Aquaculture 540 (2021) 736698

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Triploid Atlantic salmon and triploid Atlantic salmon × brown trout hybrids have better freshwater and early seawater growth than diploid counterparts

Thomas W.K. Fraser [a,*], Hilde Lerøy [b], Tom J. Hansen [a], Jon Egil Skjæraasen [c], Valentina Tronci [b], Cindy P. Pedrosa [b], Per Gunnar Fjelldal [a], Tom Ole Nilsen [b,d]

[a] Reproduction and Developmental Biology, Institute of Marine Research (IMR), Matre Aquaculture Research Station, 5984 Matredal, Norway
[b] Department of Biological Sciences, University of Bergen, 5007 Bergen, Norway
[c] Demersal Fish, Institute of Marine Research (IMR), Nordnes, 5817 Bergen, Norway
[d] Norwegian Research Center, NORCE Environment, 5838 Bergen, Norway

ARTICLE INFO

Keywords:
Smoltification
Bimodality
Hypo-osmoregulation
Sterile
Interspecific hybridisation

ABSTRACT

The use of reproductively sterile triploid salmonids would enhance the environmental sustainability of the aquaculture industry by preventing genetic exchange between escapees and wild conspecifics. To this end, we assessed smoltification and early seawater performance (241 days) following a yearling production cycle (i.e. spring smolts) in diploid and triploid female Atlantic salmon (*Salmo salar*) × male brown trout (*Salmo trutta*) hybrids compared to purebred diploid and triploid salmon. During freshwater rearing ($n = 180$/group), hybrids demonstrated a degree of bimodality in body size, significantly ($p < 0.05$) more so in diploid than triploid hybrids (11 and 37% in the lower mode, respectively) that was not seen in purebred salmon of either ploidy. This resulted in diploid hybrids being 66% smaller on average at sea transfer, whereas no hybridisation effect was seen in triploids, and both triploid groups were significantly heavier (16–43%) than diploid salmon. Irrespective of ploidy, lower mode hybrids grew poorly and showed low survival in seawater, suggesting they had failed to undergo smoltification. However, the upper mode diploid hybrids showed a similar Na^+/K^+-ATPase (NKA) enzyme activity surge during the spring as in diploid and triploid salmon, despite a higher ratio of the freshwater to seawater mRNA abundance of the NKA subunits (*nkaα1a* and *nkaα1b*) and a reduced plasma cortisol surge. At the end of the experimental period, both hybrids weighed significantly less than their salmon counterparts although the hybrid effect was again greater in diploids (71% smaller) than triploids (6% smaller). In addition, both triploid groups were on average heavier (15–22%) than diploid salmon. As such, both triploid Atlantic salmon and triploid hybrids can show enhanced growth performance from juveniles up to post-smolts compared to diploid salmon in an aquaculture setting.

1. Introduction

Due to artificial selection for on-farm performance and the subsequent loss in suitability for a life in the wild, escapees from aquaculture threaten both the genetic integrity and survivorship of natural populations. The situation in Norway, which is the largest farmed Atlantic salmon (*Salmo salar*) producer in the world, is particularly worrisome as genetic introgression between domesticated escapees and wild individuals has been found at levels up to 42% within natural river systems, with the highest values generally being associated with the most intensively farmed areas (Karlsson et al., 2016). Therefore, there is an urgent need to prevent genetic exchange between domestic and wild populations to improve the industries sustainability. Although land-based systems could theoretically prevent escapees, up-front costs are typically higher (Liu et al., 2016) and it would require a significant amount of new infrastructure before the current net pen production levels could be reached. Alternatively, the production of sterile fish would allow for the continued use of current infrastructure whilst preventing genetic exchange between escapees and wild fish.

Triploidy, the situation whereby an individual has three complete

* Corresponding author.
E-mail address: thomas.fraser@hi.no (T.W.K. Fraser).

https://doi.org/10.1016/j.aquaculture.2021.736698
Received 10 December 2020; Received in revised form 26 March 2021; Accepted 27 March 2021
Available online 29 March 2021

chromosome sets, is relatively easy to induce in salmonids, is not considered a genetic modification, and results in functionally sterile salmonids (Piferrer et al., 2009). The technology to mass produce triploids has existed since the 1970's, however, their farm performance has often been more inconsistent than the natural diploid state with greater frequencies of skeletal deformities and higher mortalities (e.g. McGeachy et al., 1996; Fraser et al., 2013). This inconsistency is generally attributed to the physiological differences between the two ploidy, as triploids have lower temperature optima (Sambraus et al., 2017a, 2018) and altered nutritional requirements (e.g. phosphorus Fjelldal et al., 2016 and histidine Taylor et al., 2015). Therefore, there has been a general reluctance by the industry to use triploids without further improvements to their farm performance (Benfey, 2015), although they have been used for several decades in all-female stocks in Tasmania (Australia) to prevent the growth reduction that occurs with sexual maturation (Amoroso et al., 2016).

An alternative approach to providing sterility in salmonids is producing interspecific hybrids. Of particular interest, crosses between the sympatric Atlantic salmon and brown trout (*Salmo trutta*) are commonly reported in the field (Verspoor, 1988) and their performance has occasionally been investigated with regards to their potential for heterosis or "hybrid vigour". Such hybrids were found to have poor performance in culture, being 46% smaller than Atlantic salmon after 27 months in seawater (Refstie, 1983) and not to be 100% sterile (Wilkens et al., 1993). Studies on returning migrants provide further anecdotal evidence that hybrids may have lower post-migration fitness than Atlantic salmon (Adams et al., 2014). Here, it would be interesting to determine whether this is due to the brown trout component. For example, post-migration, brown trout may frequently move between the marine and freshwater environment, whereas Atlantic salmon migrate far out to sea with constant high salinity and only return to spawn (Klemetsen et al., 2003). These differences in environmental range most likely explain why, compared to Atlantic salmon, brown trout show a reduced spring surge in indicators of smoltification, the process by which salmonids increase their hypo-osmoregulatory ability as a pre-adaption to life in seawater (Tanguy et al., 1994). Therefore, one may suspect the brown trout genetic component may make hybrids less suited to extended periods in full strength seawater compared to Atlantic salmon.

Although diploid Atlantic salmon × brown trout hybrids appear unsuited for aquaculture, their performance can be improved by triploidisation. For example, we recently found triploid hybrids to be 40–50% larger than diploid hybrids during freshwater rearing (Fraser et al., 2020) whereas triploid hybrids were found to be on average 33% larger than diploid salmon after 376 days in seawater, although there were no diploid hybrids or triploid salmon for comparison (Galbreath and Thorgaard, 1997). In addition to these growth improvements, triploidising hybrids provides further assurances of sterility (Galbreath and Thorgaard, 1995) and increase larval viability (Scheerer and Thorgaard, 1983). The reasons behind the ploidy effect on hybrid growth performance are unclear, but as the pressure-shock used to induce triploidy leads to a doubling of the maternal chromosome set (Piferrer et al., 2009), triploid Atlantic salmon female × brown trout male hybrids are genetically two thirds Atlantic salmon and one third brown trout, whereas diploid hybrids are equal parts salmon and trout. Therefore, triploid hybrids may share more characteristics with Atlantic salmon that are generally more suited to Norwegian farming conditions than brown trout (Gjedrem and Gunnes, 1978).

In the current study, we assessed smoltification development (gill Na^+/K^+-ATPase enzyme activity, relevant mRNA abundance, and plasma cortisol) and seawater growth in diploid and triploid Atlantic salmon × brown trout hybrids to assess their potential for future farming purposes compared to Atlantic salmon counterparts. We used a cross between an Atlantic salmon female with a brown trout male as this cross is generally considered more viable than the reciprocal cross (Álvarez and Garcia-Vazquez, 2011). Our hypothesis is that diploid hybrids would show both reduced growth and a lower spring surge in smoltification markers compared to diploid salmon, but any hybridisation effect would be reduced in triploid hybrids as their DNA content is biased towards salmon.

2. Material and methods

2.1. Ethics

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

2.2. Fish stock and rearing conditions

The incubation and early life rearing of the fish stock was previously described in a multiyear class study (Fraser et al., 2020, see the 2017 year class). In brief, on the 17th January 2017 (day 0) eggs from one domesticated Mowi strain Atlantic salmon were divided into two equal parts and fertilised with either sperm from one first generation offspring of wild (River Vosso, Norway) Atlantic salmon or one non-anadromous (lake Tunhovd, Eastern Norway) brown trout from a recently domesticated stock. After fertilisation, half the eggs from each cross were subject to a hydrostatic pressure shock of 655 bar for 6 min and 15 s (TRC-APV, Aqua Pressure Vessel, TRC Hydraulics inc., Dieppe, Canada) exactly 37.5 mins after fertilisation at 8 °C to induce triploidy. Ploidy confirmation (n = 48–50 from each group) was later achieved using blood cell diameter and reported in Fraser et al. (2020). In brief, triploids have larger cell volumes as they have a larger cell nuclei due to the increased amount of genetic material, yet they maintain the same nuclei to cell volume ratio as diploids (Benfey et al., 1984). The mean red blood cell diameter of triploids was approx. 18% larger than diploids, irrespective of hybridisation, with no overlap between individual mean values of putative diploids and triploids (min-max of individual means [µm, n = 92–1250 measured cells per individual: diploid salmon, 12.7–14.0; diploid hybrid 12.5–14.2; triploid salmon, 15.1–16.4; triploid hybrid, 14.6–16.1).

