

Triploid and diploid Atlantic salmon show similar susceptibility to infection with salmon lice *Lepeophtheirus salmonis*

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Abstract

BACKGROUND: Sea lice infection is the most expensive disease factor for Atlantic salmon sea-cage farming. For triploid salmon to be accepted as a commercial possibility, investigation of susceptibility of triploid salmon to sea lice infection is a fundamental milestone. The susceptibility of diploid and triploid salmon to infection with *Lepeophtheirus salmonis* was examined in a tank trial in Scotland, a tank trial in Norway and a cage trial in Scotland.

RESULTS: Following a single infection challenge, results indicated a significant correlation between fish size and the number of attached sea lice. Triploid fish were larger than diploids at the smolt stage. In the tank trials, no difference was found between infection levels on diploids and triploids after a single infection challenge. The tank trial in Scotland continued with a second infection challenge of the same fish, which also showed no infection differences between ploidies. A borderline correlation between first infection and re-infection intensity was found for PIT-tagged diploid salmon examined after each challenge. No significant difference in louse infection between diploid and triploid salmon (~2 kg) was found in the cage trial undertaken under commercial conditions.

CONCLUSION: This study concludes that triploid Atlantic salmon are not more susceptible to sea louse infection than diploid fish.

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Keywords: sea lice; ploidy; abundance; infection challenge

1 INTRODUCTION

Sea lice, in particular the salmon louse *Lepeophtheirus salmonis*, are considered the most damaging parasites of marine farmed salmonids,¹ costing the global aquaculture industry over €300 million per year, with the Scottish industry alone losing €33 million annually, corresponding to 5% to 6% of the production value.^{2,3} In addition to the direct costs of sea lice control there are production losses, due to downgrading, and reduced growth costs as well as the consequences of immune suppression, which can lead to infection by other pathogens. These factors in turn have detrimental outcomes in terms of fish welfare and profitability. Additional costs, connected with the possibility of lice derived from farmed salmon infecting local wild salmon stocks, also have to be considered, according to the farming context.⁴

Due to increasing drug resistance in sea lice,^{5–7} which affects susceptibility to many available treatments such as organophosphates, pyrethroids, avermectins and topical disinfectants,⁸ much research has been dedicated to the development of new control methods and the understanding of the sea louse life-cycle.^{9,10} These include management practices such as integrated pest management, which encourages synchronized fallowing and lice treatment at different farms in a particular system.¹¹ Other control strategies being investigated

for marine copepod parasites include the development of new therapeutants¹² and vaccines¹³ and the use of controls involving aspects of chemical ecology.^{14–16} These strategies, however, are not likely to be commercially available within the short to medium term. Encouraging results have also been recently obtained with respect to breeding programmes for genetic resistance to sea lice in commercial Atlantic salmon, *Salmo salar*, populations, with heritability of up to 0.3 reported.^{17,18} Differences in susceptibility to sea lice infection and abundance of sea lice on wild and farmed strains of Atlantic salmon have been investigated, with as much as 70% variation between the highest and lowest infected family strains, showing the potential of selective breeding and family selection.^{18–20} It was reported, that susceptibility to louse infection

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could be dependent on a Major Histocompatibility Complex (MHC) genotype, although a major effect is unlikely.²¹ The effect may be due to variation in quantitative trait loci associated with MHC class II regions through linkage disequilibrium.²⁰ It is, however, untested that QTLs (quality trait loci) moderate the effect of MHC genes, but the discovery of a genetic element that conclusively affects susceptibility to lice is an exciting development. However, results from studies of salmon susceptibility to both *Lepeophtheirus salmonis* and the branchiuran *Argulus coregoni* suggest that there are many interacting factors that contribute to the extent of a louse infection on an individual, thus the role of genetic factors may be small. Selection for salmon resistance, however, is a long-term goal and only reduced sensitivity to sea lice is likely to be achieved.

Finally, another relevant strategy is the use of cleaner fish, such as wrasse (*Ctenolabrus* and *Labrus* spp.) in polyculture.^{2,22–26} In line with the principles of integrated pest management, the best solutions to the sea lice problem are likely to involve the coordinated use of a broad range of these and other strategies.

