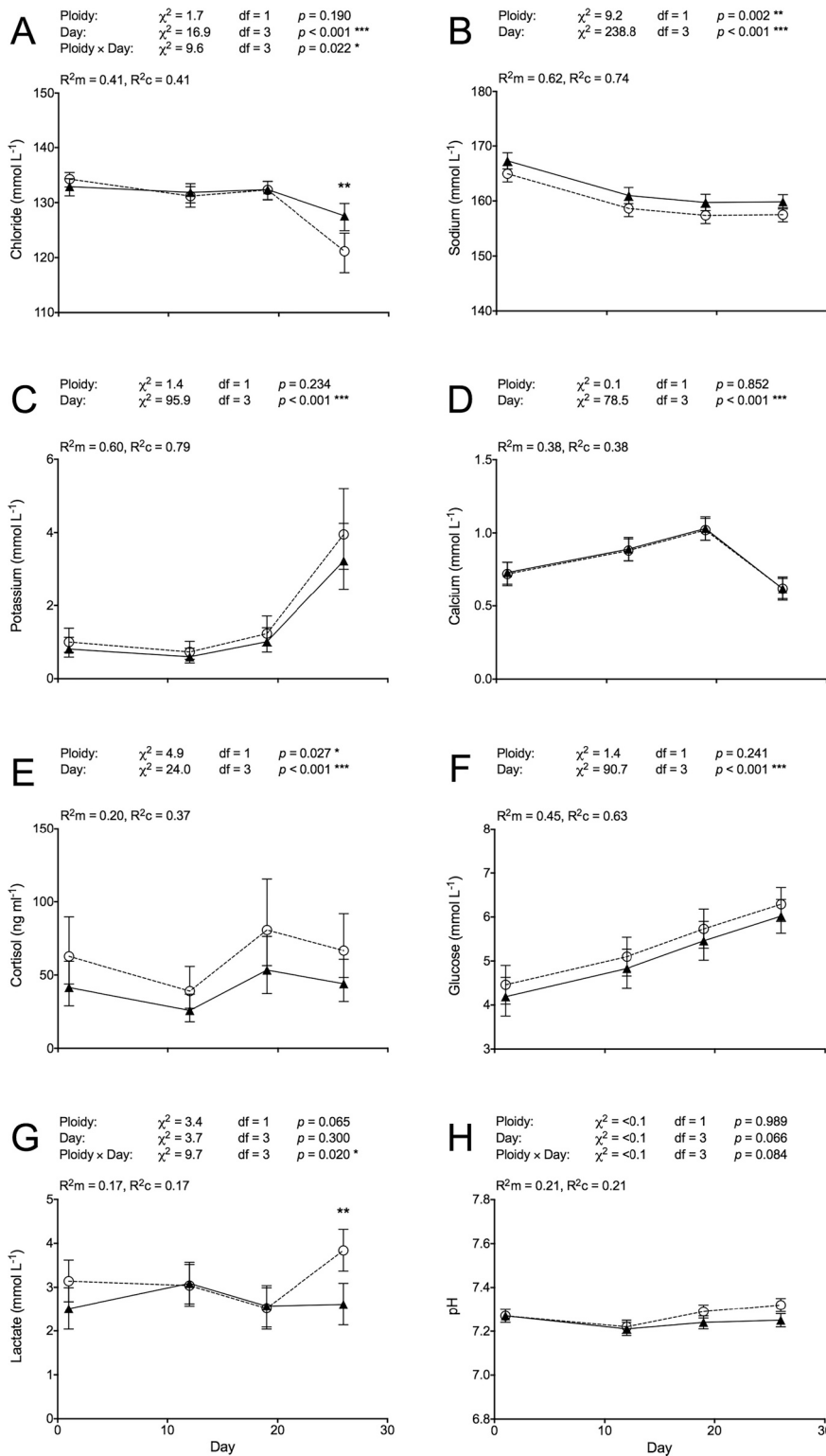
Fig. 2. Smoltification physiology in diploid (triangles, solid line) and triploid (circles, dashed line) Atlantic salmon. Data and statistics are from linear mixed effect models (LME) with mean ± 95% CI. An asterisk indicates a significant ploidy effect within time point (post hoc, least square means, * $p < 0.05$, ** $p < 0.01$,*** $p < 0.001$).

condition. At the end of the study, the ploidy effect had marginally increased after rearing on the various salinity treatments compared to the situation at the start of the experiment, but marginally decreased in those fish maintained on freshwater (see Fig. S4).

Within the biopsy fish, there was no association between gill NKA enzyme activity and body mass on day 65 (LME, $\chi^2 = 0.43$, df = 1, $p =$

0.512).