The resulting four groups, diploid salmon, triploid salmon, diploid hybrid, and triploid hybrid, were all reared under the conditions found in Fig. 1 that is typical for the production of yearling (i.e. 1+) smolts. Each group was incubated in a single tray before being moved to single fiberglass tanks at first feeding (1 × 1 × 0.43 m). At first feeding on day 100, the number of fish was reduced to 800 per tank. Mortality between fertilisation and first feeding was 21, 28, 48, and 16% for the diploid salmon, triploid salmon, diploid hybrid, and triploid hybrid, respectively. On day 233, 180 fish per group were implanted with a passive integrated transponder (PIT tag, 2 × 12 mm RFID solutions, Stavanger), had their fork length and weight recorded, and were equally distributed between 3 tanks for common garden rearing (1 × 1 × 0.43 m, n = 60/group/tank). On day 381, 72 fish from each group (n = 24/tank) were removed and equally distributed into an additional 6 tanks (1 × 1 × 0.43 m, n = 48/tank with 12/group and 4/group/original tank) used to assess the development of smoltification. As there was a notable bimodal size distribution in the diploid and triploid hybrids on day 381, we selected larger hybrids (Fig. S1) to assess smolt development as the smaller size fraction (< 13–14 cm) still had parr markings and were considered unlikely to undergo smoltification. Subsequent analysis demonstrated diploid hybrids below 13.2 and triploid hybrids below 14.5 cm showed minimal growth from February onwards (Fig. S1) as expected if the fish are unlikely to smoltify. The fish selected for smoltification analysis remained on freshwater throughout. Those fish not used to assess smoltification were maintained in their original three tanks (n = 120/tank with 30/group) in order to assess early seawater growth and survival. Subsequently, the water inflow to the tank was changed to full strength seawater over a 5 day period beginning on day

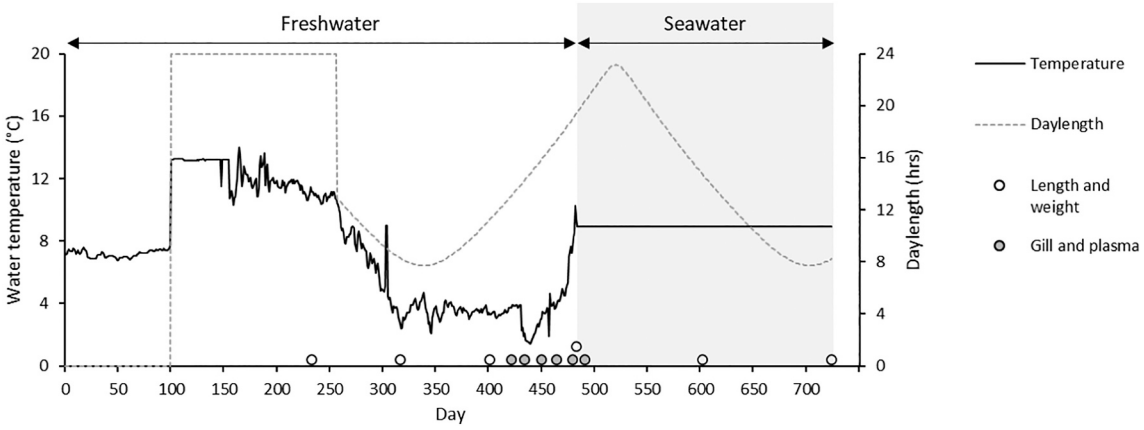

Fig. 1. Environmental conditions and sampling times throughout the experiment. Daylength in the facility is based on civil twilight (60° N).

484 (20 ppt on day 484, 28 ppt on day 486, and 35 ppt on day 489). These fish remained on 35 ppt up until the end of the reported period (day 724) after 241 days in seawater. On day 602, all three tanks of fish were transferred into one common garden large (6 m ø). Throughout, fish that appeared moribund were removed from the study and considered to have died.

2.3. Sampling procedures

All fish were anaesthetised in buffered 100 mg/L MS222 (Finquel®) on days 233, 316, and 401 and measured for PIT number, body weight (to 1 g), and fork length (to 0.1 cm). Those fish used to assess seawater growth were also anaesthetised and measured on days 483, 602, and 724. The condition factor (CF) was calculated as CF = weight [g]/ length^3 [cm] × 100. Specific growth rate (SGR, % day^{-1}) was calculated as ($e^q - 1$)100 where q = [ln(W_2) − ln(W_1)] ($t_2 - t_1$)$^{-1}$ and W_2 and W_1 are average body weight (g) at times t_1 and t_2, respectively.

On day 316, 16 fish tank^{-1} (n = 12 group^{-1}, n = 4 group^{-1} tank^{-1}) were removed from the study for use in another project. On days 421, 434, 450, 464, 479, and 491, all the fish within one of the six tanks used to assess smoltification were heavily sedated in buffered 300 mg/L Finquel® by turning off the inflow water and adding a stock solution of buffered Finquel® directly into the tank. Following one minute of sedation, the fish were quickly netted out of the tank and blood was collected from the caudal vein into heparinised syringes. Due to high human resources, blood was collected from all 48 fish within 5 mins of the original tank disturbance. Part of the blood sample was used to determine haematocrit via microhematocrit centrifugation whereas the remaining blood was centrifuged (16,000 *g* for 2 mins at 4 °C) and the plasma collected and stored on dry ice before being transferred to the −80 °C freezer. For all fish blood sampled, the second gill arch on the left side of the fish was also collected into ice-cold 1 mL SEI buffer (250 mM sucrose, 10 mM EDTA and 50 mM imidazole, pH 7.3) and frozen on dry ice, while gill filaments from the front right arch were collected and immediately frozen on dry ice before being stored at −80 °C. The sex of each fish was assessed by visual examination of the gonads.

2.4. Plasma analyses

Plasma samples were analysed for plasma ions (sodium, chloride, potassium, and calcium), pH, lactate, and glucose using a Radiometer ABL90 flex plus according to the manufacturers protocol (Bergman Diagnostika, Norway). Plasma cortisol was assessed using an ELISA as described in the kit protocol (RE 52061, IBL).

2.5. Gill Na^+/K^+–ATPase activity

Gill NKA enzyme activity was measured according to McCormick (1993). In brief, gill filaments were thawed, homogenised in 100 μL of SEI buffer plus 25 μL of SEID buffer (SEI buffer plus 0.1% deoxycholic acid (Calbiochem)) and centrifuged at 4 °C, at 5000 g, for 60 s. and analysed using a kinetic assay mixture that utilizes the hydrolysis of ATP, which is enzymatically coupled to the conversion of NADH to NAD+ by pyruvate kinase and lactic acid dehydrogenase with or without the addition the specific NKA inhibitor ouabain. Subsequently, duplicates of 10 μL of supernatant were mixed with 200 μL of the assay medium without inhibitor whereas another set of duplicates were mixed with assay buffer containing the NKA inhibitor (0.5 mM ouabain (Sigma Aldrich)). Kinetic assay readings were carried out at 340 nm for 10 min (reading every 10 s) at 25 °C in a spectrophotometer (Tecan Spark, Bergman Diagnostika). Total protein concentration was determined using a BCA Protein Assay Kit (Thermo Fisher Scientific Pierce™, Norway), with each sample of supernatant run in triplicate. NKA enzyme activity was determined as the ouabain sensitive fraction of the ATP hydrolysis and expressed as μmol ADP mg protein^{-1} h^{-1}.

2.6. Gill mRNA abundance

RNA isolation was carried out on frozen gill tissue (20–25 mg) from 8 random samples group^{-1} day^{-1} on days 434, 450, 464, 479, and 491. Initially, the tissue was homogenised in RLT Plus buffer (Qiagen) using ceramic spheres (Bertin Technologies BERT03961–1-103) and the Precellys 24 tissue homogenizer (Bertin Technologies) at 5000 rpm for 15 s. Total RNA (tRNA) was then isolated using the Qiagen RNA kit in the QIA symphony SP automatic system. tRNA concentration and purity were measured using a NanoDrop One/OneC Microvolume UV–Vis Spectrophotometer (Thermo Fisher Scientific) with purity confirmed to be above 1.9 and 2 for the 260/280 nm and 260/230 ratios. RNA integrity of 24 samples (6 from each group) was assessed using an RNA 6000 Nano LabChip kit using the Agilent 2100 Bioanalyzer (Agilent Technologies). All samples had a RIN value of >7.5. Complementary DNA was reversely transcribed using 1.5 μg of total RNA using oligo (dT_{20}) primer and the Superscript III kit (Fisher Scientific) using a MicrolabSTARlet Liquid Handling Workstation (Hamilton Robotics).

