

## RESEARCH ARTICLE

# Resistance to *Saprolegnia parasitica* infection: A heritable trait in Atlantic salmon

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## Abstract

A controlled *Saprolegnia parasitica* infection model was used to challenge 1158 fish representing 105 pedigreed Atlantic salmon families to evaluate the possibility of selecting for *Saprolegnia* resistance in a commercial breeding programme. Fish were infected in five study tanks and observed for 40 days post-infection for lesion score and survival. Survival analysis of the top 10 resistant and bottom 10 susceptible families indicated that the hazard of dying following *Saprolegnia* infection was 1509% higher in susceptible families. In all fish, a 10 g increase in weight correlated with a 7.8% increase in the hazard of dying while sex did not affect mortality. Resistance to *Saprolegnia* was estimated to have a heritability of 0.25, indicating that selection is possible. Genetic and phenotypic correlations indicated that the 11-point scoring system, developed in this study to quantify *Saprolegnia* infection severity, had a high negative correlation with survival as days to mortality at  $\geq -0.922(\pm 0.005)$ , suggesting that the scoring method could help assess lesion development in studies where mortality is not the primary biological endpoint.

## KEYWORDS

challenge, family, genetic selection, lesion scoring, Saint John River, *Saprolegnia parasitica*

## 1 | INTRODUCTION

Atlantic salmon (*Salmo salar*) production has grown by ~66% from ~1.73 to 2.61 MT with a value of 17.06 B USD over the 8 years between 2011 and 2019 (FAO, 2021). This is aided by effective farming technologies and disease control that have continued to develop for both the freshwater phase (from eggs to 100–200 g smolt) in hatcheries and the seawater phase (from smolt to ~5 kg harvest). The industry must expand horizontally to cover more areas and vertically to become more efficient to maintain this same impressive growth trajectory. The most recent production increases and economic growth have been attributed to the seawater phase, but have been fuelled by increases in production of healthy high-quality smolts in

the freshwater phase where continual improvements on survivability and disease control must also be made.

One of the freshwater diseases, *Saprolegnia* spp., belong to the class Oomycota containing several species of fungus-like microorganisms that are pathogenic to aquatic animals and plants. *Saprolegnia* can present significant challenges and considerable losses for returning wild Atlantic salmon stocks in freshwater (Neitzel et al., 2004), but also for the aquaculture industry (Hussein & Hatai, 2002) in closed controlled environments. Outbreaks of *Saprolegnia* can trigger high losses of both eggs and fry which leads to an economic impact in the freshwater phase as well as a loss in numbers to stock for the seawater phase.

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Various treatments have been administered for *Saprolegnia*. Malachite green was the predominant treatment but it has been banned due to its high toxicity including carcinogenicity, mutagenicity and teratogenicity (Sudova et al., 2007). Formalin solution (37% formaldehyde) is the current most effective treatment (Bly et al., 1996; Gieseker et al., 2006; Walser & Phelps, 1994; Waterstrat & Marking, 1995), but it has a Classification as a 1B carcinogen by the European Commission, indicating a potential causal relationship (European Commission, 2014 with an expectation that it will be banned as a treatment option). Other less effective treatments have also been available as a bath treatment with relative success, such as Bronopol (2-bromo-2-nitro-1,3-propanediol) (Oono & Hatai, 2007; Pottinger & Day, 1999), salt (NaCl) (Ali, 2005; Edgell et al., 1993; Taylor & Bailey, 1979; Waterstrat & Marking, 1995), boric acid (Ali et al., 2014), hydrogen peroxide (Barnes et al., 1998; Waterstrat & Marking, 1995), copper sulphate (Marking et al., 1994; Sun et al., 2014) and iodophors (Walser & Phelps, 1994) among others. Biological control using bacterial isolates of *Pseudomonas fluorescens* in the feed looks promising to treat fry but will be limited to exogenous feeding life stages (González-Palacios et al., 2019). Research efforts continue to seek alternatives and develop new screening tools to advance progress as the industry must consider concerns associated with safety, toxicity and applicability of the potential treatment options (Stueland et al., 2005; Tedesco et al., 2019).