In recent years there has been a dramatic increase in the production of farmed Atlantic salmon, raising concerns about the environmental impact of these activities. One particular area of concern is escapees, which have been documented to cause genetic changes in native populations as a result of interbreeding.^{27,28} Although considerable technological advances have been made in the design of cages, escapes through natural disaster, human error or mechanical failure are small, but inevitable risks and salmon being on-grown in the marine environment are reproductively competent. For these reasons, the production of sterile fish to mitigate the environmental impact of escapees and potential inter-breeding with wild stocks, is receiving ever-increasing attention. The use of triploid salmon in commercial Atlantic salmon aquaculture is the only commercially acceptable means of sterility to address the environmental impacts of escapees.^{29–31} Differences in performance, physiology, behaviour and morphology between triploid and diploid fish are well described,^{31–35} and these differences could conceivably contribute to differential susceptibility to sea lice infection. A range of factors contribute to the susceptibility of Atlantic salmon to infection and may signal host suitability to the parasite, such as nutritional condition and size/morphology.^{36,37}

Triploid fish have three sets of chromosomes instead of two, which leads to larger, but fewer cells in all tissues and organs.³² Behavioural differences between fish of different ploidies have been described, with reduced aggressiveness, inferior overall performance in sub-optimal conditions, feeding at deeper water depths and lower responsiveness to environmental stimuli reported for triploid Atlantic salmon.^{32,33} Studies have also suggested that triploids could be more susceptible to pathogen or parasite infection due to reduced immune activity as compared to diploids.^{38–42} For example, triploid Atlantic salmon have been found to be more susceptible than their diploid counterparts to infection by *Gyrodactylus salaris*, a monogenean ectoparasite.⁴³ The authors suggested this might be due to compromised complement-dependent immune pathways in triploid salmon. It is uncertain, however, as to what extent the observations in these various studies relate to ploidy *per se* rather than to the interaction of ploidy with particular genotypes. In many of the previously published trials, fish size was unaccounted for. Results from tank and cage trials have shown that large fish tend to be more heavily infected.^{36,44,45} Given that triploid smolts show higher growth potential than diploid salmon smolts,³⁵ the size

of the fish can be a potential confounding factor with respect to comparison of sea lice infection levels.

Salmonid fish are capable of generating an immune response to salmon lice,⁴⁶ however, no acquired protection against re-infection has been observed. Various studies^{47–49} tested individually tagged diploid salmon in separate challenges with sea lice and demonstrated that the infection level for a single salmon in one challenge is a poor predictor of its infection level in a subsequent challenge. Persistent infection may lead to compromised host immunity and tissue damage followed by a period of hypo-responsiveness and delayed healing.⁴⁹ It has been suggested that weakening of the animal could be expected to be more pronounced in triploid fish compared to diploid fish due to reduced immune activity.⁴¹

The aim of the present study was to compare the susceptibility of triploid and diploid Atlantic salmon to infection by the salmon louse *Lepeophtheirus salmonis* in several experimental and commercial settings in Scotland and Norway. In addition, a re-infection trial was undertaken to determine if a correlation existed between the outcomes of infection events for individual fish.

