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Vertebral deformities in interspecific diploid and triploid salmonid hybrids

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Abstract

Vertebral deformities in salmonid interspecific hybrids, some of which were triploidised, were assessed across three separate year classes during the freshwater life stage. Initially, eggs from a farmed Atlantic salmon *Salmo salar* were crossed with the sperm from a *S. salar*, arctic char *Salvelinus alpinus* or brown trout *Salmo trutta*. For *S. salar* × *S. trutta*, half the eggs were triploidised. In a second- and third-year class, the eggs from a farmed *S. salar* were crossed with the sperm from either a *S. salar* or a *S. trutta*, and half of each group was triploidised. In the two initial-year classes, all hybrids were larger than the *S. salar* controls, and triploid *S. salar* × *S. trutta* were larger than diploid counterparts. In the third-year class, the *S. salar* × *S. trutta* were smaller than the *S. salar*, in contrast to the initial 2 year classes, although the triploid hybrids were still larger than the diploids. In the third-year class, a high degree of spontaneous triploidy was also observed in the putative diploid groups (between 16 and 39%). Vertebral deformities were consistently higher in pressure-shocked triploids than diploids, irrespective of hybridisation, but there was no consistent effect of hybridisation among experiments. Although this study was not able to explain the contrasting results for vertebral deformities between year classes, triploid *S. salar* × *S. trutta* can demonstrate impressive freshwater growth that could be of interest for future farming programmes.

KEYWORDS
compression, ploidy, radiology, spinal deformity

1 | INTRODUCTION

Interspecific hybrids can be used in aquaculture to increase growth rates, transfer/combine desirable traits between species or induce sterility (Bartley *et al.*, 2001). Studies in cultured fish have found a number of salmonid hybrids to be viable, in terms of hatching and survival, but their traits are not commercially advantageous (Blanc & Chevassus, 1979; Gray *et al.*, 1993; Scheerer & Thorgaard, 1983). Nonetheless, these studies on hybrid performance were mainly carried out in the 1980s and 1990s. There is little knowledge on how these hybrids would perform in more modern farming facilities with enhanced husbandry practices that generally lead to improved survival and growth.

One of the disadvantages of using salmonid hybrids is a general trend of lower viability, in terms of early survival and hatching rates, compared to purebred controls. Nonetheless, hybrids can be triploidised to produce fish with three complete chromosome sets, and this can increase a hybrid's viability and developmental stability

(Chevassus *et al.*, 1983; Scheerer & Thorgaard, 1983). Furthermore, triploid hybrids can have better performance characteristics, such as growth and disease resistance, than either the purebred triploid or the diploid hybrid salmon themselves. For example, triploid Atlantic salmon *Salmo salar* (L. 1758) × brown trout *S. trutta* (L. 1758) had better survival and growth (Galbreath & Thorgaard, 1995), whereas disease resistance was higher in triploid rainbow trout *Oncorhynchus mykiss* (Walbaum 1972) × arctic char *Salvelinus alpinus* (L. 1758) (Dorson *et al.*, 1991) and seawater tolerance was earlier in triploid chum salmon *Oncorhynchus keta* (Walbaum 1792) × chinook salmon *Oncorhynchus tshawytscha* (Walbaum 1972) (Seeb *et al.*, 1993), compared to their respective diploid hybrids.

A further benefit of triploid salmonids is that they are functionally sterile (Benfey & Sutterlin, 1984). This is of particular interest, as the high levels of genetic introgression between domestic escapees and feral populations that exist today (Glover *et al.*, 2017) are a major environmental issue as it contributes to the decline of wild *S. salar* populations (McGinnity *et al.*, 2003). Although the methods to produce triploid salmonids have been available for more than 40 years (*e.g.*, Benfey & Sutterlin, 1984), their uptake has been limited to Tasmanian (Australia) all-female *S. salar* farming where they are used to prevent early sexual maturation (Amoroso *et al.*, 2016). More recently, licences have been approved for commercial triploid *S. salar* production in Norway (Kjøglum *et al.*, 2016) and Canada (DFO, 2016), and triploidy is used as a safeguard against environmental contamination following approval for production of genetically modified *S. salar* (DFO, 2019). Nonetheless, the commercial uptake of triploid use has been slow because of a higher incidence of lower-jaw deformities (Amoroso *et al.*, 2016; Fraser *et al.*, 2015; Sutterlin *et al.*, 1987) and vertebral compression/fusion (Fjelldal & Hansen, 2010) compared to diploids. Of note, Whitt *et al.* (1972) also observed an increased occurrence of jaw deformities in interspecific sunfish hybrids compared to the parental species. Therefore, due to performance and welfare concerns regarding the use of triploids (Fraser *et al.*, 2012), particularly skeletal development, it would be of interest to investigate the level of vertebral deformities in triploid salmonid hybrids to see if they show similar or altered levels to diploid *S. salar*.

This study assessed vertebral deformities in salmon hybrids from 3 year classes using the hypothesis that triploids would have more vertebral deformities than diploids. Hybrids were produced by crossing a single female *S. salar* with either male *S. trutta* or *S. alpinus*. These hybrid crosses were chosen as the *S. salar* × *S. trutta* cross is known to be the most likely candidate for commercial aquaculture because of its viability (Galbreath & Thorgaard, 1994), and the female *S. salar* × male *S. trutta* cross is considered more viable than the female *S. trutta* × male *S. salar* cross (McGowan & Davidson, 1992). The *S. salar* × *S. alpinus* cross was not triploidised but is known to be viable from previous studies (Fleming *et al.*, 2014) and may be of interest to the aquaculture industry. Therefore, the latter cross is included as an observation for a general comparison of potential hybridisation effects on vertebral deformities. Endpoints included body size and condition, as well as radiological results from pre-smolts.

2 | MATERIALS AND METHODS

2.1 | Ethical consideration

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

2.2 | Fish stocks

For all year classes, each group was incubated in a single incubation tray before being moved to single fibreglass tanks at first feeding (1 × 1 × 0.43 m) for the remainder of the study. All groups were fed standard commercial diets for diploid *S. salar* (Skretting AS, Stavanger, Norway).