Real-time polymerase chain reaction (RT-PCR) was used to determined gene transcription of *nkaα1a* and *nkaα1b* (see Nilsen et al., 2007 for primer sequences). RT-PCR was carried out in a CFX-96 Real-Time PCR detection system platform (Bio-Rad) using the following PCR conditions: 3 min at 95 °C, 34 cycles of 15 s at 95 °C and 1 min at 60 °C with a standard melting curve at the end (10 s at 95 °C, 5 s at 65–95 °C with increments of 0.5 °C, and 5 s at 95 °C).

For each assay, duplicate two-fold cDNA dilution series from pooled

samples (1:5–1:160) were used to determine both amplification efficiencies for each oligo pair and optimal dilution for cDNA template. Samples were run in 10 μL duplicates using 6.25 μL iTaq Universal SYBR Green Supermix (Bio-Rad), 0.25 μL of each primer, and 2.5 μL of diluted cDNA (dilution 1:50). Each plate included a non-template control as well as a common sample used for the intercalibration of assays among plates. The relative transcription levels of the genes were normalized following the efficiency corrected method (Pfaffl et al., 2004) using *ef1α* as an endogenous reference gene (see Olsvik et al., 2005 for primer sequence).

2.7. Statistics

Mortality was assessed using a cox proportional hazards model. Here, data on the time the individual was within the experiment in days (continuous), group (4 levels; diploid salmon, diploid hybrid, triploid salmon, triploid hybrid), and the outcome at the time of leaving the study (dead/alive) were included. Notably, the majority of the hybrid mortalities were in seawater and mostly in those fish that were < 15 cm in body length at seawater transfer (see results). As this is similar to historic reports of a size threshold for smoltification in cultured salmonids (e.g. Thorpe, 1977), we assigned fish of <15 cm at sea transfer (day 483) as being parr, and ran a separate model on those considered smolts (i.e. > 15 cm) only. Significance was assigned at $p < 0.05$.

To assess growth we first compared each time point separately as an all-inclusive longitudinal model to compare ploidy, hybridisation, and any interaction, over time based on repeated measures (individual fish) was difficult to achieve due to bimodal body size distributions and size related mortalities (see results) within some groups leading to poor model fit. Therefore, we used the non-parametric Kruskal-Wallis test to assess group differences in length, weight, and body condition within each time point separately and used the Dunn's test with a Bonferroni correction for multiple comparisons as a post-hoc to compare groups (4 levels; diploid salmon, diploid hybrid, triploid salmon, triploid hybrid). We used two approaches, initially we modelled body size based on all individuals measured at a given timepoint, whereas in a second analysis we assessed body size at all time points in only those fish that survived until the end of the study (i.e. without those fish that failed to undergo the parr-smolt transformation).

Based on our hypothesis, we expected the following results with regards to smoltification markers; diploid salmon = triploid salmon > triploid hybrid > diploid hybrid. To test our hypothesis, where possible we employed general additive models (GAMs) and compared three models; the first model allowed the response to vary over time dependent on group (4 levels, diploid salmon, diploid hybrid, triploid salmon, triploid hybrid), the second model allowed the response to vary over time dependent on ploidy (2 levels, diploid, triploid) and hybridisation (2 levels, salmon, hybrid), whereas the third model allowed the response to vary over time, but to be consistent across all groups. The three models were compared using the GCV score, with the model with the lowest score being considered to have the most appropriate data fit. Post-hoc tests were done using lsmeans within the "emmeans" library. Model diagnostics of the residuals were carried out following examination of standardised vs fitted residuals, histograms, and/or q-q plots. Where normality could not be achieved (i.e. potassium and pH), we used the non-parametric Kruskal-Wallis test to look for general group (4 levels), ploidy (2 levels), and hybridisation effects (2 levels) within the entire dataset, with Dunn's test as a post-hoc. Following this, we used the same approach, but within each time point separately. Some endpoints were natural log or tukey transformed to ensure normality of the model residuals (see R script for details).

Fish used in the physiology study that had a fork length of <13.2 cm in February 2018 ($n = 5$ diploid hybrids, 1 from days 421, 434, and 464, and two from day 479) were excluded as they were considered likely to remain as parr and not undergo the parr-smolt transformation (see above, Fig. S1). In addition, for some endpoints (1 for *nkaα1a* and *nkaα1b* (the same sample for each) and 5 for plasma sodium and 2 for chloride (one of which was the same sample)) we checked models with and without outliers (based on a zscore of ±3.0) in order to achieve normality of the model residuals, with the exception of plasma sodium whereby to achieve normality in the residuals we used a cut-off of ±2.5 for the zscore (see R script for details).

3. Results

3.1. Mortality

By the end of the experiment, both diploid and triploid hybrids had significantly higher accumulated mortality than diploid salmon (Fig. 2A). Mortalities were generally low in freshwater (< 8% in all groups), but the diploid hybrids still had significantly lower survival than diploid salmon during this period (Fig. 2A). The majority of the hybrid mortalities were in seawater and then mostly in fish <15 cm in body length at seawater transfer (Fig. 2B). Nevertheless, a separate model on fish considered to be smolts at sea transfer (i.e. only those >15 cm) still found significantly lower survival in diploid hybrids compared to diploid salmon, even when accounting for body length at sea transfer, but there was no difference in survival between triploid hybrids and diploid salmon (Fig. 2C).

3.2. Growth

Regarding hybridisation, salmon where always significantly heavier than hybrids in diploids, but the opposite was generally true in triploids except for days 233 and 483 when there was no hybridisation effect and the final timepoint when salmon were significantly heavier than hybrids (Fig. 3). In addition, triploid salmon were generally longer than diploid salmon throughout, although this difference was not significant at all timepoints. For body condition, hybrids had either equal or greater values than salmon in freshwater, but had significantly lower values at the end of the study, irrespective of ploidy (Fig. S2).

Due to size dependent mortality in seawater, we also assessed models that compared only those fish that survived until the final timepoint (i.e. mainly the upper mode hybrids vs diploid and triploid salmon). Subsequently, we found that the diploid hybrids that survived until the end of the study were equal in body weight as diploid salmon up until sea transfer, but still showed lower growth in seawater (Fig. S3).

3.3. Frequency of lower mode (parr) vs upper mode (smolt) fish on day 401

Based on body length data and survival in seawater, we approximated that diploid hybrids of <13.2 cm and triploid hybrids of <14.5 cm on day 401 were mostly likely to have remained as parr (see Fig. S1). This represented 11 and 37% of the diploid and triploid hybrid populations on day 401, respectively, i.e. a significant difference between ploidy (G-test, $p < 0.001$).

3.4. Smoltification indicators

Significant effects of time on all parameters and ploidy × hybrid interactions on the NKA enzyme activity, subunit expression, and plasma cortisol were observed. As expected during the parr-smolt transformation, we observed a significant decrease in the NKA subunit ratio, an increase in plasma cortisol, an increase in gill enzyme activity, and a decrease in body condition over time beginning from days 450, 450, 464, and 479, respectively. For the ploidy × hybrid interactions, in support of our hypothesis, the diploid hybrids had a significantly higher NKA subunit ratio (Fig. 4A) and *nkaα1a* expression (Fig. 4B) on day 434, but this effect disappeared with time. In contrast, triploid salmon had higher *nkaα1b* values than hybrids on day 421, but by day 491 diploid salmon had a higher expression than all other groups (Fig. 4C). There