2 EXPERIMENTAL METHODS

2.1 Experiment 1: Tank sea lice challenge and re-challenge in Scotland

2.1.1 Fish stock

On 25 November 2009, 78 female two-sea-winter broodstock from the five generation Landcatch Natural Selection (LNS) Atlantic salmon breeding programme were stripped and milt collected from 26 unrelated males at Landcatch Ltd, Ormsary, UK. A sub-sample of 70 g of eggs (~300 eggs) per female was removed, fertilized by a different unrelated male, each male being crossed with three different females (i.e. male 1 crossed with females 1–3, male 2 with females 4–6, etc.), giving three half-sibling families per male. Following fertilization egg batches were sub-divided into two (150 eggs/cross), pooled into batches of six females and two males (1800 eggs/pool) and water hardened at 10 °C. Triploidy was induced in one batch by applying a hydrostatic pressure shock (in-house custom built vessel) 30 min post-fertilization at 655 bar (9500 psi) for 5 min³⁵ leaving the other half of the batch untreated as diploids controls. Fertilization rates were approximately 85%. The process was repeated a further five times. Eggs were incubated at ambient water temperature (6 °C) in six separate silos per ploidy (~11 000 ova/ploidy) until eyeing, before transfer to on-growing hatchery (Gairloch Hatchery, Rosshire, Scotland). Survival to hatch (March 2010) was ~65% (~5600 fry/ploidy), at which point silos were pooled per ploidy and split between two tanks per ploidy, and fry on-grown to 2 g before transfer to Institute of Aquaculture freshwater facilities (Niall Bromage Freshwater Research Facility) in July 2010. Fish were reared in two circular 1.8 m³ tanks per ploidy, under simulated natural photoperiod and fed a commercial diet (Skretting) during daylight hours to manufacturer's recommendations using 6 L Arvotec T Drum feeders controlled by a computer aided PC system. Ambient water temperature ranged from 1.5 °C in winter to 15.5 °C in summer). Mortality during freshwater from first feeding to smolt was < 1.5% in both ploidy, and presence of externally visible deformity were < 1% in both ploidy at time of sea transfer. At end of freshwater rearing diploid and triploid smolts weighed 66.5 ± 3.0 g

and 91.8 ± 2.8 g, respectively ($P > 0.05$). Fish were transferred to Institute of Aquaculture marine facilities (Machrihanish Marine Environmental Research Laboratories, MERL) in mid-April 2011 and stocked in two 3 m^3 stock tanks (one/ploidy).

On 17 June 2011, after three months in seawater, 200 fish from each ploidy (mean weight \pm standard deviation (SD) of 108.6 ± 20.6 g and 144.2 ± 22.9 g, respectively, for diploids and triploids) were intramuscularly PIT-tagged (8 mm passive inductive transponder-tags (PIT-tags), Trovan Ltd, Identify UK Ltd, Hesse, UK) and transferred to 600 L tanks (two replicate tanks per ploidy, 100 fish/tank, stocking density of 20 kg m^{-3}). Fish were acclimated in the new tank system for three weeks prior to the start of the sea lice challenge.

2.1.2 Sea lice challenge and sampling

On the 4 July 2011, fish (mean weight: diploids 107.4 ± 21.2 ; triploids 143.9 ± 22.8) were crowded and challenged with sea lice, *Lepeophtheirus salmonis*, copepodids (30 lice fish⁻¹, or 5 lice L⁻¹ water). Water temperature during the trial was 14 ± 1 °C with a 12 h light:12 h dark light regime. Fish were fed to satiation. Following successful settlement (infection abundance ~ 10 lice fish⁻¹), sea lice numbers were recorded on the 12 July 2011 (chalimus I & II) following light anaesthesia with MS222 (50 ppm). The fish (mean weight: diploids 105.5 ± 22.4 ; triploids 142.8 ± 25.7) were subjected to a second infection on the 19th July 2011 with the same dose of sea lice copepodids, simulating a second wave of infection. A control group of "naïve" sibling diploid fish (mean weight of 188.3 ± 27.0 g), previously uninfected, was also infected at the same time in two replicate tanks. After settlement, sea lice numbers were again recorded on 23 July 2011 (chalimus I & II for second sea lice challenge; chalimus IV and pre-adults for initial challenge). Lice recordings were conducted by a single scientist at all times to avoid variance between counting due to human error. All experiments were carried out in accordance with the Animal (Scientific Procedures) Act 1986 UK under the approval of the local ethical review board.