3.5. Post-smolt biology under different salinities

Salinity effects on plasma and gill biochemistry were apparent for all endpoints except plasma glucose in diploids (see Tables 1 and 2 for the
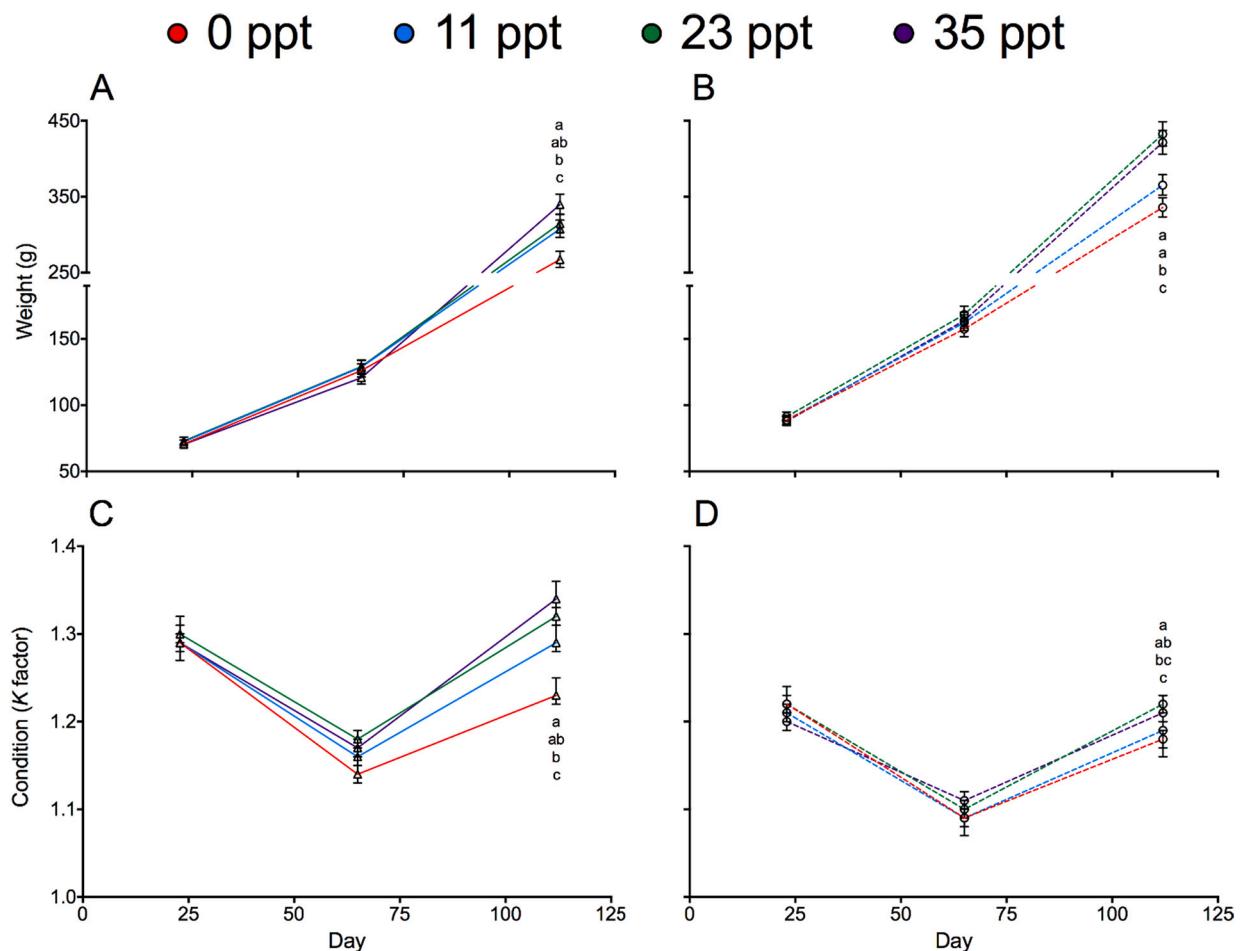
Fig. 3. Body weight and condition over time in diploid (solid lines) and triploid (dashed lines) Atlantic salmon reared under one of four salinity regimes. Data are from linear mixed effect models (LME) with mean ± 95% CI. Different lowercase letters indicate a significant salinity effect within timepoint (post hoc, least square means, $p < 0.05$).

models and values, respectively. Figs. S5–13 in the supplementary material provide visual representation of the data provided in Tables 1 and 2). In general, irrespective of ploidy, NKA enzyme activity was higher in those fish maintained on and above 23 ppt than those below, and there was no difference between 0 and 11 ppt. Regarding plasma ions, Cl^- and Na^+ were generally positively associated with salinity whereas K^+ and Ca^{2+} showed no consistent trends. Of the other parameters, plasma pH was generally negatively associated with salinity whereas cortisol generally remained higher in freshwater fish up until day 65, compared to the other salinity treatments. Consistent salinity trends on lactate were not apparent.

Significant ploidy × salinity and/or the 3-way interaction with time, were found for all endpoints except NKA enzyme activity and plasma K^+. The largest ploidy effects were apparent in those fish maintained on freshwater in that triploids developed lower plasma Cl^-, Na^+, and pH from day 65 onwards, but had elevated glucose throughout. In contrast, the same plasma ion disturbances were generally not apparent or sometimes transiently reversed in the various salinity treatments from day 65 onwards, and there was little to no ploidy effect on plasma glucose or pH. Nevertheless, one general ploidy trend irrespective of salinity was for triploids to have higher lactate values than diploids during the initial post-smolt period, although this effect persisted for longer/appeared later in the higher salinity treatments.