2.2.1 | 2013 year class

On 11 December 2013, the eggs from one *S. salar* (*ca.* 5.1 kg) of the domestic Aquagen strain were divided into three parts and fertilised with the sperm from (a) three two-seawinter *S. salar* from Aquagen (*ca.* 7.1 kg), (b) three non-migratory domestic Hardangervidda *S. trutta* (*ca.* 0.54 kg) or (c) three wild (Skogseidvatnet) *S. alpinus* (*ca.* 0.86 kg). Some of the *S. salar* × *S. trutta* embryos were given a pressure shock to induce triploidy (see later). This resulted in four groups consisting of 1050–1846 fertilised eggs each: purebred diploid *S. salar*, diploid *S. salar* × *S. trutta*, triploid *S. salar* × *S. trutta* and diploid *S. salar* × *S. alpinus*. The fish were reared under the conditions found in Figure 1a. At first feeding on 30 April 2014, the number of fish stocked in each tank was 223–706. The mortality between fertilisation and first feeding was 71, 52, 67 and 88% for the diploid *S. salar*, diploid *S. salar* × *S. trutta*, triploid *S. salar* × *S. trutta* and diploid *S. salar* × *S. alpinus*, respectively. On 5 November 2014, between 43 and 50 fish from each group were killed using an overdose of anaesthetic (200 mg l^{-1}, Finquel), measured for fork length and weight and radiographed.

2.2.2 | 2017 year class

On 17 January 2017, eggs from one *S. salar* of the domesticated Mowi strain were divided into two equal parts and fertilised with either the sperm from one *S. salar* or one *S. trutta*. The male *S. salar* was the first-generation offspring from wild *S. salar* from the River Vosso in western Norway. The male *S. trutta* was from a domestic stock that originated from Lake Tunhovd in eastern Norway. After fertilisation, the batches of *S. salar* and *S. salar* × *S. trutta* were both split into two equal parts, with one half given a pressure shock (see

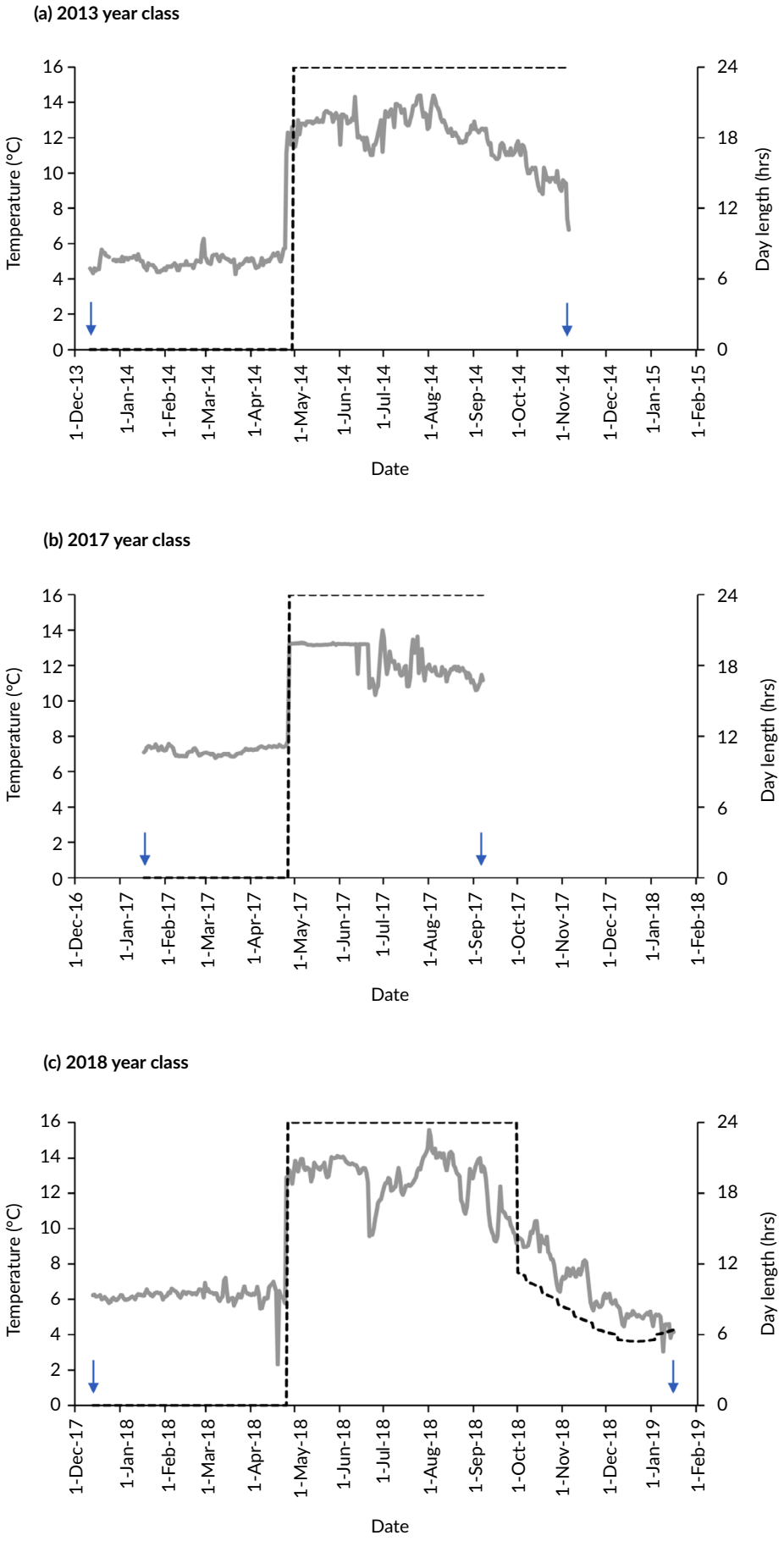

FIGURE 1 Daily inflow water temperature (solid line) and day length (dotted line) for each year class. (a) 2013, (b) 2017 and (c) 2018 year classes. All year classes were incubated at 5–7°C under total darkness before being transferred to 12–13°C and continuous light at first feeding. In each figure, the earliest arrow represents the time of fertilisation, whereas the latest arrow represents the time of sampling