2.2 Experiment 2: Tank sea lice challenge in Norway

2.2.1 Fish stock

Fish used in this trial were produced at Matre Research Station, Norway. On 3 November 2009, $\sim 200\,000$ eggs were produced from 12 Atlantic salmon females (12 000–22 500 eggs female⁻¹). Eggs were fertilized by three different males (Aquagen stock, Trondheim, Norway), each male being crossed with four different females (male 1 crossed with females 1–4, male 2 with females 5–8, etc.), giving four half-sibling families per male and three groups of full-sibling families. Hydrostatic pressure (TRC-APV, Aqua Pressure Vessel, TRC Hydraulics Inc., Dieppe, Canada) was used to create triploids (37.5 min post-fertilization, 9500 psi (655 bar) for 6 min 15 s at 8 °C),⁴⁸ giving a total of 12 groups per ploidy. Thereafter, each group was incubated in isolation in an UV-treated, flow-through system in darkness. On the 22 July 2010, all fish within each ploidy were mixed and randomly allocated to six fibreglass tanks (total of 12 tanks; $2 \text{ m} \times 2 \text{ m}$, $n = 6$ tanks ploidy⁻¹). On the 27 October 2010, all fish were injected intraperitoneally with 0.1 mL of a multivalent oil-adjuvant vaccine (Minova 6 Vet., Norvax(r), Intervet International B.V., Boxmeer, The Netherlands) using a vaccination pistol (DosysTM 173 classic, Socorex Isba S.A., Renes, Switzerland). Fish were transferred to seawater on the 4 May 2011, with 90 random fish from each ploidy (mean weight of 79 ± 20 and 91 ± 24 g in diploid and triploid, respectively) being allocated

to three $1.5 \text{ m} \times 1.5 \text{ m}$ tanks, with 30 fish from each ploidy in each tank (total of 60 per tank). Fish were reared under continuous light (LL) from May 2010 until October 2010, at which point the photoperiod was switched to simulated natural. Seawater temperature was 8.8 ± 1.0 °C.

2.2.2 Sea lice challenge and sampling

Lice used for the infection were produced from an outbred laboratory strain that had been maintained at approximately 9 ± 1 °C at the Institute of Marine Research Bergen hatchery.⁵⁰ Fish (diploids 313.3 ± 35.9 g, triploids 345.6 ± 41.6 g) were crowded (all three tanks to approximately half of their volume) and challenged on 18 August 2011. Copepodids (10 days post-hatch, estimated total of 13 250 copepodids, 74 lice fish⁻¹) were added to the tanks and tank water level returned to normal after 1 h exposure time. The challenged fish were examined on 16 September 2011 (29 days post-infection). Fish were sacrificed by lethal anaesthesia, their lengths and weights measured and lice numbers counted (stages: pre-adult 1 and 2). The experimental protocol was approved by the Norwegian Animal Research Authority.

2.3 Experiment 3: Natural sea cage infection in Scotland

2.3.1 Fish stock

On 28 November 2008, 45 females and 15 males from two seawinter (unrelated) Atlantic salmon broodstock were stripped of gametes by Landcatch Natural Selection Ltd, Ormsary, UK. A sub-sample of ~ 180 eggs female⁻¹ was removed, fertilized by a different unrelated male, each male being crossed with three different females (i.e. male 1 crossed with females 1–3, male 2 with females 4–6, etc.), giving three half-sibling families per male. Fertilization rates varied between 79.1 and 91.1%. Triploidy was induced as in Experiment 1³¹ with a total of six females and two males shocked at any one time. Post-water hardening eggs were stocked into 20 L silos (3/ploidy). Eggs incubated at Landcatch hatchery (Ormsary, UK) until transfer at the eyed stage to the Inchmore Hatchery, Invermorriston, Marine Harvest Scotland on 12 March 2009. On hatching (25 April 2009) family batches of alevins were pooled and split between two first feeding tanks/ploidy. First feeding fry (diploid (2N) = 2453 fish; triploid (3N) = 2166 fish; 31 May 2009) were exposed to continuous light (LL) and fed for 24 h using Arvotec automatic feeders until the summer solstice (21 June 2009), and were subsequently reared under an ambient photoperiod regime to produce S1+ smolts and were fed during daylight hours. At ~ 5 g, fry were transferred to two freshwater pens (1/ploidy, Glenfinnan, Marine Harvest Scotland) and reared until smoltification. Mortality from first feeding to smolt was 9.4% and 8.5% for diploids and triploids, respectively, with externally visible deformity $< 1\%$ in both ploidy. On 11 June 2010, diploid ($n = 1213$) and triploid ($n = 986$) smolts were transferred into seawater at Marine Harvest Ardnish fish farm with an average weight of 66.1 ± 3.6 g and 86.1 ± 7.4 g for diploids and triploids, respectively ($P > 0.05$). Both groups were transferred into single net pens ($10 \text{ m} \times 10 \text{ m} \times 10 \text{ m}$). On 18 January 2011, fish were graded into two sizes per ploidy (mean weight of 2109 ± 0.05 g and 1541 ± 0.04 g for diploid large and small grades; 2305 ± 0.1 g and 1695 ± 0.7 g for triploid large and small grades, respectively). Fish were stocked into a total of eight $5 \text{ m} \times 5 \text{ m} \times 5 \text{ m}$ pens corresponding to two replicate pens per ploidy and grade. All groups were exposed to continuous light (LL) using a single 400 W metal halide (BGB Engineering Ltd, Grantham, UK) submersible light per pen in order to prevent maturation according to standard