3.6. Vertebral deformities

As we hypothesised, vertebral deformities were not affected by

ploidy (GLMER, $\chi^2 = 0.029$, df $= 1$, $p = 0.865$). The prevalence of fish with one or more deformed vertebra was 21% (95% CI, 17–25) in both ploidy and there was no ploidy effect on the number of deformed vertebrae per deformed fish (Kruskal-Wallis, $\chi^2 = 0.61$, df $= 1$, $p = 0.435$) with a median value of 2 (IQR, 2) for both. The distribution of deformed vertebra along the vertebra was similar, although diploids generally had a slightly higher prevalence of deformities from vertebra 1–5, whereas triploids tended to have slightly higher values between vertebra 21–27 and 31–55 (Fig. S14).

3.7. Cataracts

Triploids had a significantly (GLMER, $\chi^2 = 12.2$, df $= 1$, $p < 0.001$) higher prevalence of fish with cataracts than diploids (mean % and 95% CI: Diploid, 84, 74–90; Triploid, 98, 91–99) and a significantly higher (POLR, $\chi^2 = 25.9$, df $= 1$, $p < 0.001$) mean cataract score in those fish with cataracts (mean and 95% CI: Diploid, 2.1, 2.0–2.3; Triploid, 2.8, 2.6–3.0).

3.8. Post-smolt maturation

Maturity was only found in males and the prevalence of pubertal post-smolts was significantly lower (GLM, $\chi^2 = 31.8$, df $= 1$, $p < 0.001$) in triploids compared to diploids (mean % and 95% CI: Diploid, 15, 11–20; Triploid, 2, 1–4).

Table 2

Gill Na$^+$/K$^+$-ATPase (NKA) and plasma physiology in diploid and triploid Atlantic salmon reared in one of four salinities. Data are means ±95% CI from the models found in Table 2. Values in bold indicate a significant ploidy effect within day (post hoc, least square (LS) means, $p < 0.05$). Different upper (diploid) or lowercase (triploid) letters within time point indicate a significant salinity effect within ploidy (LS mean, $p < 0.05$).