Genetic improvement through selective breeding for disease resistance offers a reasonable and sustainable alternative to increase the resistance of a population and reduce mortality in the event of disease outbreaks. To enable selection, collection of reliable data from controlled challenge models is critical to measure the specific trait of interest while controlling all other variables to estimate heritabilities and breeding values of improved performance. Host resistance is usually measured by survival (time or days post-infection to mortality) or mortality (dead/alive) following a natural outbreak or an experimental challenge. To date, research focused on variability across families and heritability of *Saprolegnia* resistance in fish is lacking. A single study (Nilsson, 1992) suggested possible heritable resistance to *Saprolegnia* from a naturally occurring infection in Arctic char. Therefore, for the present study, a reliable large-scale challenge model was used to test the resistance to *Saprolegnia parasitica* infection in a commercial broodstock of Atlantic salmon presmolts from 105 families. This is the first experimental study that establishes the heritability of *Saprolegnia* resistance in pedigreed Atlantic salmon broodstock along with genetic and phenotypic correlations between *Saprolegnia*-related traits and recorded body weights.

## 2 | METHODS

### 2.1 | Fish

In 2017, 105 families (partial factorial mating design using 62 sires and 74 dams) were retained at the Huntsman Marine Science Centre (St. Andrews, New Brunswick, Canada) as part of a Saint John River stock commercial Atlantic salmon breeding programme

with Mowi Canada East. This programme has been selecting on quality traits (e.g., seawater growth and fillet colour) and welfare traits (e.g., sea lice and bacterial kidney disease) while maintaining genetic variation since 2010. This specific year class represents the second generation in the breeding programme with contribution from multiple parental year classes (contribution from 88 full- or half-sibling families).

The 105 families were reared in individual family tanks until approximately 10 g average weight, at which time individual Atlantic salmon were evaluated and 12 individuals were PIT tagged per family (August/September 2018). PIT-tagged fish were placed into three communal tanks for holding prior to acclimation and study tank allocation.

Study fish were allocated among five tanks to complete the challenge based on the anticipated individual fish size and desired stocking density. A total of 105 families were designated such that 63 families were placed into each of the five tanks, resulting in approximately four individuals per family per each of three tanks. Weights at the time of PIT tagging were used to designate individuals into one of three tanks per each family so that each of the three tanks had a similar size range of individuals by family. Individuals were assessed (weight/length) and sorted into prechallenge tanks on 09 and 10 January 2019.

### 2.2 | Experimental system

Experimental tanks were made of fibreglass and circular with 115 cm diameter. Freshwater was supplied to each tank ( $5 \pm 0.5$  L min<sup>-1</sup>) using a single-pass flow-through design at ambient water temperature, ranging from ~8.5 to 10.5°C throughout the study. Water temperature, oxygen saturation and flow rate in each tank were assessed and recorded daily. The fish were offered commercial feed (Nutra Fry, Skretting) and fed at 1% of their body weight unless otherwise specified below. The photoperiod within the study room was adjusted to be 12:12-h light:dark periods.

### 2.3 | *Saprolegnia* strain, sporulation and production

The *Saprolegnia parasitica* strain was isolated from a local Atlantic salmon commercial production hatchery. The fungus was characterized by the amplification of internal transcribed spacers (ITS) using primers P-ITS1 (5'-TCC-GTA-GGT-GAA-CCT-GCG-G-3') and P-ITS4 (5'-TCC-TCC-GCT-TAT-TGA-TAT-GC-3') according to White et al. (1990). Characterized *Saprolegnia parasitica* was subsequently maintained on cornmeal agar (CMA) plates and transferred biweekly until the study infections were all completed.