Table 1. Summary of size and sea louse infection statistics (non-parametric ANOVA) for the diploid and triploid salmon in the three trials

Trial/ploidy	Fish parameters						Louse parameters					
	n	Number of individuals assessed/replicate	Mortality (%)	Louse Prevalence (%)	Length (cm)	Weight (g)	Calculated surface area (cm ²)	Abundance	P-Value	Range	Density (abundance/surface area)	P-Value
<i>Tank trial Scotland</i>												
Diploid	2	193	3.5	97.4	23.3 ± 1.3	108.7 ± 20.6	200.2 ± 18.9	7.6 ± 7.2 a	0.906	0–105	0.038 ± 0.035 a	0.990
Triploid	2	187	6.5	98.4	26.1 ± 1.2	144.2 ± 22.9	254.4 ± 16.9	10.4 ± 8.7 a		0–58	0.040 ± 0.033 a	
<i>Tank trial Norway</i>												
Diploid	3	93	0.0	100	29.4 ± 1.4	313.3 ± 35.9	442.6 ± 29.9	7.0 ± 3.4 a	0.594	1–17	0.016 ± 0.008 a	0.828
Triploid	3	86	0.0	100	30.7 ± 1.1	345.6 ± 41.6	468.8 ± 33.5	7.7 ± 3.1 a		3–17	0.016 ± 0.007 a	
<i>Cage trial Scotland</i>												
Diploid	4	293	NA	43.4	54.0 ± 3.6	1952.5 ± 445.0	1300.7 ± 166.3	0.6 ± 0.9 a	0.388	0–6	0.0005 ± 0.0007 a	0.543
Triploids	4	210	NA	56.3	54.8 ± 4.0	1967.3 ± 495.9	1321.0 ± 187.5	0.8 ± 1.0 a		0–7	0.0007 ± 0.0008 a	

NA, not available.

industry practice for Atlantic salmon. During seawater grow-out, the water temperature ranged between 6 to 15 °C. Salmon were fed a range of different commercial diets (Skretting Optiline) according to manufacturer's tables. Sea lice infections occurred naturally.

2.3.2 Sampling

On 7–8 March 2011, fish from all pens (1170 and 959 diploid and triploid salmon, respectively) were anesthetized using MS222 (50 ppm), however, only 814 triploids were assessed due to recorder error. Unbalanced numbers between ploidies originated from an original miscount of fish transferred from the hatchery to the freshwater pens. Fish length, weight and experimental group were recorded before the lice were counted from each fish (lice attached to the fish as well as lice in the anaesthetic bath). Sea lice developmental stages were also recorded.