Parameter	Salinity							
	0		11		23		35	
Day	Diploid	Triploid	Diploid	Triploid	Diploid	Triploid	Diploid	Triploid
NKA (µmol ADP mg protein^{-1} h^{-1})								
33	3.94 (3.32–4.67)^{AB}	3.61 (3.12–4.17)^{ab}	3.31 (2.82–3.88)^B	3.60 (3.11–4.16)^b	4.50 (3.87–5.24)^A	4.69 (4.03–5.46)^a	4.00 (3.44–4.66)^{AB}	4.02 (3.45–4.67)^{ab}
65	4.03 (3.56–4.56)^B	4.12 (3.62–4.69)^b	4.54 (4.01–5.14)^B	4.43 (3.89–5.03)^b	6.56 (5.82–7.40)^A	6.81 (6.02–7.71)^a	7.28 (6.45–8.21)^A	7.83 (6.92–8.87)^a
112	5.10 (4.50–5.77)^B	5.04 (4.49–5.66)^b	5.32 (4.70–6.02)^B	5.60 (4.97–6.31)^b	6.81 (6.02–7.71)^A	5.60 (4.90–6.40)^a	7.31 (6.32–8.45)^A	8.02 (6.94–9.27)^a
Chloride (mmol l^{-1})								
33	**123.6 (121.9–125.2)^B**	**128.6 (126.9–130.2)^c**	130.7 (129.0–132.4)^A	131.1 (129.4–132.7)^{bc}	131.9 (130.2–133.5)^A	133.8 (132.2–135.5)^{ab}	132.6 (131.0–134.3)^A	135.3 (133.6–136.9)^a
65	**133.4 (131.7–135.1)^B**	**127.8 (126.1–129.4)^c**	136.7 (135.0–138.4)^{AB}	135.7 (134.0–137.4)^b	**137.4 (135.8–139.1)^A**	**141.5 (139.8–143.2)^a**	137.9 (136.3–139.6)^A	139.7 (138.0–141.4)^a
112	**134.7 (133.0–136.3)^B**	**126.4 (124.9–127.8)^b**	137.7 (136.2–139.1)^{AB}	137.0 (135.5–138.5)^a	139.7 (138.1–141.3)^A	140.2 (138.7–141.7)^a	139.9 (138.3–141.5)^A	139.4 (137.9–140.9)^a
Sodium (mmol l^{-1})								
33	164.1 (162.9–165.4)^{BC}	164.7 (163.5–165.9)^{ab}	163.3 (162.1–164.5)^C	163.5 (162.3–164.7)^b	**166.8 (165.6–168.0)^{AB}**	**164.4 (163.2–165.6)^{ab}**	**169.1 (167.9–170.3)^A**	**166.4 (165.2–167.6)^a**
65	158.9 (157.6–160.1)^B	**155.9 (154.7–157.1)^b**	158.9 (157.7–160.1)^B	157.8 (156.6–159.0)^b	161.1 (159.9–162.3)^{AB}	161.8 (160.6–163.0)^b	163.8 (162.6–165.0)^A	163.1 (161.9–164.3)^a
112	**157.8 (156.7–159.0)^B**	**153.9 (152.8–155.0)^c**	159.8 (158.6–160.9)^{AB}	158.0 (156.9–159.2)^b	161.0 (159.8–162.2)^A	161.1 (159.9–162.2)^a	162.5 (161.3–163.6)^A	162.2 (161.1–163.3)^a
Potassium (mmol l^{-1})								
33	0.47 (0.30–0.74)^B	0.38 (0.24–0.60)^b	0.37 (0.23–0.58)^B	0.32 (0.21–0.51)^b	0.68 (0.43–1.07)^A	1.33 (0.85–2.10)^a	0.87 (0.55–1.38)^A	0.99 (0.63–1.57)^a
65	3.56 (2.78–4.35)^B	2.66 (1.87–3.44)^b	4.61 (3.82–5.39)^{AB}	4.25 (3.46–5.04)^{ab}	4.48 (3.70–5.27)^A	4.57 (3.79–5.36)^a	2.72 (1.94–3.51)^B	3.49 (2.70–4.27)^b
112	3.92 (3.72–4.12)	4.17 (3.98–4.35)	3.98 (3.79–4.17)	4.10 (3.91–4.29)	4.06 (3.86–4.26)	4.27 (4.09–4.46)	4.07 (3.87–4.27)	4.10 (3.92–4.29)
Calcium (mmol l^{-1})								
33	**0.54 (0.44–0.64)^B**	**0.92 (0.82–1.02)**	0.88 (0.78–0.98)^A	0.96 (0.86–1.06)	**0.65 (0.56–0.75)^{AB}**	**0.83 (0.73–0.93)**	**0.64 (0.54–0.74)^B**	**0.85 (0.75–0.95)**
65	1.11 (1.01–1.21)^A	1.09 (0.99–1.19)	1.07 (0.97–1.17)^{AB}	1.06 (0.96–1.16)	**0.86 (0.76–0.96)^B**	**1.18 (1.08–1.28)**	0.99 (0.89–1.09)^{AB}	1.15 (1.05–1.25)
112	1.21 (1.11–1.30)	1.19 (1.10–1.28)	1.22 (1.13–1.31)	1.26 (1.17–1.36)	1.32 (1.22–1.41)	1.29 (1.20–1.37)	1.38 (1.29–1.48)	1.32 (1.23–1.41)
Cortisol (ng ml^{-1})								
33	68.5 (47.7–98.4)	99.4 (69.2–142.8)	94.4 (65.7–135.6)	90.9 (63.3–130.5)	65.3 (45.5–93.8)	60.8 (42.3–87.3)	71.8 (50.0–103.1)	103.3 (71.9–148.3)
65	118.6 (81.9–171.8)^A	104.1 (72.5–149.5)	49.6 (34.5–71.2)^B	66.6 (46.0–96.5)	33.0 (23.0–47.5)^B	54.3 (37.8–78.0)	34.4 (23.9–49.3)^B	62.2 (43.3–89.3)
112	35.6 (24.9–50.8)	60.6 (43.3–84.9)^{ab}	38.6 (27.5–54.0)	29.9 (21.2–42.1)^b	**33.7 (23.7–48.2)**	**74.1 (52.9–103.8)^a**	21.8 (15.3–31.1)	28.9 (20.6–40.5)^b
Glucose (mmol l^{-1}) (general ploidy effect at 0ppt with triploid > diploid and a general salinity effect in triploids, 0ppt > 11ppt = 23ppt = 35ppt)								
33	5.51 (5.21–5.81)	5.93 (5.63–6.23)	5.53 (5.23–5.83)	5.54 (5.24–5.84)	5.11 (4.81–5.41)	5.16 (4.86–5.46)	5.48 (5.18–5.78)	4.91 (4.61–5.21)
65	4.51 (4.20–4.82)	4.64 (4.34–4.94)	4.15 (3.85–4.45)	4.04 (3.73–4.35)	4.06 (3.76–4.36)	4.34 (4.04–4.64)	4.47 (4.17–4.78)	4.66 (4.36–4.96)
112	4.52 (4.22–4.81)	5.15 (4.88–5.43)	4.36 (4.08–4.64)	4.30 (4.02–4.58)	4.31 (4.02–4.61)	4.29 (4.01–4.57)	4.29 (4.00–4.59)	4.34 (4.06–4.62)
Lactate (mmol l^{-1})								
33	**2.86 (2.53–3.19)^B**	**3.71 (3.38–4.04)**	**2.89 (2.56–3.22)^B**	**3.51 (3.18–3.84)**	**3.37 (3.05–3.70)^{AB}**	**4.19 (3.86–4.52)**	4.04 (3.71–4.37)^A	3.63 (3.30–3.96)
65	3.66 (3.32–4.00)^A	3.27 (2.94–3.60)^{ab}	2.67 (2.34–3.00)^B	2.95 (2.61–3.29)^b	**2.49 (2.16–2.82)^B**	**3.83 (3.50–4.16)^a**	**2.92 (2.60–3.25)^{AB}**	**3.57 (3.25–3.90)^{ab}**
112	2.16 (1.84–2.48)	2.48 (2.18–2.77)	2.33 (2.04–2.63)	2.23 (1.92–2.53)	2.32 (2.00–2.64)	2.45 (2.15–2.75)	2.38 (2.06–2.70)	2.04 (1.74–2.34)
pH								
33	7.30 (7.27–7.33)^A	7.28 (7.25–7.31)^a	7.23 (7.20–7.27)^{AB}	7.28 (7.25–7.31)^a	7.19 (7.15–7.22)^B	7.17 (7.13–7.20)^b	7.15 (7.11–7.19)^B	7.20 (7.17–7.24)^{ab}
65	**7.15 (7.11–7.19)**	**7.24 (7.20–7.27)^a**	7.17 (7.13–7.21)	7.16 (7.11–7.20)^{ab}	7.13 (7.09–7.17)	7.08 (7.03–7.12)^b	7.07 (7.03–7.12)	7.08 (7.03–7.12)^b
112	**7.18 (7.14–7.21)^A**	**7.28 (7.25–7.31)^a**	7.14 (7.10–7.17)^{AB}	7.16 (7.12–7.20)^b	7.08 (7.04–7.12)^{BC}	7.12 (7.09–7.16)^b	**7.00 (6.94–7.04)^C**	**7.08 (7.04–7.12)^b**