Before sporulation, fungal mycelium was cut from the CMA and transferred to sterile Petri dishes containing ~15 ml Sabouraud dextrose broth (SDB) supported with 1% Pen-Strep (P4333, Sigma, ON, Canada) and incubated at 21°C for 3–4 days to obtain fungal growth for the sporulation process. Sporulation was triggered as described by Diéguez-Urbeondo et al. (1994). Briefly, fungal

mycelia were collected from the SDB cultures and washed three times in sterile-filtered freshwater. The rinsed fungal mycelia were split into smaller portions and returned to Petri dishes containing ~25 ml of freshwater for 14 h at 21°C. The *Saprolegnia* biflagellate zoospores were then collected by passing the contents of these final Petri dishes through two layers of a sterile cheesecloth into a sterile beaker. The concentration of the resulting stock solution was evaluated by counting the collected zoospores with a haemocytometer.

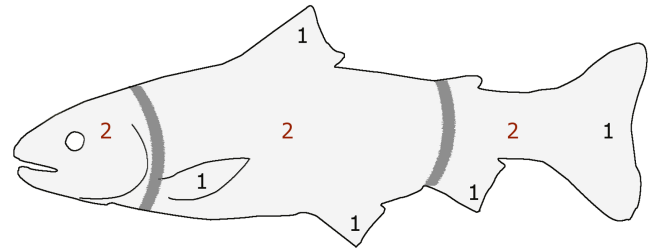
## 2.4 | Challenge model

The study fish had nearly 2-month acclimation period post-transfer prior to infection. No feed was offered for the 24 h prior to infection and 48 h post-infection. The exposure was applied by using the Ami-Momi procedure as described by Hatai and Hoshiai (1993). The Ami-Momi procedure presented a safe and efficient method to induce infection in the tested population. Briefly, 10 fish were netted at a time from the initial holding acclimation tank and were shaken for 2 min in the air using a fan-shaped scoop net. Fish were then returned to another tank preloaded with the fungal spores at 25,000 spores·l<sup>-1</sup> until all the fish were transferred. Using the number of individuals placed into each tank and calculating the biomass from the expected size increase after the 09/10 January measure, the tank water depth was adjusted in each receiving tank to achieve an equal density of 20 kg m<sup>-3</sup> in each of the five tanks.

The Ami-Momi procedure prepares the fish for an external infection by removing the surface mucus layer, disrupting the natural physical barrier and inducing a transient cortisol level increase. Water flow in the receiving tank was stopped immediately prior to dosing and restored at 48 hrs post-infection. The infection occurred in a staggered manner to facilitate the logistics of completing Ami-Momi, conducting the sporulation and infection, assessing mortalities across the challenge period and collecting fish samples. The first challenge began on 04 March 2019 on designated tank E then tanks D (05 March 2019), C (06 March 2019), B (15 March 2019) and A (15 March 2019) were subsequently challenged. An independent batch of fungal spores was prepared for each tank in the manner described above. The target temperature was 10°C for the challenge period with temperature maintenance occurring by the use of heaters in individual tanks. Target oxygen saturation was 100% and flow rate was 4–5 L/min when resumed after the 48-h period.

## 2.5 | Fish assessment

All moribund fish, mortalities and survivors were assessed for fungal growth and scored to a maximum of 11 points. There was a score of 1 point for each fin type displaying fungal growth for a total of 5 points and 2 points per body region (head, body and tail) when fungus or lesion were present on the skin in these areas (Figure 1). Lesions extending across multiple body regions were scored in



**FIGURE 1** Scoring *Saprolegnia* lesions using an 11-point scoring method. The presence of *Saprolegnia* on any of the fin types was scored as 1 and the presence of *Saprolegnia* on the body regions scored as 2. A lesion crossing body areas was scored in both areas. All numbers added to create a total score for each individual Atlantic salmon

each of the affected areas. Mortalities and moribund fish were collected twice daily. Total body weight, fork length and sex were also recorded at the time of mortality, fish removed as moribund or at study termination.