2.4 Calculations and statistical analysis

Due to the observed differences in body size, adjustment of lice numbers with respect to calculated fish surface area, as defined by O'Shea *et al.*,⁵¹ was performed for all the observed/directly counted sea lice numbers using the following formulae, and averaging the result of both length and weight:

$$\text{Fish-length (cm)} : S = 0.72 L^{1.88}$$

$$\text{Fish-weight (g)} : S = 14.93 W^{0.59}$$

where S is the surface area, L is length (in centimetres) and W is weight (in grams).

All datasets were checked for normality/goodness-of-fit and homogeneity of variance using the Ryan–Joiner test and Levene's test respectively (Minitab, Version 16.1.0). Due to non-normality and unequal variances, all mortality, length and weight comparisons were undertaken using Mann–Whitney U tests. The lice data were analysed with non-parametric analysis of variance (ANOVA) tests, permutational multivariate analysis of variance (permanova software, Department of Statistics, University of Auckland, 2005),^{52,53} due to lack of normality. T -Statistics, based on

distances, were used to carry out pair-wise *a posteriori* tests to identify possible tank/pen effects in all three trials using the permanova software and as described by the Department of Statistics, University of Auckland (2005).^{55,56} Spearman's rank correlation coefficient was used to compare the infection levels of the challenged fish (Minitab, Version 16.1.0.). Mortality comparisons were carried out using two-tailed unpaired t -tests (GraphPad InStat, Version 3.10).

3 RESULTS

3.1 Experiment 1: Tank sea lice challenge and re-challenge in Scotland

Triploid fish were significantly larger (weight diploids 108.7 ± 20.6 g, triploids 144.2 ± 22.9 g, $P < 0.001$, Table 1) than diploid fish at initial infection, which made it important to correct lice numbers for fish size. During the course of the trial there were mortalities over both infections for both diploids (3.5%) and triploids (6.5%) (Table 1), which have been excluded from the results, as accurate louse numbers could not be established for these fish (mortality was not significantly different between diploids and triploids, $P = 0.169$).

No significant difference in infection severity was observed between triploid and diploid fish before or after correcting for fish body size (lice abundance: $P = 0.906$; lice density: $P = 0.990$) (Table 1). Within ploidies, tank effects were observed for sea lice abundance and density, however, no significant ploidy × replicate interactions were found.

Triploid fish were significantly larger than diploid fish at re-infection and lice numbers were therefore corrected for fish size (Table 1). No significant differences in sea lice abundance or density were found for either the chalimus count from the second infection wave, or the count of remaining lice from the first infection wave, or when comparing overall lice abundance on the fish (Table 2). No significant difference in infection intensity, measured by the abundance of chalimus between control naïve diploid (uninfected prior to second challenge) and pre-infected diploid fish was found ($F = 4.27$, $P = 0.105$). The correlation between initial and repeat infection levels (measured as lice density) was marginally significant for individual diploid fish, (Spearman's $\rho = 0.158$; P -value = 0.0429; $R^2 = 0.0249$) (Figure 1a),

Table 2. Effects of ploidy on successive sea louse infection challenges in the re-infection tank trial (assessed by non-parametric ANOVA)

Trial/source	F	P-Value
First challenge louse abundance	0.670	0.581
First challenge louse density	0.274	0.834
Second challenge louse abundance	0.839	0.498
Second challenge louse density	0.612	0.560
<i>Overall statistics</i>		
Overall louse abundance	0.587	0.585
Overall louse density	0.129	0.880