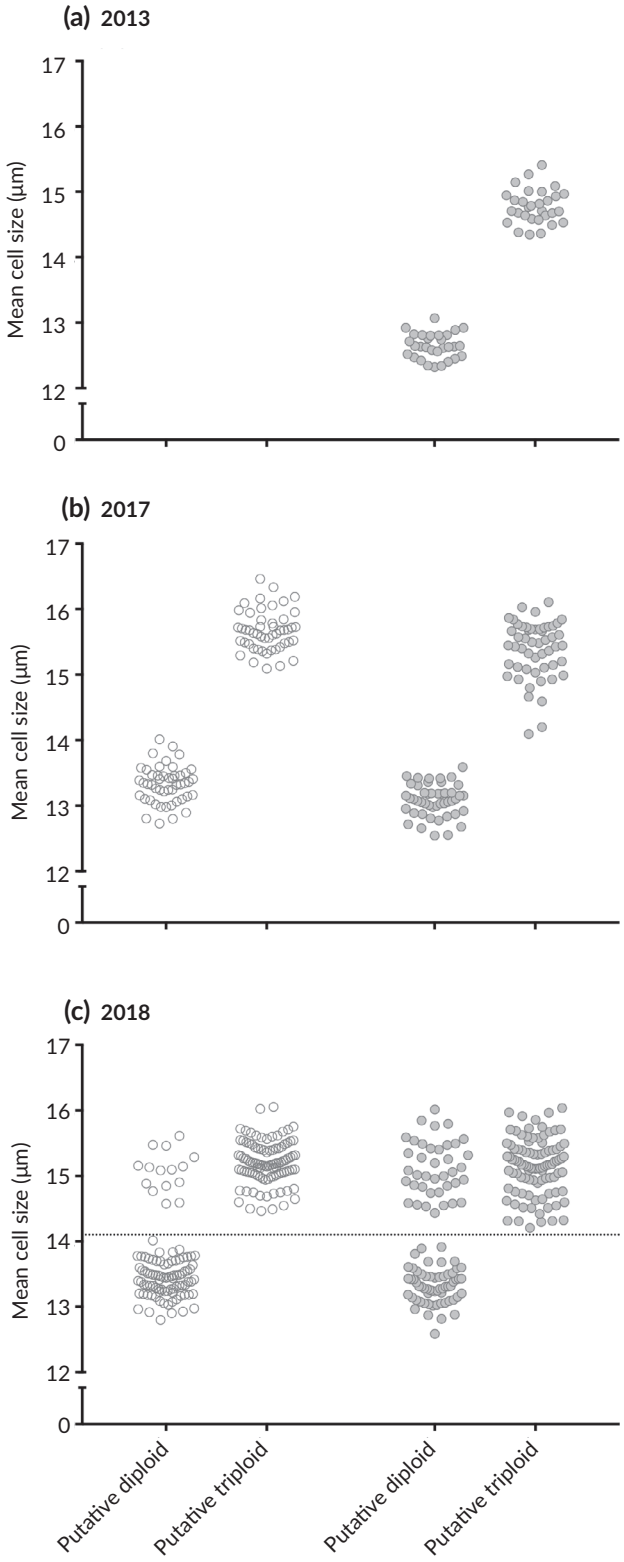

FIGURE 2 Red blood cell size in putative diploid and triploid fish from 3 year classes of Atlantic salmon *Salmo salar* × brown trout *Salmo trutta* hybrids. (a) 2013, (b) 2017 and (c) 2018 year classes. In (a), there were no triploid *S. salar*, and the diploid *S. salar* were not assessed. In (c), the putative diploids with a mean cell size >14.1 µm (dotted line) were considered spontaneous triploids (○) *S. salar*, (◉) *S. salar* × *S. trutta*

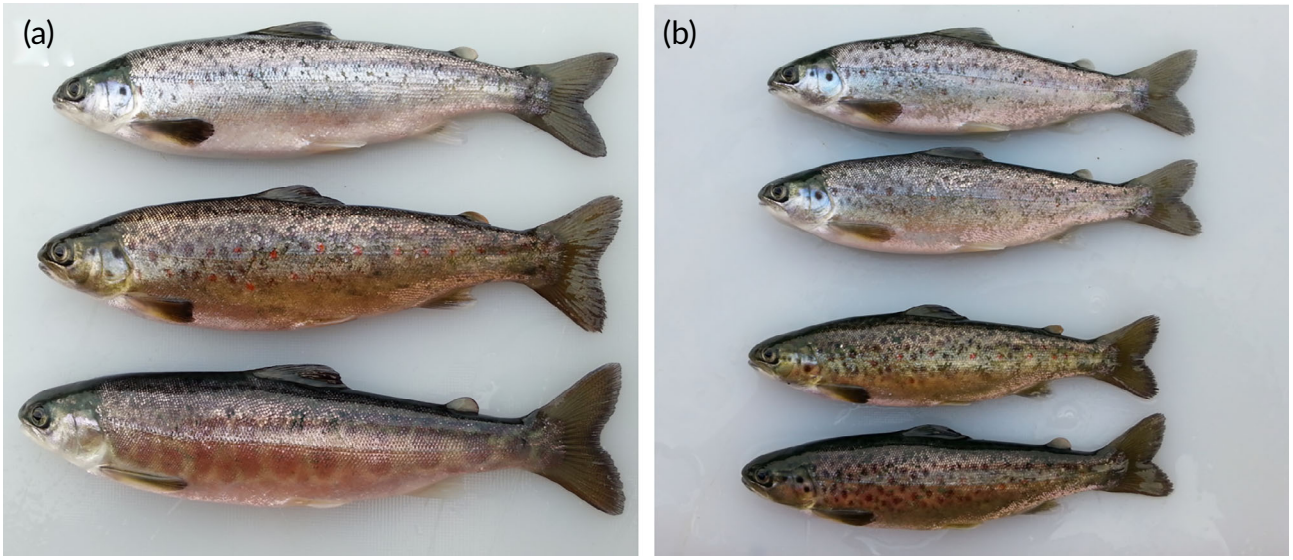

FIGURE 3 External appearance of interspecific salmon hybrids. (a, from top to bottom) Atlantic salmon *Salmo salar*, *S. salar* × brown trout *Salmo trutta* and an *S. salar* × arctic char *Salvelinus alpinus*. In 2017, (b) triploid (top 2) *S. salar* × *S. trutta* had the silvery appearance of the purebred *S. salar*, but with a slightly yellowish hue and a few red spots, whereas the diploid (bottom 2) *S. salar* × *S. trutta* had the external appearance of *S. trutta*, but with a reduction in the number of red spots. In 2018, all hybrids, irrespective of ploidy, had the external appearance of *S. trutta*