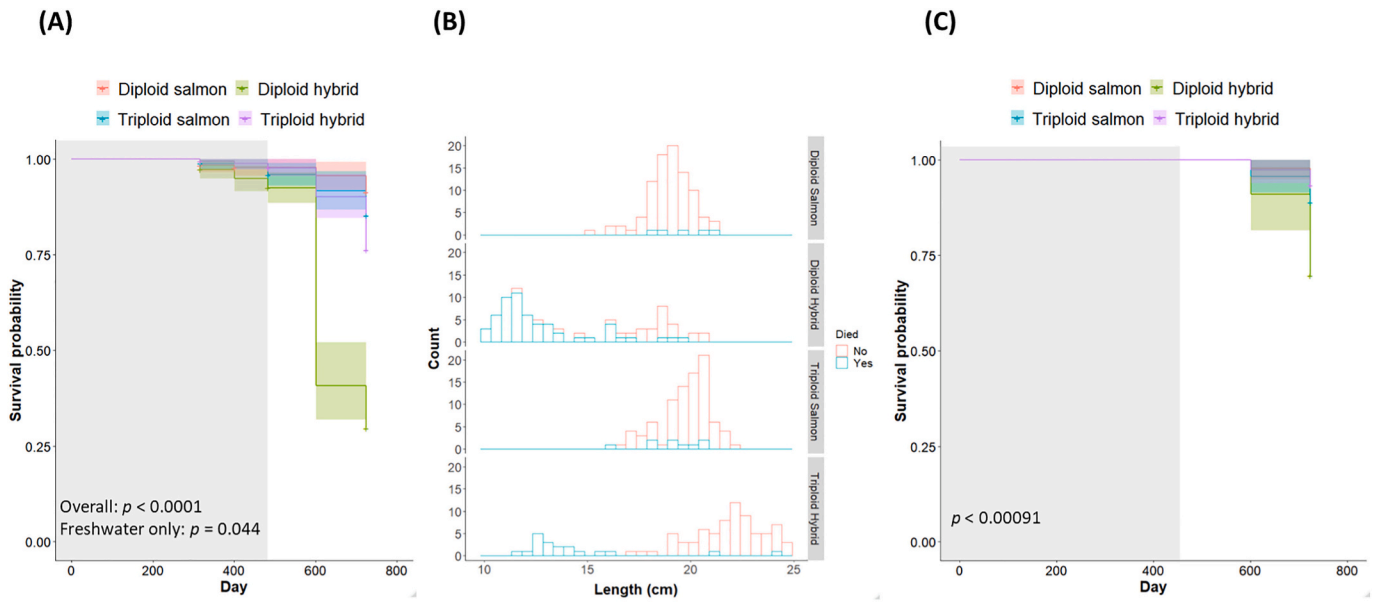

Fig. 2. Mortality assessment in diploid and triploid Atlantic salmon and Atlantic salmon × brown trout hybrids. (A) Survival models with confidence intervals for all fish throughout the experiment (overall) and during freshwater only (the shaded area). (B) Histogram of mortality in seawater related to size at sea transfer. (C) Mortality in seawater discounting fish that were < 15 cm at sea transfer. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was only a tendency for diploid hybrids to have lower gill NKA enzyme activity than diploid salmon ($P = 0.06$) whereas the triploid hybrid did have significantly higher activity than the triploid salmon and the diploid hybrid (Fig. 4D). Plasma cortisol generally increased throughout smoltification, but was significantly lower in the diploid hybrids on days 450, 464, and 491 compared to all other groups (Fig. 4E). For body condition, there was no ploidy × hybrid interaction, but triploids had significantly lower values than diploids and hybrids had significantly higher values than salmon (Fig. 4F).

3.5. General physiology during smoltification

Plasma sodium, chloride, potassium, and calcium showed transient trends over the smoltification period (Fig. 5A–D, respectively), but only plasma potassium showed a significant group effect with triploid hybrids having significantly lower values than all other groups irrespective of time. In addition, hybrids, independent of ploidy and time, had significantly higher plasma chloride than purebred salmon.

Haematocrit, and plasma glucose and lactate (Fig. 6A–C, respectively) showed transient trends during smoltification, with ploidy × hybrid interactions in haematocrit and glucose. For haematocrit, triploids had lower values than diploids in both salmon and hybrids, but hybrids only had significantly lower values than salmon in triploids independent of time. For glucose, triploid salmon had significantly lower values than all other groups, with no hybridisation effect in diploids. In lactate, hybrids had significantly lower values than salmon irrespective of ploidy with the difference increasing over smoltification. Plasma pH showed no notable time trends, or any effect of ploidy, hybridisation, or group (Fig. 6D).

4. Discussion

We assessed growth and smoltification in diploid and triploid Atlantic salmon × brown trout hybrids. We found support for our hypothesis that hybrids would perform comparatively better as triploids than diploids, although some of the advantages of triploid hybrids were greater than expected based on the idea that performance is solely related to the genetic contribution of the parental species alone.

Work in the freshwater life stages of salmonids has demonstrated bimodal size distributions in year-class cohorts of Atlantic salmon and to a lesser extent in brown trout (Tanguy et al., 1994). In salmon, lower mode individuals prolong their stay in freshwater for another season whereas those in the upper mode undergo the parr-smolt transformation and migrate to sea (Thorpe, 1977). The cut-off in body size for Atlantic salmon varies somewhat between studies but is generally regarded to be around 15 cm in wild fish (Hutchings and Jones, 1998). However, salmon parr from the lower mode can survive for many days at salinities of ≥30% ppt, albeit growth is at best transiently disturbed and the risk of mortality is high (Duston, 1994). In brown trout, quicker growing individuals migrate whereas the slower growers become residents (Jonsson, 1985). The mean smolt size of wild trout populations in Norway ranges from 11 to 23 cm with the smallest individuals being around 7 cm (L'Abée-Lund et al., 1989). However, as with salmon parr, resident trout can survive for extended periods in seawater (Tanguy et al., 1994). We found the diploid and triploid hybrids to show bimodal size distribution at our earliest time point and this continued up to sea transfer. Furthermore, most of the lower size mode became moribund within 4 (diploid) and 8 (triploid) months of being exposed to seawater, suggesting they had not undergone the parr-smolt transformation. There was no bimodal distribution in the diploid and triploid salmon, which was expected based on modern rearing practices. Therefore, our hybrids generally had a lower scope for growth in freshwater compared to Atlantic salmon, as seen in some studies on Atlantic salmon x brown trout hybrids (Refstie and Gjedrem, 1975; Bakke et al., 1999; Fraser et al., 2020), but not all (Galbreath and Thorgaard, 1994; Oke et al., 2009; Fraser et al., 2020). However, as triploid hybrids had a higher frequency of smolts, a larger size threshold for smoltification, and the suspected parr lasted longer in seawater, it appears the inclusion of the paternal and/or brown trout genome drove this hybridisation effect of bimodality.

We found some support for our hypothesis that hybridisation would reduce the magnitude of smoltification as the diploid hybrid had a significantly higher NKA mRNA abundance ratio and lower plasma cortisol surge than all other groups, and a significantly lower NKA enzyme activity than triploid hybrids. This complies with the premise that domestic (Tanguy et al., 1994) and wild (Urke et al., 2010, 2013)

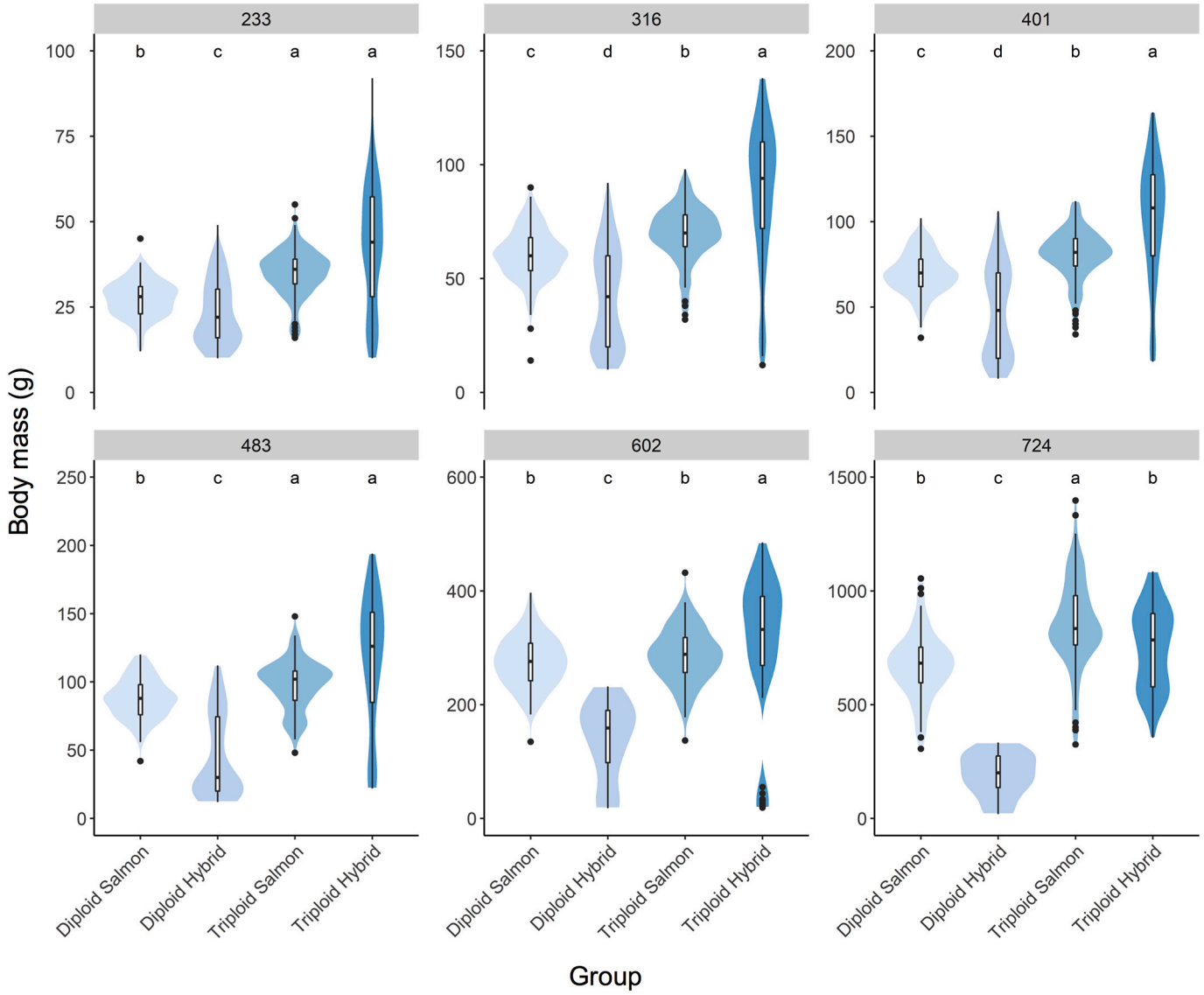

Fig. 3. Body mass over time (in days) in diploid and triploid Atlantic salmon and Atlantic salmon × brown trout hybrids. Statistical results are from Kruskal-Wallis tests within day and different subscript letters indicate significant effects of group within day (post-hoc Dunn's test, $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