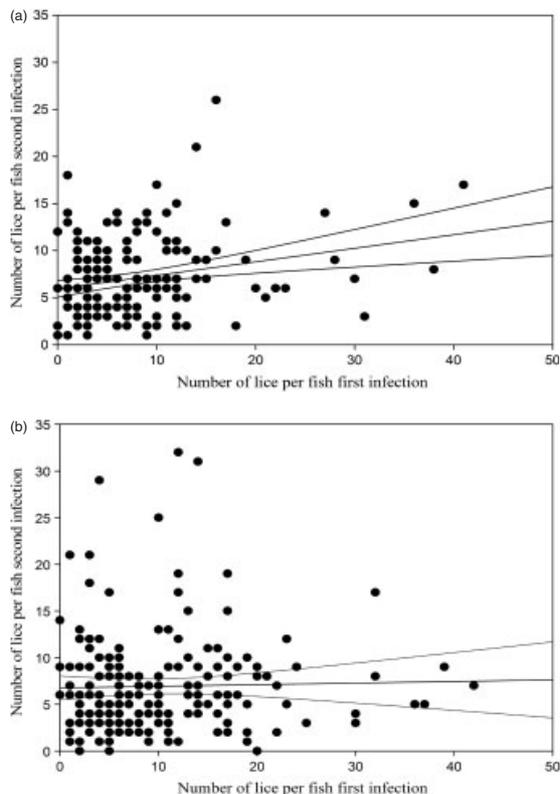


Figure 1. (a) Linear regression of sea louse abundance on individually tagged diploid salmon comparing a first and second infection with sea lice in a tank trial. (b) Linear regression of sea louse abundance on individually tagged triploid salmon comparing a first and second infection with sea lice in a tank trial. Note: fitted regression line is not sensitive to removal/inclusion of highest infected fish from first infection.

with just 2.5% of the observed variation in the second infection caused by the first infection. No significant correlation was found for individual triploid fish (Spearman's $\rho = 0.012$; P -value = 0.867; $R^2 = 0.0002$) (Figure 1b).

Mortality was significantly higher ($P = 0.0009$) in pre-infected diploid fish subjected to a second infection (25 fish, 13.0%) than naïve diploid fish similarly infected (seven fish, 3.5%), most likely caused by combined handling stress and louse attachment.

3.2 Experiment 2: Tank sea lice challenge in Norway

Triploid fish were significantly larger than diploid fish at initial infection (Table 1). No difference in infection intensity was observed between triploid and diploid fish before ($P = 0.594$) or after

($P = 0.828$) correcting for fish body size (Table 1). Within ploidies, tank effects were observed for sea lice abundance and density, however, no significant ploidy \times replicate interactions were found.

3.3 Experiment 3: Natural sea cage infection in Scotland

The lice abundance in the cage trial was much lower than in the tank trials (Table 1) (mean lice fish⁻¹ \pm standard error (SE); diploid 0.64 ± 0.88 ; triploid 0.84 ± 0.98). Prevalence of sea lice (49.9%) was lower than that seen in the artificial sea lice challenge conditions. No difference in infection intensity was observed between triploid and diploid fish before or after correcting for fish body size (Table 1). Within ploidies, cage effects were observed for sea lice abundance and density, however, no significant ploidy \times replicate interactions were found.

4 DISCUSSION

Farmed escaped Atlantic salmon have successfully introgressed and caused genetic changes in native Atlantic salmon populations.^{27,28} The feasibility of using triploid salmon in commercial production is currently being investigated in terms of a number of key farm traits including survival to hatch, size at hatch, deformity prevalence, early stage growth performance,³⁵ smoltification, survival and growth in salt water, heart morphology and severity of cataract.⁵⁴ Evidence for cellular and physiological differences between diploid and triploid salmon, but also evidence for different behavioural patterns^{32,33} is changing views about the farming of triploids leading to improved guidelines for farming triploid salmon. One aspect of particular interest is the potential for differential susceptibility to disease between diploids and triploids. In order for triploid salmon to become more widely used by the industry, it is essential that their susceptibility to infection by sea lice with respect to diploid stocks is established.

This study has examined the potential for differential susceptibility to sea lice between diploid and triploid salmon. No difference in susceptibility between ploidies was found in the tank trials performed in Scotland and Norway and in the cage trial in Scotland. This finding contrasts with an earlier study looking at the susceptibility of Atlantic salmon to another ectoparasite, *Gyrodactylus salaris*, which suggested that triploid salmon had higher infection levels.⁴³ This latter study, however, did not account for different fish sizes resulting from different ploidies.

The fish used in the present tank experiments were obtained from two different selected stocks and the trials were carried out in separate locations (Norway and Scotland) with different fish sizes and infection intensities. Since the findings from the tank trials were similar, this study provides evidence that diploid and triploid salmon do not differ in susceptibility to sea lice infection pressure.