3.9. Organ indexes

Based on body weight, triploids had significantly heavier hearts (LME, $\chi^2 = 6.3$, df = 1, $p = 0.012$), no effect on liver (LME, $\chi^2 = 2.0$, df = 1, $p = 0.157$), but a lighter viscera in larger, not smaller, fish (i.e. ploidy × weight interaction. LME, $\chi^2 = 11.2$, df = 1, $p < 0.001$) (Fig. S15A-C).

The ploidy effect on the heart was lost when tested against body length rather than weight. However, diploids still had a significantly heavier viscera (LME, $\chi^2 = 24.7$, df = 1, $p < 0.001$), although now there was no interaction with length (Fig. S15D-F). There was no salinity effect on relative heart or viscera size (data not shown, but see Supplementary material), but there was a significant salinity effect on relative liver size

when correcting for body length (LME, $\chi^2 = 8.7$, df $= 3$, $p = 0.033$), but not weight. The freshwater fish had the lowest relative liver size at a given length, which was significantly lower than the 23 and 35 ppt treatments (LS means, $p < 0.05$). Males had significantly relatively larger livers at a given weight (LME, $\chi^2 = 6.0$, df $= 1$, $p = 0.014$) and length (LME, $\chi^2 = 6.1$, df $= 1$, $p = 0.013$) than females, but there were no sex effects on the relative heart or viscera size (data not shown, but see Supplementary material).

4. Discussion

4.1. Ploidy mismatch in smoltification markers

Smoltification is a critical stage in Atlantic salmon as it confirms good growth, swimming performance, and high survival at sea (McCormick, 2012). Therefore, it is important to transfer fish within the "smoltification window". As in the current study, smoltification is generally traced through gill NKA enzyme activity, mRNA abundance of genes relating to ion transporting proteins (Nilsen et al., 2007), and/or ion regulation (Handeland and Stefansson, 2001). Our results suggested both ploidy had similar increases in the NKA enzyme activity surge during smoltification, although we found a ploidy mismatch in mRNA abundance. For instance, although triploids generally had higher NKA enzyme values, they showed a reduction in *nkaα1b* (seawater subunit) and *nkcc1a*, and an increase in *nkaα1a* (freshwater subunit), at the final timepoint before the initiation of the various salinity treatments. These alterations in mRNA abundance would suggest triploids were beginning to desmoltify. This leads to loss of pre-developed seawater adaptive mechanisms and is easily identified through a rapid loss of NKA enzyme activity and high mortalities and poor growth in seawater (Stefansson et al., 1998). Since there was no ploidy effect on gill NKA enzyme activity and we observed high growth and low mortalities in all salinity treatments, we believe that the observed α-isoform switching was only an early sign of the desmoltification process in triploids. Similar contradictions between NKA enzyme activity and mRNA abundance have been observed previously (van Rijn et al., 2020), although not explored in triploid salmonids before.