later) to induce triploidy. This resulted in four groups: diploid *S. salar*, triploid *S. salar*, diploid *S. salar* × *S. trutta* and triploid *S. salar* × *S. trutta*. Each of the four groups was reared under the conditions found in Figure 1b. At first feeding on 26 April 2017, there were between 1520 and 3460 fish per tank, and the number of fish per tank was adjusted to 800. The mortality between fertilisation and first feeding was 21, 28, 48 and 16% for the diploid *S. salar*, triploid *S. salar*, diploid *S. salar* × *S. trutta* and the triploid *S. salar* × *S. trutta*, respectively. On 7 September 2017, 180 fish per tank had their fork length and weight recorded, and 50 fish per group were killed using an overdose of anaesthetic (200 mg l^{-1}, Finquel) and radiographed.

2.2.3 | 2018 year class

On 13 December 2017, eggs from one female of the Aquagen strain were divided into two equal parts and fertilised with either sperm from one Aquagen *S. salar* or one wild *S. trutta* from the Matre River that had been captured and reared in captivity from the smolt stage. After fertilisation, the batches of *S. salar* and *S. salar* × *S. trutta* were both split into two equal batches, with one half given a pressure shock (see later) to induce triploidy. This resulted in four groups: diploid *S. salar*, triploid *S. salar*, diploid *S. salar* × *S. trutta* and triploid *S. salar* × *S. trutta*. Each of the four groups was reared under the conditions found in Figure 1c. At first feeding on 25 April 2018, there were between 355 and 1450 fish per tank, and the number of fish per tank was adjusted to 211 on 15 June 2018. Mortality between fertilisation and first feeding was 46, 82, 42 and 86% for the diploid *S. salar*, triploid *S. salar*, diploid *S. salar* × *S. trutta* and the triploid *S. salar* × *S. trutta*, respectively. Between 15 and 16 January 2019, 100 fish from each group were killed using an overdose of anaesthetic (200 mg l^{-1}, Finquel); a blood sample was collected from the caudal vein; and their fork length and weight were recorded before radiographing.

2.3 | Triploidisation

Thirty-seven minutes and 30 s after fertilisation at 8°C, those eggs to be triploidised were subjected to a hydrostatic pressure of 655 bar for 6 min and 15 s (TRC-APV, Aqua Pressure Vessel, TRC Hydraulics Inc., Dieppe, Canada) to induce triploidy. Thereafter, the ploidy level was assessed by blood cell diameter taken from blood smears (Benfey *et al.*, 1984). In 2013 and 2017, blood smears were taken from subsamples of fish before radiography. In 2018, those fish used for radiography were the same as those used for blood smears.

In both 2013 and 2017, those fish that had undergone pressure shock showed higher mean blood cell diameters with no overlap compared to those fish that remain untreated, suggesting 100% triploidy (Figure 2a,b). In 2018, the blood cell diameters suggested spontaneous triploidy within the diploids, 16% in *S. salar* and 39% in the diploid *S. salar* × *S. trutta* (Figure 2c).

2.4 | Radiography and deformity classification

In 2013 and 2017, the vertebral columns were radiographed (Porta 100 HF, Eickemeyer Medizintechnik für Tierärzte KG, Tuttlingen, Germany) using a 35 × 43 cm image plate in a rigid cassette (Dürr Medical, Bietigheim-Bissingen, Germany) with 40 kV and 10 mA at a

TABLE 1 Body size of salmon hybrids of Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and arctic char *Salvelinus alpinus* in 3 year classes

		S. salar			*S. salar* × *S. trutta*			
Year class	Parameter	Diploid	Triploid (pressure shock)	Triploid (spontaneous)	Diploid	Triploid (pressure shock)	Triploid (spontaneous)	*S. salar* × *S. alpinus*
2013	Mass (g)	40 (36–47)[a]	na	na	43 (31–50)[a]	72 (58–78)[c]	na	45 (36–55)[b]
	Fork length (cm)	15.0 (14.2–15.8)[a]	na	na	15.0 (13.4–15.5)[a]	17.6 (16.3–18.2)[c]	na	15.3 (14.3–16.2)[b]
	Condition (*K* factor)	1.22 (1.19–1.25)[a]	na	na	1.30 (1.23–1.34)[c]	1.31 (1.27–1.34)[c]	na	1.26 (1.22–1.30)[b]
2017	Mass (g)	28 (23–31)[a]	36 (31–39)[b]	na	22 (16–31)[c]	44 (28–58)[d]	na	na
	Fork length (cm)	12.6 (12.0–13.0)[a]	13.6 (12.9–14.0)[b]	na	11.2 (10.2–12.5)[c]	14.6 (12.6–15.9)[d]	na	na
	Condition (*K* factor)	1.41 (1.36–1.45)[a]	1.42 (1.38–1.45)[a]	na	1.52 (1.43–1.64)[b]	1.44 (1.36–1.54)[c]	na	na
2018	Mass (g)	151 (127–176)[a]	158 (134–178)[a]	166 (96–190)[a]	53 (29–65)[b]	100 (76–114)[c]	79 (59–101)[c]	na
	Fork length (cm)	23.8 (22.5–25.0)[a]	24.1 (22.8–25.0)[a]	24.3 (20.4–25.6)[a]	16.9 (13.9–18.0)[b]	20.9 (18.7–22.0)[c]	19.2 (17.7–20.7)[c]	na
	Condition (*K* factor)	1.14 (1.11–1.18)[a]	1.13 (1.10–1.15)[b]	1.14 (1.12–1.18)[a]	1.10 (1.06–1.14)[c]	1.10 (1.07–1.13)[c]	1.09 (1.05–1.12)[c]	na

Note. Data are medians (25th–75th percentiles) as the data lacked normality. Different superscript letters indicate significant group effect within year class (*post hoc* Dunn's test, $P < 0.05$). n = 41–53, n = 180 and n = 100 group^{-1} in 2013, 2017 and 2018, respectively. na: not applicable.

distance of 70 cm (1 s exposure). The image plate was scanned (CR 35 VET, Dürr Medical, Bietigheim-Bissingen, Germany), and the resulting image was converted into a TIFF file (Vet-Exam Plus Software, version 4.14.0). In 2018, fish were radiographed with a direct radiology system (Canon CXDI-410C Wireless, Canon Inc., Kawasaki, Japan) using a portable X-ray unit (Portable X-ray Unit Hiray Plus, Model Porta 100 HF, JOB Corporation, Yokohama, Japan) at 88 cm distance with 40 kV and 10 mA (1 s exposure). Vertebral deformities were evaluated according to the classification of Witten *et al*. (2009), and the regional classification was done according to Kacem *et al*. (1998).