Although no differences in sea lice infection between ploidies were observed, there is the possibility that different families may show different susceptibility, similarly to growth and condition performance effects comparing diploid and triploid families of Atlantic salmon.³⁵ In all experiments presented in the current study, a high number of families were used, representative of a commercial cage population. Genotype \times Environment (G \times E) interactions could not be tested in the present study given the use of three different stocks in different locations and different sizes at the time of challenge. Importantly, there is the possibility of potential different inheritance patterns between ploidy due to the increased chromosome copies in triploids, and determining maternal or paternal inheritance patterns would be essential to determine heritability for selective breeding.

Previous results from tank and cage trials have shown that large fish tend to be more heavily infected.^{36,44,45} Although triploid fish were significantly larger than diploids in the tank trials in Norway and Scotland, no indication was found that larger fish had a higher lice burden than smaller animals. The different light regimes used (tank trial Scotland: 12 h light:12 h dark light regime; tank trial Norway: simulated natural light August–September; cage trial Scotland: continuous artificial submerged light) could play a role in the attachment success of the sea lice on the fish. It was shown that *Lepeophtheirus salmonis* may use phototactic cues, such as shadow and potentially light reflection from the scales of host fish, to colonise the host.^{55,56} Thus, a constant light regime may aid lice attachment compared to day/night rhythms.

In order to reflect a more natural situation, where fish already infected with sea lice are re-infected with fresh lice over the production cycle, a re-infection trial was performed. For the second wave of infection, no differences were found between triploid and diploid salmon. The conclusions of Halačka *et al.*⁴² and Johnson *et al.*⁴¹ demonstrated that triploid fish could show lower immune activity than diploids and might therefore be more susceptible, which was not supported by the results of the current tank study, in terms of sea lice infection. When comparing sea lice infection success on naïve (no prior infection) and pre-infected diploid salmon, no significant differences were observed following a second challenge, with equivalent numbers of chalimus attached to fish. The infection levels of naïve fish were more overdispersed (variance > mean) compared to pre-infected fish, this being indicative of higher aggregation of lice in the naïve stock. Even if the overall sea lice infection level is low, a few individuals will show very high, potentially lethal numbers of sea lice. This aggregation of sea lice may arise from host factors, such as attractiveness, susceptibility and selectivity, or patchiness of sea lice occurrence.⁵⁷ Comparing infection levels for initial infection and re-infection, a significant correlation was found for diploid fish. For triploid fish, no correlation was found. Neither triploid nor diploid fish in this trial, subject to initial infection, became refractory to subsequent sea lice infection.

A significantly higher mortality was observed for pre-infected diploid fish subject to a second infection. This may indicate that the worst affected fish may have received a second severe infection, with possibly lethal consequences to the already weakened animals. The question remains, of whether the few highly infected fish, which had to be taken out of the trial due to lethal lice loads, would have shown a similar pattern of infection in subsequent infections.

The current tank trials were performed with high mean lice numbers, which rarely occur in commercial fish farms under a strict pest management strategy. This study was extended by examining corresponding infection levels on a commercial salmon production site. Following current salmon production as well as environmental guidelines and good management practice, sea lice numbers at the site were kept at a low level (range 0–7 lice fish⁻¹). Under farm conditions, which include a variety of additional factors not seen in controlled tank trials, no significant difference in susceptibility was observed between triploid and diploid salmon in sea cages. Observed cage effects within the ploidies may have been due to positioning of pens with respect to environmental factors. For example, anecdotal evidence suggests that the upstream pens of a cage group might have higher lice loads than pens located in the middle of a cage group. Tidal rhythms, salinity, water flow and turbulence associated with high current velocities have also

been described to affect sea louse behaviour in the wild.⁵⁸ Further research under commercial conditions is required to identify the main environmental parameters that explain louse burden differences.

Overall, this study clearly demonstrates that there are no intrinsic differences in susceptibility to sea lice infection between diploid and triploid Atlantic salmon.

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