## 2.6 | Statistical analysis

### 2.6.1 | Survival analysis

Time-to-event data were collected from all tanks throughout the observation period. All fish that died due to *Saprolegnia* infection were considered a 'failure' or survived to the end of the observation period were denoted as 'censored'. Furthermore, survival was recorded as time in days to mortality post-infection. Those individuals alive at study termination had a similar day of mortality. Mortality was recorded as dead/alive (alive at the time of study termination). Any fish that died in the first 48 h post-infection were not included in the dataset as this was considered a result of the exposure process. The duration of each observation period post-infection was ended after a minimum of 2 consecutive days passed with no mortalities and by 40 days post-infection (dpi) for each tank. Survival analysis was completed using StataCorp ver14 statistical software (StataCorp, 2015). The Cox proportional hazard model was used after validating the model assumptions to highlight the family effect and to study the effect of start weight and sex on survival time post-infection.

The effect of start weight and sex on survival time was tested by including all fish ( $n = 1158$ ) regardless of the family number and stratifying by tank using the Cox proportional hazard model. Stratification was used to allow separate estimates of the baseline hazard for each tank, different baseline hazards yields the following general hazard function formula for the  $j$ th tank:

$$h_j(t) = h_{0j}(t)e^{\beta X}$$

The incidence rate for each family was estimated as the number of failures divided by total time at risk (sum of number of days lived by each fish in the family) for each individual family. The survival analysis for a subset of data was completed including two groups of the

10 highest and 10 lowest incidence rate families to highlight the effect of family on survival time using the same Cox proportional hazard model stratified by tank. The model included 222 fish (114 and 108 from the most susceptible and resistant families, respectively). A graphical representation of these individual families was plotted before statistical analysis using Kaplan–Meier survivor function to demonstrate the differences between individual families.

## 2.7 | Fixed effects, heritabilities and correlations

Fixed effects included in the model per trait were tested for significance prior to estimation of heritabilities. Significance of a fixed effect was assessed using a linear model per effect per trait. Fixed effects tested were tank, challenge start date and sex. Table 1 shows that both tank and challenge start date were significant for survival, mortality and score, but not for start weight. Since tank and challenge start date were partially confounded, only tank was fitted. Sex was not significant for any trait and, therefore, was not fitted in the model.

Heritabilities and genetic and phenotypic correlations were estimated using the WOMBAT software (Meyer, 2007) and a multivariate animal model with the following general form:

$$y = Xb + Zu + e$$

where  $y$  is a vector of observations for all traits (survival, mortality and score);  $b$  is the vector of fixed effects of tank and challenge date;  $u$  is a vector of random animal genetic effect;  $X$  and  $Z$  are incidence matrices that relate observations to fixed and random effects and  $e$  is the vector of random residual effects. Heritabilities ( $h^2$ ) for each trait were calculated as:

$$h^2 = \frac{\sigma_a^2}{\sigma_p^2}$$

where  $\sigma_a^2$  is the additive genetic variance and  $\sigma_p^2$  is the phenotypic variance. Genetic correlations ( $r_g$ ) between traits  $i$  and  $j$  were calculated as:

$$r_g = \frac{\text{Cov}_{ij}}{\sqrt{\sigma_i^2 \sigma_j^2}}$$

TABLE 1 Statistical comparison ( $p$  values) of potential effects (Tank, Start Date and Sex) and traits in a *Saprolegnia parasitica* challenge of Atlantic salmon. Traits were Start Weight, Survival as days to mortality, Mortality as dead/alive and Score as an 11-point *Saprolegnia* scoring system

Potential effects	Traits			
	Start weight	Survival	Mortality	Score
Tank	.3520	<.0001	<.0001	<.0001
Start date	<.0001	<.0001	<.0001	<.0001
Sex	.0005	.0967	.0538	.0227

where is the covariance between traits  $i$  and  $j$  and  $\sigma_i^2$  and  $\sigma_j^2$  are the additive genetic variances of traits  $i$  and  $j$ , respectively.

## 3 | RESULTS

### 3.1 | Daily husbandry during infection and observation period

Water temperature was recorded daily for the duration of the challenges across all five tanks. There was some temperature variation among tanks due to difficulty in precisely adjusting individual tank heaters with the overall water temperature being  $9.6^\circ\text{C} \pm 0.52$ . The mean water temperature in tanks A and B (infected 9 days later) was  $0.54^\circ\text{C}$  higher than tanks C–E with the largest variation occurring immediately post-infection (Figure 2).