2.5 | Statistical analysis

The data were transferred to R version 3.5.2 (R Development Core Team 2018, http://www.r-project.org). Significance was assigned at $P \leq 0.05$ unless otherwise stated. The data were checked for normality using the Shapiro–Wilk test. Body mass, fork length, body condition [a.k.a. condition or *K* factor; body mass (g)/fork length (cm^3) × 100] and the number of deformed vertebrae per deformed fish between groups were compared using Kruskal–Wallis tests for each year class separately, using Dunn's test for *post hoc* analyses. For deformity prevalence, a general linear model with a binomial distribution was used to compare the differences between groups, and the *G*-test with a Bonferroni correction was used for multiple comparisons (significance was assigned at $P < 0.008$ for 2013 and 2017 year classes and $P < 0.003$ for the 2018 year class) as the *post hoc* analysis.

3 | RESULTS

3.1 | Phenotypic appearance

The external appearance of the various salmon hybrids can be seen in Figure 3a. In both the 2013 and 2017 year classes, the *S. salar* × *S. trutta* hybrids had a distinct external appearance based on whether they were diploid or triploid. Triploid *S. salar* × *S. trutta* had a phenotype more like *S. salar* but with a slightly yellowish hue and a few red spots, whereas diploid *S. salar* × *S. trutta* had a phenotype more similar to *S. trutta* but with a reduction in the number of red spots (Figure 3b). In 2018, *S. salar* × *S. trutta* all had a *S. trutta* phenotype, irrespective of ploidy.

3.2 | Body size

In 2013 and 2017, the triploid *S. salar* × *S. trutta* was significantly heavier and longer and had a higher condition factor (*i.e*., fatter) than the diploid *S. salar*, with similar results when compared to the diploid *S. salar* × *S. trutta* (Table 1). In 2017, the triploid *S. salar* × *S. trutta* was also significantly heavier and longer than triploid *S. salar*. The *S. salar* × *S. alpinus* was significantly heavier and longer and had a higher condition factor than the diploid *S. salar* but intermediate between the diploid and triploid *S. salar* × *S. trutta*.

In 2018, the pressure-shocked triploid *S. salar* × *S. trutta* was significantly heavier and longer than the diploid counterparts but smaller than the diploid and pressure-shocked triploid *S. salar* (Table 1). The spontaneous triploid *S. salar* × *S. trutta* was also significantly heavier and longer than the diploid *S. salar* × *S. trutta* but no different from the pressure-shocked