Previous studies have suggested triploidy may alter the timing and length of the smolt window. For example, in contrast to our findings, Leclercq et al. (2011) and Smedley et al. (2016) both provided anecdotal evidence that triploids smoltified earlier than diploids in under-yearlings, and yearlings, respectively. Taylor et al. (2012) also reported triploid underyearling smolts smoltified one month earlier than diploid counterparts based solely on body colouration. However, body colouration indicative of smolting can occur independently of increased gill NKA enzyme activity (Saunders et al., 1985). Furthermore, in yearling smolts, ploidy has not been found to affect the timing of smoltification based on NKA enzyme activity (Taylor et al., 2012, 2019; Sambraus et al., 2017; Fraser et al., 2021) or *nkaα1a* and *nkaα1b* abundance (Fraser et al., 2021). Therefore, there is little evidence to support the idea triploidy alters the timing or magnitude of the NKA enzyme activity surge during smoltification in Atlantic salmon. However, we did observe a significant elevation of plasma cortisol in triploids during smoltification, which was not evident in a similar study on yearlings (Fraser et al., 2021). As cortisol plays a significant role in smoltification, the biological consequences of this requires further investigation. Furthermore, temporal changes throughout smoltification and the expected period of desmoltification in the 0 ppt treatment were observed in cortisol, Cl^-, Na^+, and Ca^{2+}, and could be indicative of alterations in the timing of peak smolt and/or the onset of desmoltification. Alternatively, the increase in plasma lactate around peak smolt in triploids may suggest they are aerobically compromised during this period. This may then explain the ploidy related ionic disturbances around peak smolt, as maintaining transmembrane electrical gradients through NKA enzyme activity is considered one of the most energetically costly processes performed by a cell and may be compromised by mechanisms that increase gill gas

exchange (termed the osmorespiratory compromise, Hvas et al., 2018; Giacomin et al., 2020).

4.2. Improved triploid growth at 23 ppt

Based on the current literature, one could hypothesise that triploidy may limit salinity tolerance due to a reduced aerobic scope (Riseth et al., 2020) or alternatively, polyploidy alters salinity optima/tolerance based on trends in invertebrates and plants (Chao et al., 2013; Zhang and King, 1993). Our results lean towards the later, as triploid growth was not impaired at 35 ppt, but appeared enhanced at 23 ppt, compared to diploids. Although some studies suggested a large metabolic cost of osmoregulation in fishes, possibly up to a third of all costs, many others suggest it is minimal and only a few percent of the total energy budget (reviewed in Ern et al., 2014). Similarly, although there is evidence that triploidy reduces the aerobic scope of salmonids, this effect is relatively small and appears temperature dependent (Riseth et al., 2020). This may explain why many studies have failed to find any such ploidy effect (Lijalad and Powell, 2009; Bowden et al., 2018). Therefore, it appears unlikely that the energetic demands of osmoregulation coupled with the expected lower aerobic scope of triploids at the temperatures we used, impaired triploid performance. Nevertheless, more thorough studies that include measures of feed intake and feed conversion ratios are required to further clarify this. Although not included in this study, we previously found triploidy does not affect feed intake at 10 °C (Hansen et al., 2015) or 12 °C (Sambraus et al., 2017) in post-smolts maintained on seawater. Studies on feed conversion ratios in seawater have found either no ploidy effect (Fraser et al., 2013) or seasonal differences (Smedley, 2016). Salinity has been found to alter feed intake/conversion in fish (Likongwe et al., 1996), but was not found in Atlantic salmon (McCormick et al., 1989; Usher et al., 1991), although this has not been confirmed in triploids.

The mechanism(s) by which triploidy may alter salinity tolerance is unclear. Our most notable ploidy effects at 23 ppt were transiently elevated plasma lactate, Cl^-, Ca^{2+}, and cortisol in triploids. Unfortunately, it is difficult to relate these parameters to improved growth. Nevertheless, a notable trend is that triploid salmon generally grow as well or better in freshwater, but poorer or equal in seawater (e.g. Fraser et al., 2013). Some of these effects may be due to triploids having altered nutritional (Taylor et al., 2015; Fjelldal et al., 2016) and temperature requirements (Sambraus et al., 2018) that may limit performance. Our data suggests salinity may be another important variable. It is noticeable that of the two studies in which triploids were significantly larger than diploids at commercial harvest size (O'Flynn et al., 1997; Oppedal et al., 2003), the most impressive performance was reported in Oppedal et al. (2003) in which triploids were on average 37% heavier. These fish were maintained in indoor tanks with typical seasonal temperature changes (4–15 °C), but salinity ranged from 20 to 31 ppt. In contrast, all other studies with harvest sized fish have used sea cages for seawater grow-out in which the salinity gradients and the position of triploids with respect to the halocline are not reported. In addition, triploids fared better in Norwegian commercial farms when produced as spring (yearling) smolts compared to autumn (underyearling) smolts (Stien et al., 2019). Here, spring smolts are often exposed to brackish water from snow melt that does not occur for autumn smolts. Our current results may also be of particular interest to those growing salmon in RAS systems throughout life to avoid pre-harvest sexual maturation (e.g. Crouse et al., 2021) as the greater environmental control may make it easier to provide triploids with the optimal conditions they need to realise their true growth potential.