### 3.2 | Challenge model

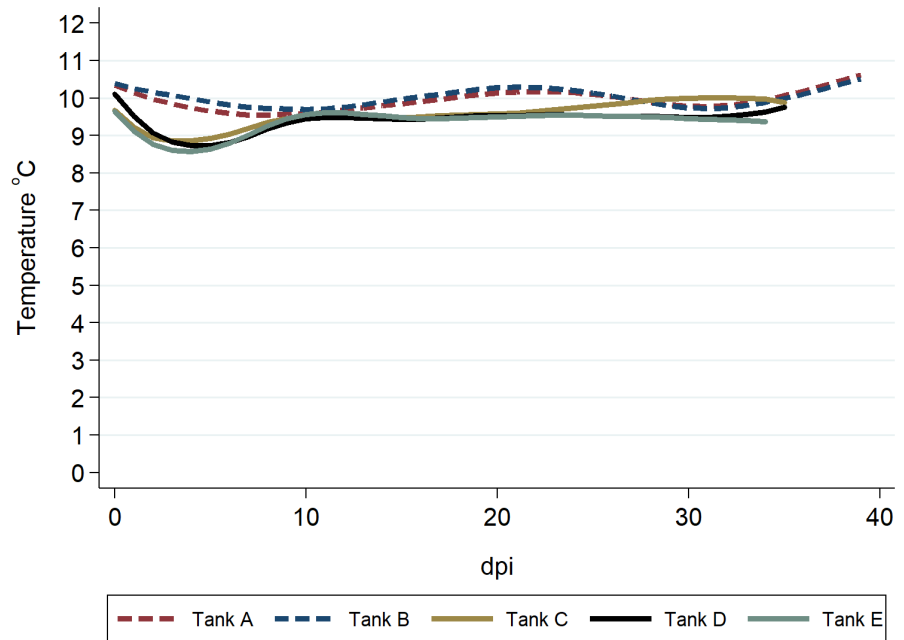
The starting weights were assessed in January before fish were sorted into tanks. The number of fish per tank and average starting weight by sex are summarized in Table 2. Prior to the challenge, the overall mean weight was  $42.7 \pm 15.5$  g, the mean fork length was  $14.8 \pm 1.9$  cm and the mean condition factor was  $1.26 \pm 0.12$ . At the time of infection, there were a total of 1158 individuals from 105 families. There were 8–12 individuals per family across three tanks, resulting in a minimum of two individuals per family per tank (e.g., 8 individuals were allocated as 3, 3 and 2 into three tanks) and a maximum of four individuals per family per tank.

Survival was recorded as time in days to mortality post-infection. Those individuals alive at study termination had a similar day of mortality. Mortality was recorded as dead/alive (alive at the time of study termination). Mortality due to *Saprolegnia* began at 2 dpi and increased to reach the peak hazard by 9 dpi (Figure 3). A total of 636 salmon died and 47 individuals were removed as moribund (discussed further together as 683 mortalities) from the starting population with mortalities reaching 25% and 50% of the total population at 10 and 19 dpi, respectively. The sum of the total number of days survived by all fish in the study population (time-at-risk) was 25,962 days and the mean incidence rate was 0.0264 fish per fish-day-at-risk. The incidence rate varied by challenge tank with mortality in tanks A (70.7%) and B (70.9%) higher than mortality rates in tanks C (58.3%), D (46.5%) and E (48.7%) (Table 3).