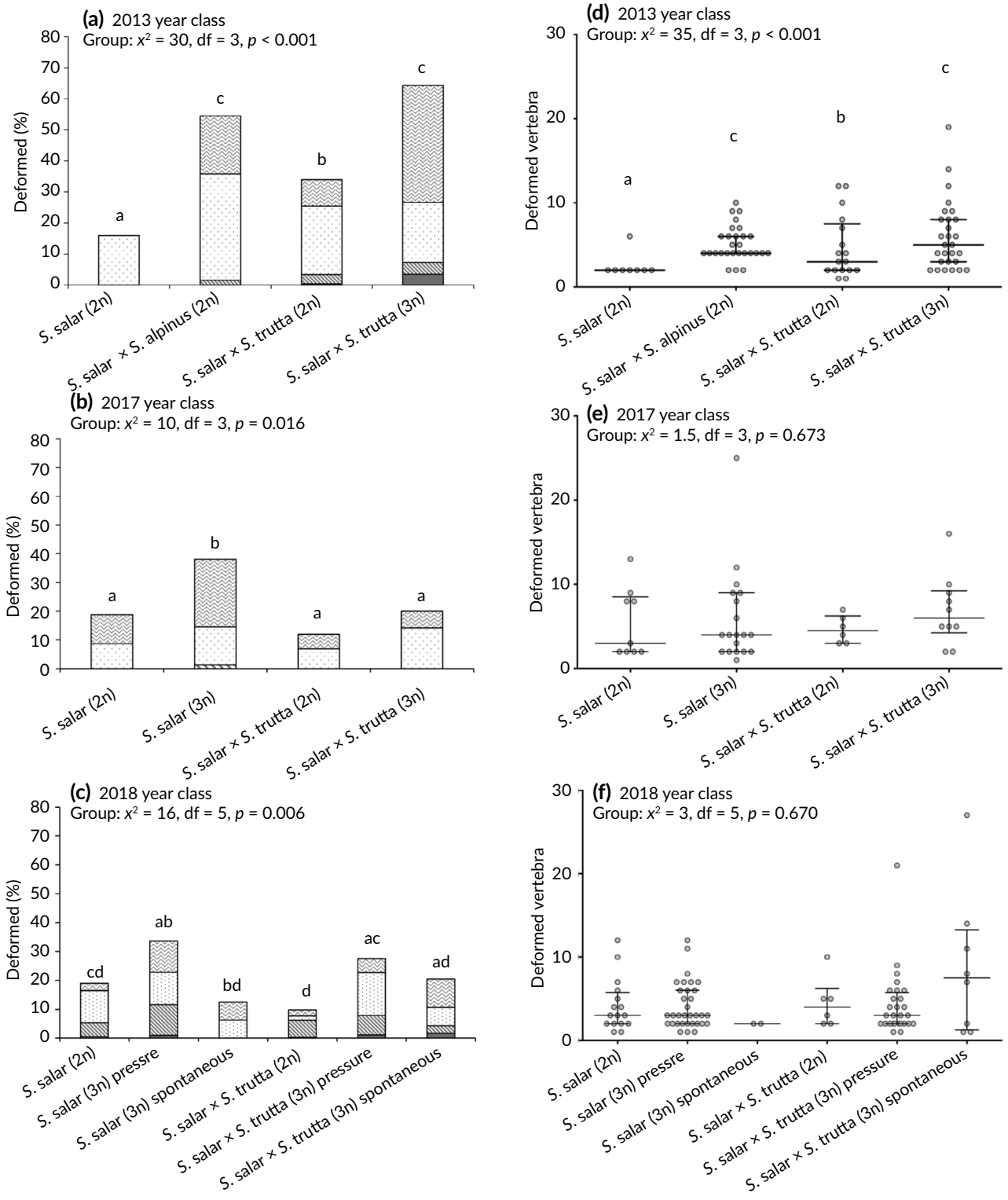

FIGURE 4 Vertebral deformities of interspecific salmon hybrids from 3 year classes. (a–c) The prevalence of fish with ≥1 deformed vertebrae in diploid (2n) and triploid (3n) Atlantic salmon *Salmo salar* and hybrids from crossing female *S. salar* with either male brown trout *Salmo trutta* or arctic char *Salvelinus alpinus*. (d–f) The number of deformed vertebrae per deformed fish. In (a–c), the fill within each bar represents the prevalence of each deformity classification, and the statistics are from general linear models. Note that the grouping "other" refers to the sum of elongated, vertically shifted, hyper-radiodense and/or internal dorsal or ventral shifted vertebrae (types 9, 12, 17 and 19, respectively, in Witten *et al.*, 2009). In (d–f), the statistics are from Kruskal–Wallis tests. Lowercase letters indicate significant differences between individual groups based on *post hoc* tests (a–c, *G*-test; d–f, Dunn's test). Total group sizes were 43–50, 48–50 and 16–99 in 2013, 2017 and 2018, respectively. (■) Other, (▧) Decreased intervertebral space, (□) Fusion, (▨) Compression

triploid *S. salar* × *S. trutta*. Similarly, the pressure-shocked triploid *S. salar* was no different in body size from the spontaneous triploid *S. salar*.

3.3 | Deformities

All the hybrids had significantly more fish with one or more deformed vertebrae than the diploid *S. salar* in 2013, but in 2017 and 2018 there was no difference between the diploid *S. salar* and any of the hybrids (Figure 4a–c). In all 3 year classes, the triploid *S. salar* × *S. trutta* had more deformed fish than the diploid *S. salar* × *S. trutta*, but this difference was not significant in 2017. In both 2017 and 2018, the triploid *S. salar* had more deformities than diploid *S. salar*. Spontaneous triploidy had no significant effect on the prevalence of deformed fish compared to pressure-shocked triploids, albeit the power of the analysis was low because of a few numbers of spontaneous triploidy. Within deformed fish, hybrids had significantly more deformed vertebrae per deformed fish than *S. salar* in the 2013 year class, but there were no group effects in 2017 or 2018 (Figure 4d–f).

Other than a decrease in fusions and an increase in decreased intervertebral space in 2018, there were no notable trends in the types of deformities observed relating to year class or groups (Figure 4a–c). Representative images of vertebral deformities within groups can be found in Supporting Information Figures S1 and S2. For the deformity location (Supporting Information Figure S3), noticeable peaks were observed in deformities in those vertebrae found under the dorsal fin (vertebrae 25–30) in triploid *S. salar* (Supporting Information Figure S1c), triploid *S. salar* × *S. trutta* and *S. salar* × *S. alpinus* (Supporting Information Figure S2a). In addition, in 2013, triploid *S. salar* × *S. trutta* had a peak in deformities within the tail region (vertebrae 48–54, Supporting Information Figure S2b) that was not seen in any other year class or group.

4 | DISCUSSION

Year-class effects were observed on the prevalence and severity of vertebral deformities in interspecific salmonid hybrids. This study rejected the hypothesis that triploidy would lead to an increase in vertebral deformities, as ploidy effects were not significant within *S. salar* × *S. trutta* in one of the 3 year classes. In all year classes, the triploid *S. salar* × *S. trutta* were larger than the diploid counterpart, and in two of the 3 year classes, the triploid *S. salar* × *S. trutta* were significantly larger than the diploid *S. salar*. Further work is required to understand the variation in year-class results as the triploid *S. salar* × *S. trutta* can show impressive growth that may be of benefit to the aquaculture industry if it persists throughout life.

With the current design, it is not possible to identify the cause of the year-class effect on deformity prevalence in hybrids. Previous research has found differences in deformity prevalence between year classes (Gjerde *et al.*, 2005; Taylor *et al.*, 2011); nonetheless, the endpoints measured in those studies (*i.e.*, inbreeding, incubation temperatures, sex/sexual maturity) explained little of the variation. Here, in the diploid *S. salar*, there was little year-class variation on the prevalence or severity of vertebral deformities. Therefore, the minor differences in age at sampling are not expected to explain the year-class effect, especially as all the fish were at the same life stage (*i.e.*, presmolts). Risk factors for skeletal deformities such as nutrition, growth rates and life stage (see Fjelldal *et al.*, 2012, for a review) were similar between the studies and appear less likely to explain the year-class effects. There was year-class variation in incubation temperature, with mean temperatures between 5 and 7°C for each year class and some daily fluctuations of ≥1°C. Here, previous work has shown that higher constant mean temperatures (Fraser *et al.*, 2015) and temperature shock (1 h with a ≥6°C temperature change, Wargelius *et al.*, 2005) can also lead to vertebral deformity development, especially in the tail region and tail fin. Nonetheless, the incubation temperatures used were within the recommended range for triploids and diploid *S. salar* (<8°C, Fraser *et al.*, 2015), and diploid *S. salar* showed no year-class variation on the prevalence of fish with one or more deformed vertebrae or any peak in deformities in the tail region.