anadromous brown trout show a reduced spring gill NKA enzyme activity surge compared to Atlantic salmon. However, although diploid hybrids did have the lowest gill NKA enzyme activity values, the difference with diploid salmon was not significant as was also found in wild populations (Urke et al., 2010, 2013). In addition, in contrast to our findings, Urke et al. (2013) failed to detect any effect of hybridisation on NKA mRNA abundance in wild diploid salmon and hybrids. This could be due to study design, as Urke et al. (2013) used a single sample time at presumed peak smolt in freshwater compared to our multiple time points. Notably, in diploids, we found hybrid differences in *nkaα1a* to be much greater earlier in smoltification compared to levels closer to peak smolt, whereas the opposite occurred for *nkaα1b*. In addition, we used a landlocked brown trout population to produce our hybrids, unlike the anadromous population in Urke et al. (2013). This may complicate comparisons, as even though landlocked trout can still show a spring surge in NKA enzyme activity (Pirhonen and Forsman, 1998) we had no purebred fish to confirm this. Finally, mRNA abundance is not always highly correlated with protein levels during smoltification (Christensen et al., 2018), but how this relates to strain is unknown.

To date, relatively little data exists on the parr-smolt transformation in triploid salmonids. Previously, Taylor et al. (2012) found triploid 0+ salmon smolts smoltified earlier than diploid counterparts based on body colouration, with others providing similar anecdotal findings (Leclerqc et al., 2011; Smedley et al., 2016). In contrast, triploidy has not been found to effect gill NKA enzyme activity in 0+ (Taylor et al., 2012) or 1+ Atlantic salmon smolts (Taylor et al., 2012; Sambraus et al., 2017b) although mortalities and plasma osmolality was higher in triploids compared to diploids when transferred to seawater at 16 vs 10 °C (Sambraus et al., 2017b). In other salmonids, although no major ploidy effect on saltwater adaptation or IGF-1 was found in non-anadromous rainbow trout (*Oncorhynchus mykiss*) (Taylor et al., 2007), diploid chinook salmon (*Oncorhynchus tshawytscha*) smolts did have higher gill NKA enzyme activity compared to triploids (Shrimpton et al., 2007, 2011), although mRNA abundance of *nkaα1a* and *nkaα1b* did not differ (Shrimpton et al., 2007) and IGF-1 was either equal (Shrimpton et al., 2007) or lower (Shrimpton et al., 2011) in triploids. We observed no ploidy effect on NKA enzyme activity or mRNA abundance in salmon, although lower glucose and haematocrit values in triploids would suggest some underlying physiological differences during the smoltification period. Therefore, it appears likely that ploidy effects are dependent on

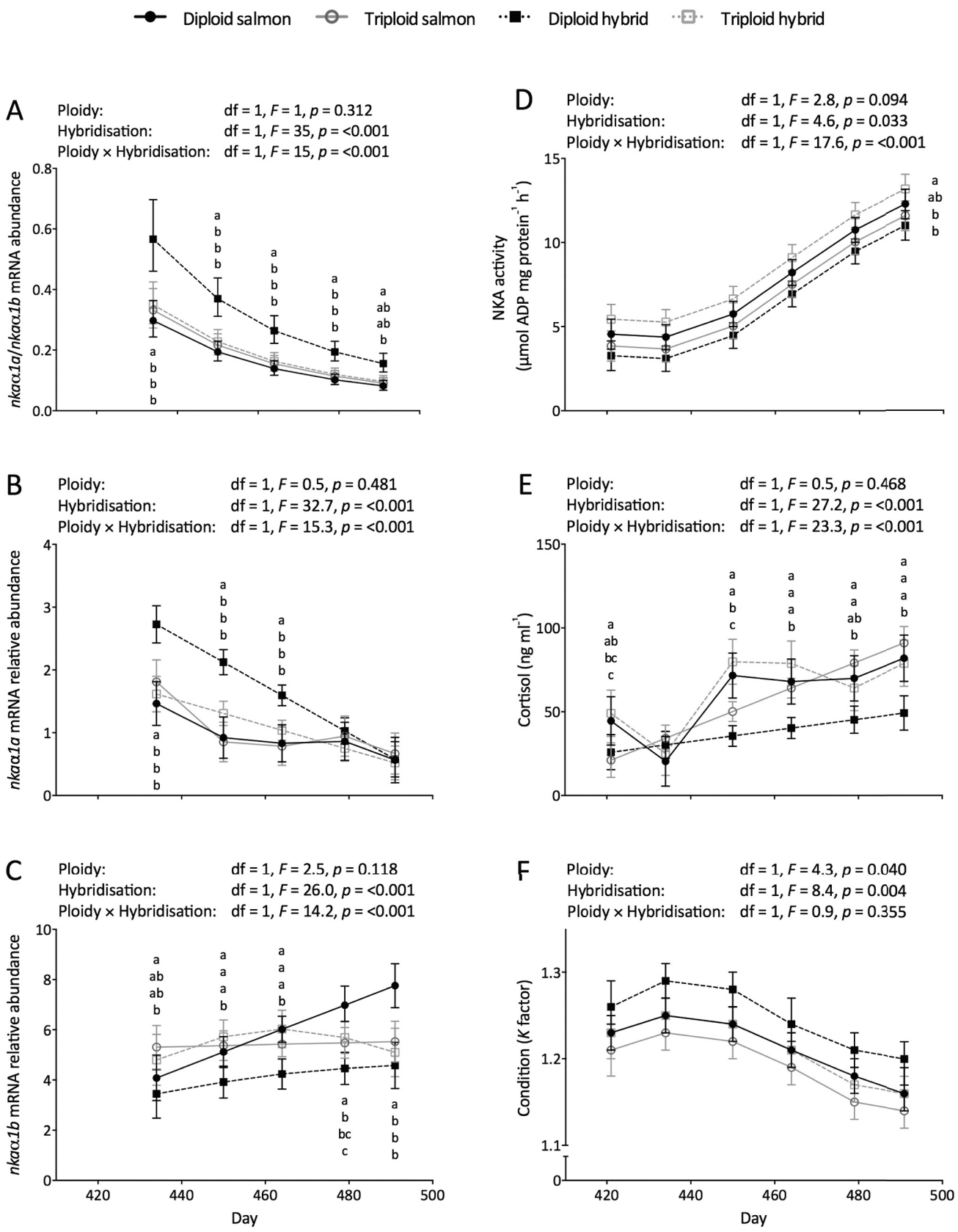

Fig. 4. Smoltification related endpoints in diploid and triploid Atlantic salmon and Atlantic salmon × brown trout hybrids. Data and statistics are from GAM models. Different lowercase letters indicate significant effects (maximum to minimum group means) within timepoint, except for D where they explain a group effect independent of time (post hoc, least square means ±95% CI, $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