### 3.3 | Impact of fish weight and sex on survival time post-infection

Individual fish were assessed for weight prior to the challenge to allow for biomass calculations to control the variability between tanks during the *Saprolegnia* infection. The tanks also included a size range of individuals within each family which resulted in similar overall tank

**FIGURE 2** Temperature profiles line plots with lowest smoother of study tanks (A-E) by days post-infection indicating slight difference in profiles of tanks A & B from C, D & E



**TABLE 2** Mean, standard errors and total numbers of starting weight (g) of all Atlantic salmon included in the *Saprolegnia parasitica* challenge by sex and tank

		Female	Male	Total mean
Tank A	$\bar{X}$	45.0	42.3	43.7
	$\sigma$	14.6	14.3	14.5
	$n$	124	108	232
Tank B	$\bar{X}$	45.9	38.7	42.3
	$\sigma$	16.1	16.0	16.4
	$n$	116	111	227
Tank C	$\bar{X}$	42.6	41.3	42.0
	$\sigma$	15.0	14.4	14.7
	$n$	125	110	235
Tank D	$\bar{X}$	43.5	44.4	43.9
	$\sigma$	14.2	15.8	15.0
	$n$	111	119	230
Tank E	$\bar{X}$	44.6	38.9	41.5
	$\sigma$	17.8	15.9	17.0
	$n$	107	127	234
Total mean	$\bar{X}$	44.3	41.1	42.7
	$\sigma$	15.5	15.4	15.5
	$n$	583	575	1158

weight across experimental tanks. Sex distribution was similar by tanks, but not determined until post-mortem examination (Table 2).

The impact of starting weight and sex on survival time was tested for all fish ( $n = 1158$ ) stratified by tank using the Cox proportional hazard model. Fish weight ranged from 9.7 to 99.8g with a mean of  $42.7 \text{ g} \pm 15.5$ . The 10th, 25th, 50th, 75th and 90th percentiles were at 22.5, 31.7, 42.4, 52.6 and 61.8g, respectively. The results from the statistical analysis indicated that the hazard is increased by 7.8%

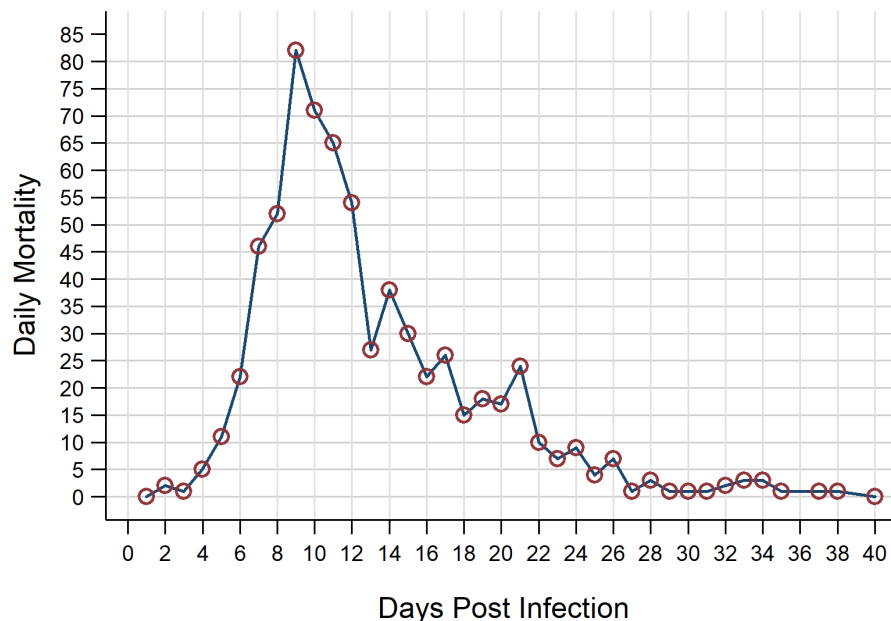
for every 10 g increase in fish weight ( $p = .002$ ) while there was no significant effect of sex ( $p = .133$ ).

### 3.4 | Impact of Atlantic salmon families on survival time post-infection

The high variability in family response to infection indicated a significant impact of family on survival post-infection to *Saprolegnia parasitica*. Survival by family ranged from 0% survivors to 91% survived. To further characterize this potential for variation by family, the survival of the 10 highest and 10 lowest incidence rate families was grouped and tested for difference in survival (Figure 4). The survival data are summarized in Table 4. Briefly, the total mortality from these same fish and families during the observation period was 126 fish. The percentage mortality in resistant and susceptible families was 9%–27% and 83%–100%, respectively. Furthermore, a Cox proportional hazard model was used to assess the effect of these highest and lowest family groups on survival time stratified by the challenge tank. The results indicated that the hazard of dying post-infection significantly increased by 1509% in the susceptible families compared to the resistant families, where the hazard ratio was  $15.09 \pm 4.19$  ( $p < .000$ ).