4.3. Brackish water of 11 ppt is not seawater

It is not uncommon for farmers to use brackish water to try and prolong the smolt window. However, as in previous studies, we find 11 ppt does not maintain elevated NKA enzyme activity above that of post-

smolts maintained in freshwater, whereas 23 ppt did. One could have expected this result given 11 ppt is approximately isoosmotic with salmonid internal fluids (Hvas et al., 2018) and may not require an increase in ion pumps to maintain homeostasis. This matches a previous study in Atlantic salmon, whereby a minimum salinity of 15 ppt was required to maintain hypo-osmoregulation after peak smoltification (Mortensen and Damsgård, 1998). Similarly, Duston (1994) found that parr not adapted for seawater entry show no increase in mortality when exposed to 10 ppt, but they do when exposed to 20 ppt.

4.4. Salinity and growth in diploids

Laboratory studies have either found minor transient effects of salinity on early post-smolt growth when followed for <3 months (McCormick et al., 1989; Duston, 1994) or that post-smolts maintained on freshwater outgrow those kept on higher salinities when followed for 10–19 months (Saunders and Henderson, 1969). Therefore, there is little evidence that more isoosmotic conditions provide the best growing conditions for Atlantic salmon via reducing the cost of osmoregulation. In contrast to most literature, we found a clear positive association between body weight and salinity after only 83 days. One may assume our relatively poor freshwater growth was associated with elevated stress as this group had higher cortisol and lactate on day 65 compared to all other salinities. Cortisol is expected to be elevated during smoltification, but decrease thereafter (Sundell et al., 2003). The peak in plasma lactate within freshwater fish would indicate higher levels of anaerobic metabolism, although it is unclear whether this is to be expected during desmoltification due to a paucity of literature. Desmoltification is expected to be completed within $500°$ of the peak smolt values (i.e. day 62 in our experiment) (Handeland et al., 2004). Of note, NKA enzyme activity never returned to pre-smolt levels and did not drop after day 65, suggesting desmoltification was completed by then.

An alternative explanation for our comparatively poor growth in freshwater maybe seasonal physiology and/or ration availability. For example, McCormick et al. (1989) demonstrated that post-smolts maintained on 13 °C seawater grew faster than those kept in freshwater when fed full rations, but the opposite occurred on reduced rations. Ytrestøyl et al. (2020) found better growth at 12 compared to 20 and 32 ppt, but they used a short day (12:12 light/dark) that limits growth through lower feed intake and alterations in metabolism that promote lipid storage over protein turnover (i.e. a winter metabolic profile, Nordgarden et al., 2003). In contrast, we used constant light that increases feed intake and improves growth through higher protein turnover and lower lipid storage (Nordgarden et al., 2003). Comparisons with the work of Saunders and Henderson (1969) are more problematic, as these authors do not report the light conditions used and they had slightly lower oxygen levels in the seawater compared to the freshwater treatments. Nevertheless, Saunders and Henderson (1969) report seasonal effects on salinity optima based on interactions with temperature that was not constant in their study. Therefore, our understanding of salinity optima in Atlantic salmon would benefit from future studies on seasonality.

4.5. Salinity effects baseline values of plasma Na$^+$, Cl$^-$, and pH

Rearing salmon on different salinities for 83 days led to notable increases in basal plasma Na$^+$ and Cl$^-$, but a reduction in plasma pH, with increasing salinity. The same patterns in plasma Na$^+$ and Cl$^-$ were found in post-smolt salmon challenged with abrupt low salinity (Bakke et al., 1991), Atlantic salmon acclimated to 0, 11, 35 ppt for three weeks (Hvas et al., 2018), coho salmon (*Oncorhynchus kisutch*) maintained on various salinities between 2.5 and 30 ppt for one year (Damsgaard et al., 2020), and in killifish (*Fundulus heteroclitus*) acclimated to 0, 11, and 35 ppt for six weeks (Giacomin et al., 2020). However, we found only transient effects on plasma Ca^{2+} and K$^+$ that followed no specific salinity trend. To date, it is unclear whether the long-term changes in Na$^+$ and Cl$^-$ ions

are due to imperfect osmoregulation or a new set point under different salinities. Similarly, Damsgaard et al. (2020) also noted a reduction in arterial and red blood cell pH with increasing salinity and suggested this is not due to a lack or regulatory ability, but more likely a new set point that is salinity dependent. Therefore, it appears changes in ion-balance in salmonids reared under different salinities are unlikely to be transient, although the biological significance is unclear.

4.6. Key welfare indicators

Skeletal deformities in triploids have long been seen as a bottleneck to their commercial acceptance (Taranger et al., 2010). We found no ploidy effect on vertebral deformities, in contrast to numerous previous studies (Fjelldal and Hansen, 2010; Leclercq et al., 2011; Fraser et al., 2013). This is most likely explained by the relatively high dietary phosphorus content throughout the study ($\geq$15.5 g/kg total phosphorus), the requirement for which is known to be higher in triploids compared to diploids (Fjelldal et al., 2016), especially during the very early stages of development (Sambraus et al., 2020). In addition, we used an incubation temperature of 6 °C which has also been found to lower the incidence of vertebral deformities in triploid Atlantic salmon parr (Fraser et al., 2015). The higher phosphorus requirement in triploids is expected to be related to larger genomes having higher per cell nucleic acid content (Neiman et al., 2012).