It is possible that genetic differences influence the prevalence of spinal deformities (Evans & Neff, 2009; Gjerde *et al.*, 2005; Habicht *et al.*, 1994; Mackay & Gjerde, 1986) although others have suggested no genetic link (Sullivan *et al.*, 2007a). Here, although a single female was used in each experiment that will have reduced the genetic variation related to the female within year class, different females and males were used to produce each year class. Previously, large maternal effects in *S. trutta* × *S. alpinus* viability were observed in contrast to much-smaller sire effects (Blanc & Poisson, 1983), whereas long-term sea lice resistance was also more related to the maternal rather than paternal contribution in *S. salar* × *S. trutta* (Bakke *et al.*, 1999). Furthermore, *S. salar* are known to show extensive interindividual chromosome polymorphisms (55–60 chromosomes) as well as some intra-individual polymorphism around chromosome arm number (*e.g.*, Grammeltvedt, 1975; Hartley & Horne, 1984), of which the effect on hybridisation success and performance is unknown. It is also noted that *S. salar* × *S. trutta* and *S. salar* × *S. alpinus* hybrids have between 68 and 69 chromosomes (Gjedrem *et al.*, 1977), which is halfway between *S. salar* (mode, 58) and either *S. trutta* (mode, 80) or *S. alpinus* (mode, 80) (Hartley & Horne, 1984). Therefore, the excess chromosomes are expected to form chromosome pairs of their own (Gjedrem *et al.*, 1977), but little is known about how this occurs in salmonids. In addition to explaining year-class variation, genetics may explain variation between hybrids within year class. For example, genetic distance is greater between *S. salar* and *S. alpinus* than between *S. salar* and *S. trutta* (Nelson, 1994) and is known to influence outbreeding depression (Edmands, 1999). Similarly, how hybridisation impacts on molecular mechanisms of body formation, such as *Hox* genes (Wang *et al.*, 2014), is currently unknown but would be of interest because of their key role in vertebral development.

Because of space limitations, no purebred *S. trutta* or *S. alpinus* are discussed in the current study, which limits the analysis. Nonetheless, any potential future use of salmonid hybrids is likely to be weighed against their performance *vs. S. salar*, for which purebreds are available in every year class, as this is the most farmed salmonid

and could be considered the industry standard. Previous work in diploid *S. salar* would suggest the prevalence of fish with one or more deformed vertebrae, 15–20%, is as expected for similar-sized fish (*e.g.*, Fraser *et al.*, 2015). For *S. trutta* (70 g), only 7% were found to have one or more deformed vertebrae (Preston *et al.*, 2017), which is lower than the values obtained for diploid *S. salar* and *S. salar* × *S. trutta*. For *S. alpinus*, there are no published reports of baseline vertebral deformities, but it was recently found that 17% (n = 110) in *ca.* 50 g fish have one or more deformed vertebrae, whereas 3% of those had between 6 and 10 deformed vertebrae per fish (Fraser *et al.*, unpubl. data). These values are similar to the levels found in diploid *S. salar* but lower than those observed in *S. salar* × *S. alpinus*. For triploid *S. salar*, data were available for only 2 year classes, and the number of deformed fish was almost double than that seen in diploid *S. salar*, but this was to be expected based on numerous publications in similar-sized fish (*e.g.*, Fjelldal & Hansen, 2010; Peruzzi *et al.*, 2018). Similarly, peaks were observed in deformity prevalence in the vertebra found beneath the dorsal fin, which is a common characteristic of triploids (Fjelldal & Hansen, 2010). The reason behind this increase in triploid deformities is still unclear. Nonetheless, triploids do have a higher dietary phosphorus requirement than diploids (Fjelldal *et al.*, 2016; Smedley *et al.*, 2016), which is a risk factor for developing vertebral deformities (Fjelldal *et al.*, 2016), and was unlikely to have been met in the current study as a standard diploid diet was used throughout. The higher phosphorus requirement in triploid *S. salar* is expected based on larger genomes having higher phosphorus requirements due to higher per cell nucleic acid content (Neiman *et al.*, 2012).

Surprisingly, triploid *S. salar* × *S. trutta* had a lower deformity prevalence than the triploid *S. salar* in the 2017 year class. Furthermore, in the 2017 and 2018 year classes, there was no difference between the deformity prevalence in triploid *S. salar* × *S. trutta* and that seen in diploid *S. salar*. Previous work has consistently shown triploid *S. salar* to have more skeletal deformities than diploids. Indeed, triploid *S. trutta* (Preston *et al.*, 2017), *O. mykiss* (Weber *et al.*, 2014) and *S. alpinus* (Fraser *et al.*, unpubl. data) also have significantly more deformed vertebrae than diploid conspecifics. It is unclear how fast-growing triploid *S. salar* × *S. trutta* were able to maintain a low deformity prevalence in 2017, as the environmental conditions were similar to those in 2013 and 2018. Nevertheless, it demonstrates that triploid *S. salar* × *S. trutta* may not necessarily require increased phosphorus nutrition to maintain a low level of vertebral deformities as seen for triploid *S. salar*, and this would be an advantage to the aquaculture industry as the ingredient is expensive and poses a threat to the environment. Nonetheless, it is noted that deformities can develop throughout the *S. salar* life cycle; therefore, future studies should assess *S. salar* × *S. trutta* up to market-sized fish.