### 3.5 | Relationship of the 11-point lesion scoring method to survival or mortality

The 11-point lesion scores were evaluated in all fish in the study population including 683 mortalities and 475 survivors. The scores for mortalities were distributed across the scale starting from a score of 2 (in 1 fish) up to 11 (in 429 fish). The scores for survivors ranged from no lesion (in 205 fish) up to 11 (in 2 fish) (Figure 5).



**FIGURE 3** Daily mortality due to *Saprolegnia parasitica* infection with Ami-Momi method in all tanks combined to highlight peak mortality occurring by 8–10 days post-infection and recovery by 40 days post-infection

**TABLE 3** Description of the survival data in an Atlantic salmon *Saprolegnia parasitica* challenge across five experimental tanks (A–E)

Tank ID	Time at risk	Incidence rate	Number of fish	Number of mortalities	Survival time		
					25%	50%	75%
A	4527	0.0362	232	164	9	13	
B	4249	0.0379	227	161	8	12	
C	5529	0.0248	235	137	13	21	
D	5862	0.0183	230	107	13		
E	5795	0.0197	234	114	12		
Total	25,962	0.0263	1158	683	10	19	

### 3.6 | Heritability of resistance to *Saprolegnia* infection between families with genetic and phenotypic correlations between traits

The heritability estimates were moderate for all traits associated with the *Saprolegnia* infection including survival ( $0.282 \pm 0.058$ ), mortality ( $0.246 \pm 0.055$ ) and the 11-point lesion scores ( $0.247 \pm 0.054$ ; Table 5). The start weight was negatively, genetically and phenotypically correlated with survival (days to mortality) indicating that a smaller salmon would be less likely to be affected by *Saprolegnia*, but the correlation was not strong and the standard error was relatively high ( $-0.260 \pm 0.140$  and  $-0.109 \pm 0.036$ , respectively; Table 5). The 11-point lesion scores were highly genetically and phenotypically correlated with both survival and mortality ( $\geq 0.959 \pm 0.019$  genetic and  $\geq 0.873 \pm 0.007$  phenotypic; Table 5).