We found an increased occurrence and severity of cataracts in triploids compared to diploids, but no effect of salinity. This result agrees with previous reports of increased cataract development in triploid Atlantic salmon (Wall and Richards, 1992; Leclercq et al., 2011), most likely due to a dietary histidine deficiency (Taylor et al., 2015). Therefore, it appears the current diets which had between 11.7 and 12.3 g/kg histidine were insufficient to prevent the formation of cataracts in triploids as expected (Taylor et al., 2015; Sambraus et al., 2017). However, the cataract scores were generally low and below those found to reduce growth in previous studies (i.e. >4, Fraser et al., 2019a). 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) and Breck and Sveier (2001) commented that salinity variation could be a factor in the osmotic origin of cataracts. However, we did not observe any effect of salinity in the current study.

We found triploidy decreased the incidence of sexual maturation in male post-smolts as in some studies on Atlantic salmon (Fraser et al., 2014a), but not others (Oppedal et al., 2003). Further work is required into what drives this inconsistency across experiments. Of note, *vgll3* is known to explain a significant amount of the variation in post-smolt maturation (Fjelldal et al., 2020) and work in wild fish would suggest the allele that promotes early puberty is dominant over the allele that promotes later maturation (Barson et al., 2015). How triploids regulate the additional third copy of *vgll3* is currently unknown, especially in heterozygous individuals, but would be of future interest. As the decision to mature occurs within the first few days after the "winter signal" has finished and the fish are still in freshwater (Fjelldal et al., 2020), this experiment should not be treated as a real test of the impact of salinity on post-smolt maturation. However, male salmon are thought to be able to terminate puberty shortly after initiation if environmental conditions change (Fraser et al., 2019b). Therefore, we have tentative evidence that salinity exposure may not trigger termination of post-smolt maturation.

4.7. Organ indexes

We found ploidy effects on organ indexes although their functional significance requires clarification. For instance, triploids had relatively larger hearts when corrected for body weight than diploids, as previously observed in some studies (Fraser et al., 2015: Fjelldal et al., 2016), but not others (Leclercq et al., 2011; Fraser et al., 2013, 2014b; Bowden et al., 2018; Smedley et al., 2016). Similarly, based on body weight, we previously found triploids to have relatively larger livers than diploids

(Fraser et al., 2014a; Fjelldal et al., 2016), whereas others have found no ploidy effect (this study, Tibbetts et al., 2013; Bowden et al., 2018; Taylor et al., 2019) or smaller relative sizes in triploids (Nuez-Ortín et al., 2017). For visceral mass, our lower relative size in triploids fits with the result of Taylor et al. (2019), whereas Cotter et al. (2002) only reported a strong trend for the same effect. Relative heart and liver size are known to be plastic in salmon and influenced by environmental factors (Gamperl and Farrell, 2004; Døskeland et al., 2016). Therefore, differences in developmental stage and environmental conditions may explain some of the reported inconsistencies between studies, especially if there are ploidy × environment interactions. In this context, we have previously found ploidy effects on relative liver size to depend on sampling time within the same study (Fraser et al., 2014a). Furthermore, triploids can have lower (this study, Fraser et al., 2013), equal (Fraser et al., 2021), or higher body condition than diploids (Fraser et al., 2015), although again these differences can change within study depending on sampling time (Fraser et al., 2021) or environmental conditions (Fraser et al., 2015), which also suggests complex ploidy × environment or ploidy × life stage interactions which make organ indexes and allometry challenging to interpret. Indeed, there was no ploidy effect on relative heart size when we used length rather than weight as a co-variate. Although body weight is generally used to determine relative organ size, it is more variable than length in that it can decrease during periods of low feed intake or during developmental processes such as smoltification leading to temporal increases in organ indexes.

Salinity and sex had significant effects on relative liver size but had no effect on the heart or viscera. The same salinity effect in liver was previously reported in Atlantic salmon maintained on 0 or 35 ppt for several months (Krogdahl et al., 2004) whereas there was no effect of salinity in Atlantic salmon kept at 12, 22 or 32 ppt from 70 to 450 g (Ytrestøyl et al., 2020). We found no salinity effect on relative heart size, unlike Ytrestøyl et al. (2020) who found an increase in the cardiosomatic index (CSI) with increasing salinity. Similarly, there was no sex effect on the hepatosomatic index in Ytrestøyl et al. (2020), but there was for CSI with males having relatively larger hearts than females. Further studies should try to understand the function significance of differences in allometry.

4.8. Conclusions

In summary, we found some mismatches in smoltification markers in underyearling triploid salmon. However, triploid post-smolts performed as well or better than diploids at all salinities tested, but may have a lower salinity optimum than diploids. This result should be confirmed in larger studies using more families to determine whether it is a general result of triploidy and not only restricted to the genetic material used in the current study. Salinity resulted in some differences in baseline plasma ions and pH, but there were few consistent ploidy effects on plasma biochemistry. Nevertheless, we found evidence that triploids can be produced without any increase in the prevalence of vertebral deformities. Finally, salinity had no impact on the prevalence or severity of cataracts in post-smolts.

Funding

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.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2021.737350.

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