Based on the current findings, it seems possible to produce *S. salar* × *S. trutta* with acceptable levels of vertebral deformities during the freshwater life stage. Nonetheless, the year-class inconsistency would suggest that hybrids have the potential to develop more vertebral deformities under certain circumstances than diploid *S. salar*, although the mechanism is unclear. Similarly, tiger trout, produced by crossing *S. trutta* with brook trout *Salvelinus fontinalis* (Mitchill, 1814), are also known to show variable growth performance to purebreds depending on rearing conditions (Blanc & Chevassus, 1986). The triploid *S. salar*, the triploid *S. salar* × *S. trutta* and the *S. salar* × *S. alpinus* had peaks in deformities around vertebrae 27–29. This is a common observation when working with triploid *S. salar* (Fjelldal & Hansen, 2010; Fraser *et al.*, 2015; Peruzzi *et al.*, 2018) but can also be apparent in farmed diploid *S. salar* to a lesser extent (Fraser *et al.*, 2013; Sullivan *et al.*, 2007b). Nonetheless, it is noted that these deformities are not observed in wild adult, migrating Atlantic salmon (Fraser *et al.*, 2014; Sambraus *et al.*, 2014). Why these vertebrae would have an increased risk of deformity is unclear; nonetheless, vertebrae 28 and 29 are some of the first to form in *S. salar* (Grotmol *et al.*, 2003), and recent work in the salmonid *O. tshawytscha* would suggest they lie within a morphologically distinct transitional region (De Clercq *et al.*, 2017) that is sensitive to temperature manipulation (De Clercq *et al.*, 2018). Deformities in the 2013 year class of triploid *S. salar* × *S. trutta* were mainly located in the tail region, but these are generally seen either in seawater fish (Fjelldal *et al.*, 2009; Fraser *et al.*, 2019) or in freshwater stages following low phosphorus treatment (Fraser *et al.*, 2019; Smedley *et al.*, 2018) or triploidisation and high incubation temperatures (Fraser *et al.*, 2015). Several groups showed peaks in deformities between vertebrae 1 and 20, such as diploid and triploid *S. salar* and *S. salar* × *S. trutta*. In time-series studies, post-cranial deformities in *S. salar* are known to occur both during freshwater and seawater life stages (Fjelldal *et al.*, 2007).

In the 2018 year class, a relatively high occurrence of spontaneous triploidy was observed. Spontaneous triploidy is known to occur in salmonids (Thorgaard & Gall, 1979), and a recent study found an average rate of 2% triploidy in putative diploid *S. salar* across Norwegian sea farms, but this could be as high as 28% within a given seacage (Glover *et al.*, 2015). Spontaneous triploidy has previously been found to be positively associated with post-ovulatory aging in *O. mykiss* (Aegerter & Jalabert, 2004) and tench *Tinca tinca* (L. 1758) (Flajšhans *et al.*, 2007). Therefore, the occurrence of spontaneous triploidy is likely an indicator of low egg quality in the 2018 year class. One may wonder whether it is the pressure shock or the triploid condition itself that increases deformity prevalence in *S. salar*. Here, recent work in *O. mykiss* found that triploids produced by crossing tetraploids with diploids resulted in triploids with lower incidences of vertebral deformities than triploids produced by conventional pressure shock of newly fertilised zygotes produced from diploid parents (Weber *et al.*, 2014). The results of this study follow the same trend, with spontaneous triploids having fewer deformed fish than pressure-shocked triploids; nonetheless, these differences were not significant in either *S. salar* or *S. salar* × *S. trutta*, but n was low.

Triploid *S. salar* × *S. trutta* were consistently larger (40–50%) than the diploid counterpart, and in 2013 and 2017, the hybrid was 44 and 36% larger than the diploid *S. salar*, respectively. In those cases where the triploid *S. salar* × *S. trutta* performed best, a ploidy effect on the external appearance was noted, as the triploid hybrid resembled *S. salar*, whereas the diploid hybrid resembled *S. trutta*. In contrast, in the 2018 year class when the triploid *S. salar* × *S. trutta* hybrid was smaller than the diploid *S. salar*, all the hybrids resembled *S. trutta*

irrespective of ploidy. Previously, Wilkins *et al.* (1994) and Fleming *et al.* (2014) also noted that some meristic and morphological traits in triploid salmonid hybrids were more related to either of the parents than the diploid hybrid. How triploid salmonids handle the extra chromosome set is relatively unknown, although dosage compensation by gene copy silencing likely occurs (Pala *et al.*, 2008), and it has been suggested that the paternal genetic contribution is of less importance in artificial triploids compared to the maternal contribution (Blanc *et al.*, 2001; Harvey *et al.*, 2017). Nonetheless, with respect to *S. salar* × *S. trutta* in the current study, the ploidy effect on external morphology in the hybrids would suggest that a parental effect on gene copy silencing may be key to performance; when triploid *S. salar* × *S. trutta* resembled the mother, it performed better in terms of growth compared to diploid *S. salar* than when it resembled the father. Of further note, in 2018 the sperm was from a wild *S. trutta*, whereas in 2013 and 2017 domestic trout strains that underwent a degree of selection were used. Therefore, further work should explore the maternal and paternal genotypes with regard to growth performance.

The variation in survival before the first feeding led to differences in fish density during certain periods within each year class that do not allow for robust comparisons of growth. Survival in salmonid hybrids is known to be variable compared to purebreds, and has been better, equal or lower (Refstie & Gjedrem, 1975: Sutterlin *et al.*, 1977). Survival in purebreds can also be more variable than preferred in hybridisation work, as observed, as it can be difficult to find mature individuals from each species simultaneously, which can impact on gamete quality. Nevertheless, because of the paucity of published literature regarding hybrid performance, this study notes that the diploid *S. salar* × *S. trutta* showed inconsistent performance, being similar in size to diploid *S. salar* in the 2013 year class but 21 and 65% smaller in the 2017 and 2018 year classes, respectively. Previously, Refstie and Gjedrem (1975) and Bakke *et al.* (1999) found diploid *S. salar* × *S. trutta* to be 74% (7.7 *vs.* 30 g) and 18–33% (5.7 *vs.* 3.8 and 8.3 *vs.* 6.8 g) smaller than purebred *S. salar*, respectively. Also *S. salar* × *S. alpinus* were 11% larger than *S. salar*, whereas Sutterlin *et al.* (1977) and Refstie and Gjedrem (1975) reported the same hybrid to be a more impressive 54% (13 *vs.* 28 g) and 69% (30 *vs.* 97 g) larger than purebred *S. salar*, respectively.

In summary, diploid and triploid interspecific salmonid hybrids showed year-class-dependent results for spinal deformities. The mechanism(s) behind the inconsistency is unclear, but triploid *S. salar* × *S. trutta* exhibited the potential for excellent freshwater growth without compromised vertebral development in 1 year class (2017). Future work should focus on the long-term performance of triploid *S. salar* × *S. trutta* under different farming conditions as well as their performance related to parentage.

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AUTHOR CONTRIBUTIONS

T.J.H. and P.G.F. designed the study, obtained funding, generated data and helped with manuscript preparation. T.W.K.F. generated data, undertook data analysis and prepared the manuscript. F.S. generated data and helped with manuscript preparation.

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SUPPORTING INFORMATION

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

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