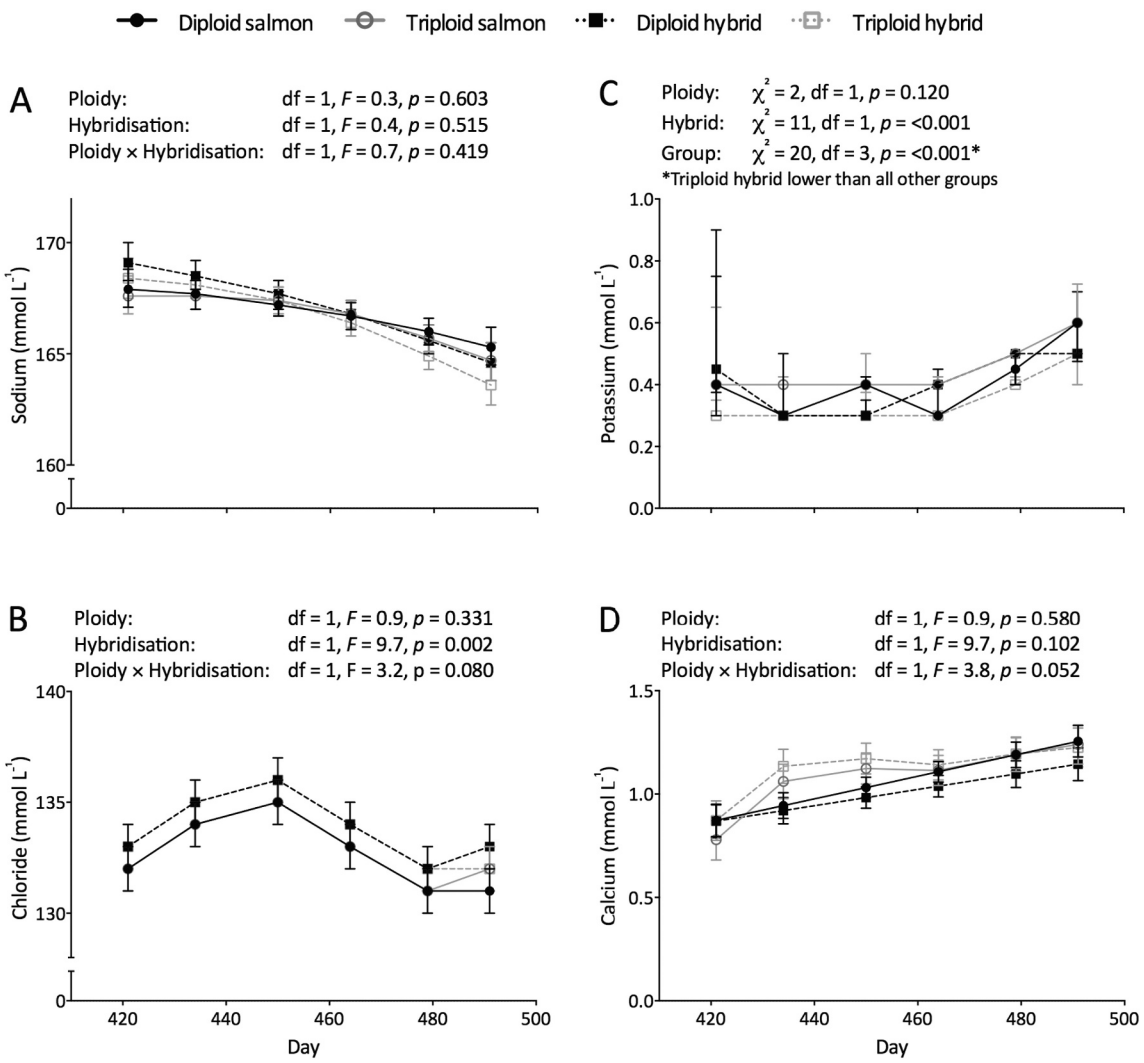

Fig. 5. Plasma ions in diploid and triploid Atlantic salmon and Atlantic salmon × brown trout hybrids. Data and statistics are from GAM models and include least square ± 95% CI for each time point with the exception of potassium for which the statistics are from a Kruskal-Wallis test and data are medians ± the 25 and 75% quartiles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

species and/or the environmental conditions used to induce smoltification.

In contrast to the expected, we found triploid hybrids to have a greater smoltification surge than triploid salmon. Unexpected effects of hybridisation have been reported to occur before in crossings of diploid anadromous (steelhead) female × non-anadromous (rainbow trout) male, in that hybrids reached peak smolt earlier than purebred steelhead trout (McLeese et al., 1994). In addition, pre-smolt Atlantic salmon x arctic char (*Salvelinus alpinus*) hybrids outperformed both purebreds in a salinity challenge (Sutterlin et al., 1977). In contrast, salmonid hybrids produced from species with notable differences in seawater tolerance have been found to have the expected intermediate values in smoltification parameters (Boeuf and Harache, 1984; Foote et al., 1992). Regarding triploid hybrids, triploidisation of a female non-anadromous strain brook charr (*Salvelinus fontinalis*) × male anadromous strain arctic char had no effect on seawater acclimation (Dumas et al., 1995). In contrast, triploid female chum salmon (*Oncorhynchus keta*) × male chinook salmon hybrids resembled chum salmon, in that they could be transferred to seawater immediately after first feeding in contrast to chinook salmon that require several months to a year before being able to tolerate seawater, but no diploid hybrids or triploid purebreds were included in the comparison (Seeb et al., 1993). Therefore, current evidence suggests hybridisation, triploidy, and/or their combination, can alter the timing of smoltification in unexpected ways, and should be researched thoroughly to ensure optimisation of production protocols.

Seawater growth and survival was comparatively poorer in diploid, compared to triploid, hybrids, as expected based on the relative contribution of brown trout DNA. Previously, Refstie (1983) also found a 20% reduction in seawater survival in diploid Atlantic salmon × brown trout hybrids compared to diploid Atlantic salmon whereas triploid hybrids had equal survival to diploid salmon (Galbreath and Thorgaard, 1997). However, as NKA enzyme activity in the diploid hybrids was comparable to diploid salmon, and to values recorded in smolts from other studies (Urke et al., 2013), a lack of seawater readiness at sea transfer appears unlikely to explain the long-term reduction in growth and survival. In addition, the triploid hybrid had higher NKA enzyme activity than the triploid salmon, yet still showed reduced long-term growth compared to triploid salmon. However, irrespective of ploidy, hybrids did show some morphological and physiological differences during smoltification with a higher body condition and plasma chloride values, but lower plasma lactate and potassium (triploid hybrids only). The difference in plasma chloride is particularly interesting given its relevance to hypo-osmoregulatory ability. Therefore, future studies should assess basal physiology throughout the life cycle to gain further insights into comparative physiology and environmental optima. Nevertheless, we can only conclude that either our use of 35 ppt

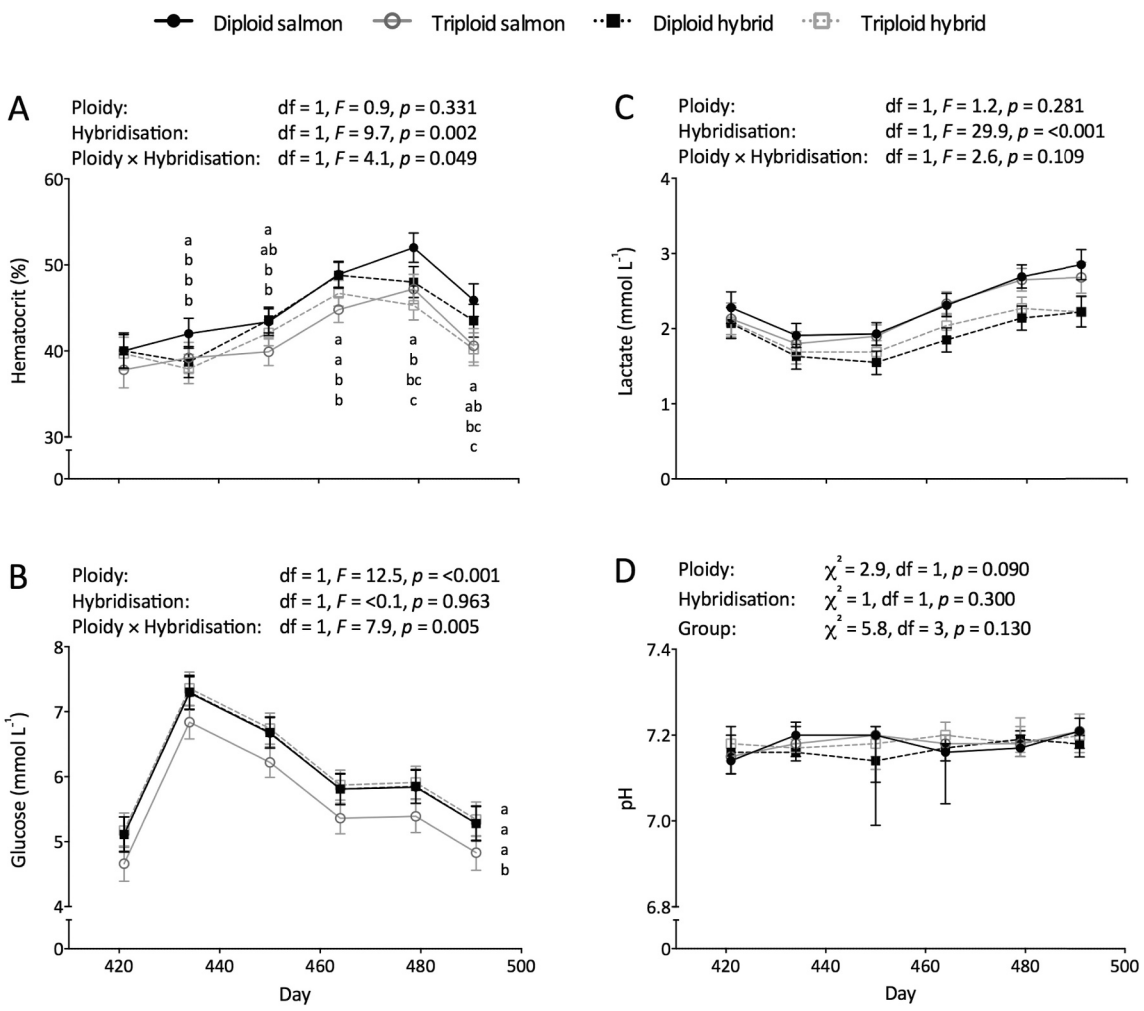

Fig. 6. Haematocrit and plasma physiology in diploid and triploid Atlantic salmon and Atlantic salmon × brown trout hybrids. Data and statistics are from GAM models and include lsmeans ±95% CI for each time point with the exception of pH for which the statistics are from a Kruskal-Wallis test and data are medians ±25 and 75% quartiles. Different lowercase letters indicate significant effects (maximum to minimum group means) within timepoint, except for B where they explain a group effect independent of time (post hoc, least square means ±95% CI, $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

favoured the performance of Atlantic salmon over hybrids, or that hybrids simply show impaired growth in larger fish.

Triploid salmon show inconsistent farm performance compared to diploids in seawater with reports of equal (O'Flynn et al., 1997; Smedley et al., 2016), better (O'Flynn et al., 1997; Oppedal et al., 2003) or poorer growth (Cotter et al., 2002; Taylor et al., 2013; Fraser et al., 2013). Indeed, a recent industrial project in Norway that utilised data from commercial trials in Northern Norway concluded triploid salmon should not be used as autumn (i.e. 0+) smolts due to wound and ulcer development, especially during the first winter, and generally higher vulnerability to parasite, virus, and bacterial infections compared to diploids (Stien et al., 2019). To date, the exact reasons behind the inconsistent seawater performance is unknown, but are generally suspected of being related to ploidy differences in environmental optima such as temperature (Sambraus et al., 2018), nutritional requirements (Taylor et al., 2015; Fjelldal et al., 2016), and/or inadequate timing of sea transfer due to differences in smoltification physiology (Taylor et al., 2012). Here, we report that when produced as 1+ smolts and grown indoors at 35 ppt with a natural photoperiod at 8.8 °C on "diploid" feed, triploid salmon performed favourably compared to diploid salmon. Therefore, with the rise of recirculating aquaculture systems that move the salmon production on-land, triploids may provide growth benefits over traditional diploid salmon if environmental conditions are maintained within their optimum range.

Funding

This work was funded by the Institute of Marine Research (internal project number 14594), Norway Royal Salmon (TRIPWELL, IMR internal project number 14930-04), and CtrlAqua SFI at NORCE.

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

We thank Ivar Helge Matre, Lise Dyrhovden, Simon Flavell, Britt Daae, and Florian Sambraus for help with sampling and/or fish husbandry.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

org/10.1016/j.aquaculture.2021.736698.

References

Adams, C.E., Burrows, A., Thompson, C., Verspoor, E., 2014. An unusually high frequency of Atlantic salmon x brown trout hybrids in the Loch Lomond catchment, west-Central Scotland. Glasg. Nat. 26, 75–81.

Álvarez, D., Garcia-Vazquez, E., 2011. Maintenance of asymmetric hybridization between Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) via postzygotic barriers and paternal effects. Can. J. Fish. Aquat. Sci. 68, 593–602.

Amoroso, G., Cobcroft, J.M., Adams, M.B., Ventura, T., Carter, C.G., 2016. Concurrence of lower jaw skeletal anomalies in triploid Atlantic salmon (*Salmo salar* L.) and the effect on growth in freshwater. J. Fish Dis. 39, 1509–1521.

Bakke, T.A., Soleng, A., Harris, P.D., 1999. The susceptibility of Atlantic salmon (*Salmo salar* L.) × brown trout (*Salmo trutta* L.) hybrids to *Gyrodactylus salaris* Malmberg and *Gyrodactylus derjavini* Mikailov. Parasitology 119, 467–481.

Benfey, T.J., 2015. Effectiveness of triploidy as a management tool for reproductive containment of farmed fish: Atlantic salmon (*Salmo salar*) as a case study. Rev. Aquac. 7, 1–19.

Benfey, T.J., Sutterlin, A.M., Thompson, R.J., 1984. Use of erythrocyte measurements to identify triploid salmonids. Can. J. Fish. Aquat. Sci. 41, 980–984.

Boeuf, G., Harache, Y., 1984. Adaptation osmotique a l'leau de mer de differentes especes (*Salmo trutta*, *Salmo gairdneri*, *Salvelinus fontinalis*) et hybride (*Salmo trutta* ♀ × *Salvelinus fontinalis* ♂) de salmonids. Aquaculture 40, 343–358.

Christensen, A.K., Regish, A.M., McCormick, S.D., 2018. Shifts in the relationship between mRNA and protein abundance of gill ion transporters during smolt development and seawater acclimation in Atlantic salmon (*Salmo salar*). Comp. Biochem. Physiol. A. 221, 63–73.

Cotter, D., O'Donovan, V., Drumm, A., Roche, N., Ling, N.E., Wilkins, N.P., 2002. Comparison of freshwater and marine performances of all-female diploid and triploid Atlantic salmon (*Salmo salar* L.). Aquac. Res. 33, 43–53.

Dumas, S., Audet, C., Blanc, J.M., de la Noüe, J., 1995. Seawater acclimation of diploid and triploid brook charr (*Salvelinus fontinalis*), diploid Arctic charr (*Salvelinus alpinus*), and their diploid and triploid hybrids. J. Fish Biol. 46, 302–316.

Duston, J., 1994. Effect of salinity on survival and growth of Atlantic salmon (*Salmo salar*) parr and smolts. Aquaculture 121, 115–124.

Fjelldal, P.G., Hansen, T.J., Lock, E.J., Wargelius, A., Fraser, T.W.K., Sambraus, F., El-Mowafi, A., Albrektsen, S., Waagbø, R., Ørnsrud, R., 2016. Increased dietary phosphorus prevents vertebral deformities in triploid Atlantic salmon (*Salmo salar* L.). Aquac. Nutr. 22, 72–90.

Foote, C.J., Wood, C.C., Clarke, W.C., Blackburn, J., 1992. Circannual cycle of seawater adaptability in *Oncorhynchus nerka*: genetic differences between sympatric sockeye salmon and kokanee. Can. J. Fish. Aquat. Sci. 49, 99–109.

Fraser, T.W.K., Hansen, T., Skjæraasen, J.E., Mayer, I., Sambraus, F., Fjelldal, P.G., 2013. The effect of triploidy on the culture performance, deformity prevalence, and heart morphology in Atlantic salmon. Aquaculture 416-417, 255–264.

Fraser, T.W.K., Hansen, T.J., Sambraus, F., Fjelldal, P.G., 2020. Vertebral deformities in interspecific diploid and triploid salmonid hybrids. J. Fish Biol. https://doi.org/10.1111/jfb.14353.

Galbreath, P.F., Thorgaard, G.H., 1994. Viability and freshwater performance of Atlantic salmon (*Salmo salar*) × brown trout (*Salmo trutta*) triploid hybrids. Can. J. Fish. Aquat. Sci. 51, 16–24.

Galbreath, P.F., Thorgaard, G.H., 1995. Sexual maturation and fertility of diploid and triploid Atlantic salmon × brown trout hybrids. Aquaculture 137, 299–311.

Galbreath, P.F., Thorgaard, G.H., 1997. Saltwater performance of triploid Atlantic salmon *Salmo salar* L. × brown trout *Salmo trutta* L. hybrids. Aquac. Res. 28, 1–8.

Gjedrem, T., Gunnes, K., 1978. Comparison of growth rate in Atlantic salmon, pink salmon, Arctic char, sea trout and rainbow trout under Norwegian farming conditions. Aquaculture 13, 135–141.

Hutchings, J.A., Jones, M.E.B., 1998. Life history variation and growth rate thresholds for maturity in Atlantic salmon, *Salmo salar*. Can. J. Fish. Aquat. Sci. 55, 22–47.

Jonsson, B., 1985. Life history patterns of freshwater resident and sea-run migrant brown trout in Norway. Trans. Am. Fish. Soc. 114, 182–194.

Karlsson, S., Diserud, O.H., Fiske, P., Hindar, K., 2016. Widespread genetic introgression of escaped farmed Atlantic salmon in wild salmon populations. ICES J. Mar. Sci. 73, 2488–2498.

Klemetsen, A., Amundsen, P.A., Dempson, J.B., Jonsson, B., Jonsson, N., O'Connell, M.F., Mortensen, E., 2003. Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. Ecol. Freshw. Fish 12, 1–59.

L'Abée-Lund, J., Jonsson, B., Jensen, A.J., Saettem, L.M., Heggberget, T.G., Johnsen, B. O., Naesje, T.F., 1989. Latitudinal variation in life-history characteristics of sea-run migrant brown trout *Salmo trutta*. J. Anim. Ecol. 58, 525–542.

Leclerqc, E., Taylor, J.F., Fison, D., Fjelldal, P.G., Diez-Padrisa, M., Hansen, T., Migaud, H., 2011. Comparative seawater performance and deformity prevalence in out-of-season diploid and triploid Atlantic salmon (*Salmo salar*) post-smolts. Comp. Biochem. Physiol. A 158, 116–125.

Liu, Y., Rosten, T.W., Henriksen, K., Hognes, E.S., Summerfelt, S., Vinci, B., 2016. Comparative economic performance and carbon footprint of two farming models for producing Atlantic salmon (Salmo salar): land-based closed containment system in freshwater and open net pen in seawater. Aquac. Eng. 71, 1–12.

McCormick, S.D., 1993. Methods for nonlethal gill biopsy and measurement of Na + ,K + -ATPase activity. Can. J. Fish. Aquat. Sci. 50, 656–658.

McGeachy, S.A., O'Flynn, F.M., Benfey, T.J., Friars, G.W., 1996. Seawater performance of triploid Atlantic salmon in New Brunswick aquaculture. Bull. Aquacult. Assoc. Can. 96, 24–28.

McLeese, J.M., Johnsson, J., Huntley, F.M., Clarke, W.C., Weisbart, M., 1994. Seasonal changes in osmoregulation, cortisol, and cortisol receptor activity in the gills of parr/smolt of steelhead trout and steelhead-rainbow trout hybrids, *Oncorhynchus mykiss*. Gen. Comp. Endocrinol. 93, 103–113.

Nilsen, T.O., Ebbesson, L.O.E., Madsen, S.S., McCormick, S.D., Andersson, E., Björnsson, B.Th., Prunet, P., Stefansson, S.O., 2007. Differential expression of gill Na$^+$,K$^+$-ATPaseα - and β-subunits, Na$^+$,K$^+$,2Cl$^-$ cotransporter and CFTR anion channel in juvenile anadromous and landlocked Atlantic salmon *Salmo salar*. J. Exp. Biol. 210, 2885–2896.

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

Oke, K.B., Westley, P.A.H., Moreau, D.T.R., Fleming, I.A., 2009. Hybridization between genetically modified Atlantic salmon and wild brown trout reveals novel ecological interactions. Proc. R. Soc. B 280, 20131047.

Olsvik, P.A., Lie, K.K., Jordal, A.-O., Nilsen, T.O., Hordvik, I., 2005. Evaluation of potential reference genes in real-time RT-PCR studies of Atlantic salmon. BMC Mol. Biol. 6, 21.

Oppedal, F., Taranger, G.L., Hansen, T., 2003. Growth performance and sexual maturation in diploid and triploids Atlantic salmon (*Salmo salar* L.) in seawater tanks exposed to continuous light or simulated natural photoperiod. Aquaculture 215, 145–162.

Pfaffl, M.W., Tichopad, A., Prgomet, C., Neuvians, T.P., 2004. Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper- excel-based tool using pair-wise correlations. Biotechnol. Lett. 26, 509–515.

Piferrer, P., Beaumont, A., Falguière, J.-C., Flajšhans, M., Haffray, P., Colombo, L., 2009. Polyploid fish and shellfish: production, biology and applications to aquaculture for performance improvement and genetic containment. Aquaculture 293, 125–156.

Pirhonen, J., Forsman, L., 1998. Relationship between Na$^+$,K$^+$-ATPase activity and migration behaviour of brown trout and sea trout (*Salmo trutta* L.) during the smelting period. Aquaculture 168, 41–47.

Refstie, T., 1983. Hybrids between salmonid species. Growth rate and survival in seawater. Aquaculture 33, 281–285.

Refstie, T., Gjedrem, T., 1975. Hybrids between Salmonidae species. Hatchability and growth rate in the freshwater period. Aquaculture 6, 333–342.

Sambraus, F., Olsen, R.E., Remen, M., Hansen, T.J., Torgersen, T., Fjelldal, P.G., 2017a. Water temperature and oxygen: the effect of triploidy on performance and metabolism in farmed Atlantic salmon (*Salmo salar* L.) post-smolts. Aquaculture 473, 1–12.

Sambraus, F., Fjelldal, P.G., Remø, S.C., Hevrøy, E.M., Nilsen, T.O., Thorsen, A., Hansen, T.J., Waagbø, R., 2017b. Water temperature and dietary histidine affect cataract formation in Atlantic salmon (*Salmo salar* L.) diploid and triploid yearling smolt. J. Fish Dis. 40, 1195–1212.

Sambraus, F., Remen, M., Olsen, R.O., Hansen, T.J., Waagbø, R., Torgersen, T., Lock, E.J., Imsland, A., Fraser, T.W.K., Fjelldal, P.G., 2018. Changes in water temperature and oxygen: the effect of triploidy on performance and metabolism in large farmed Atlantic salmon. Aquac. Environ. Interact. 10, 157–172.

Scheerer, P.D., Thorgaard, G.H., 1983. Increased survival in salmonid hybrids by induced triploidy. Can. J. Fish. Aquat. Sci. 40, 2040–2044.

Seeb, J.E., Thorgaard, G.H., Tynan, T., 1993. Triploid hybrids between chum salmon female × Chinook salmon male have early sea-water tolerance. Aquaculture 117, 37–45.

Shrimpton, J.M., Sentlinger, A.M.C., Heath, J.W., Devlin, R.H., Heath, D.D., 2007. Biochemical and molecular differences in diploid and triploid ocean-type Chinook salmon (*Oncorhynchus tshawytscha*) smolts. Fish Physiol. Biochem. 33, 259–268.

Shrimpton, J.M., Heath, J.W., Devlin, R.H., Heath, D.D., 2011. Effect of Triploidy on Growth and Ionoregulatory Performance in Ocean-Type Chinook Salmon: A Quantitative Genetics Approach Aquaculture 362–363, 248–254.

Smedley, M.A., Clokie, B.G.J., Migaud, H., Campbell, P., Walton, J., Hunter, D., Corrigan, D., Taylor, J.F., 2016. Dietary phosphorus and protein supplementation enhances seawater growth and reduces severity of vertebral malformation in triploid Atlantic salmon (*Salmo salar* L.). Aquaculture 451, 357–368.

Stien, L.H., Sæther, P.A., Kristiansen, T., Fjelldal, P.G., Sambraus, F., 2019. Første samlerapport: Velferd for triploid laks i nord-Norge (in Norwegian). Rapport fra Havforskningen Nr. 2019–2047.

Sutterlin, A.M., MacFarlane, L.R., Harmon, P., 1977. Growth and salinity tolerance in hybrids within *Salmo* sp. and *Salvelinus* sp. Aquaculture 12, 41–52.

Tanguy, J.M., Ombredane, D., Baglinière, J.L., Prunet, P., 1994. Aspects of parr-smolt transformation in anadromous and resident forms of brown trout (*Salmo trutta*) in comparison with Atlantic salmon (*Salmo salar*). Aquaculture 121, 51–63.

Taylor, J.F., Needham, M.P., North, B.P., Morgan, A., Thompson, K., Migaud, H., 2007. The influence of ploidy and saltwater adaptation, acute stress response and immune function following seawater transfer in non-smolting rainbow trout. Gen. Comp. Endocrinol. 152, 314–325.

Taylor, J.F., Leclercq, E., Preston, A.C., Guy, D., Migaud, H., 2012. Parr-smolt transformation in out-of-season triploid Atlantic salmon (*Salmo salar* L.). Aquaculture 362-363, 255–263.

Taylor, J.F., Sambraus, F., Mota-Velasco, J., Guy, D.R., Hamilton, A., Hunter, D., Corrigan, D., Migaud, H., 2013. Ploidy and family effects on Atlantic salmon (*Salmo salar*) growth, deformity and harvest quality during a full commercial production cycle. Aquaculture 410-411, 41–50.

Taylor, J.F., Waagbø, R., Diez-Padrisa, M., Campbell, P., Walton, J., Hunter, D., Matthew, C., Migaud, H., 2015. Adult triploid Atlantic salmon (*Salmo salar*) have higher dietary histidine requirements to prevent cataract development in seawater. Aquac. Nutr. 21, 18–32.

Thorpe, J.E., 1977. Bimodal distribution of length of juvenile Atlantic salmon (*Salmo salar* L.) under artificial rearing conditions. J. Fish Biol. 11, 175–184.

Urke, H.A., Koksvik, J., Arnekleiv, J.V., Hindar, K., Kroglund, F., Kristensen, T., 2010. Seawater tolerance in Atlantic salmon, *Salmo salar* L., brown trout, *Salmo trutta* L., and *S. salar* × *S. trutta* hybrids smolt. Fish Physiol. Biochem. 36, 845–853.

Urke, H.A., Kristensen, T., Arnekleiv, J.V., Haugen, T.O., Kjærstad, G., Stefansson, S.O., Ebbesson, L.O.E., Nilsen, T.O., 2013. Seawater tolerance and post-smolt migration of wild Atlantic salmon *Salmo salar* × brown trout *S. trutta* hybrid smolts. J. Fish Biol. 82, 206–227.

Verspoor, E., 1988. Widespread hybridization between native Atlantic salmon, *Salmo salar*, and introduced brown trout, *S. trutta*, in eastern Newfoundland. J. Fish Biol. 32, 327–334.

Wilkens, N.P., Courtney, H.P., Curatolo, A., 1993. Recombinant genotypes in backcrosses of male Atlantic salmon × brown trout hybrids to female Atlantic salmon. J. Fish Biol. 43, 393–399.