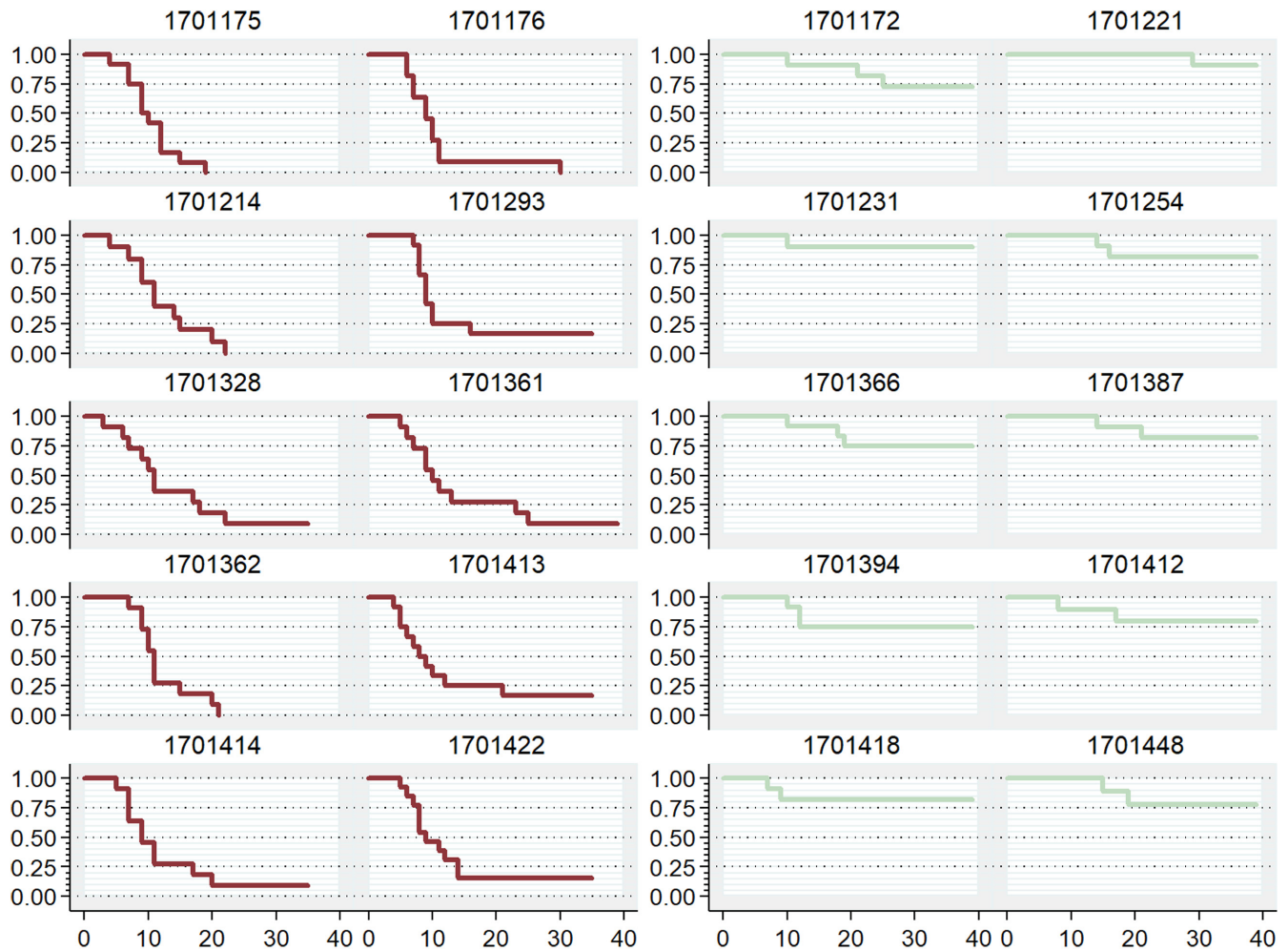
## 4 | DISCUSSION

This study presents the first *Saprolegnia* challenge test to be conducted for Atlantic salmon. The results presented here highlight five areas for discussion: (1) Challenge model and phenotype collection;

(2) Differences between top and bottom extreme families; (3) Effect of weight, sex and tank on resistance; (4) Selecting for *Saprolegnia* resistance in commercial populations and (5) Further work and challenges. These points are discussed below.

In this study, we successfully adopted the previously established Ami-Momi method described by Hatai and Hoshiai (1993) to conduct a *Saprolegnia parasitica* challenge in Atlantic salmon. Several other methods were previously tested to complete these challenge infections in pilot trials (This work was completed at Huntsman prior to this challenge and has not been published) with relative success, including cortisol implants (Pottinger & Day, 1999) and scarification (Eissa & Soliman, 1994; Gieseke et al., 2006). The Ami-Momi method was selected for this study given its consistent and practical use for the total number of individual fish challenged. The Ami-Momi method has proven effective and adequate to allow infection with no acute adverse effects on the salmon, with only one fish dying on the day of challenge and no mortalities observed during the 48-hr challenge period.

Each tank included 8–12 individuals per family from 63 of the 105 families at the start of the challenge. These families were distributed to the challenge tanks in a manner to minimize Family x Tank interaction. This approach was employed to reduce genetic



**FIGURE 4** Kaplan–Meier survival curves of the 10 most resistant and 10 most susceptible Atlantic salmon families in a *Saprolegnia parasitica* challenge selected for survival analysis

**TABLE 4** Description of the survival data in 20 families of Atlantic salmon that underwent a *Saprolegnia parasitica* challenge including 10 most resistant and 10 most susceptible families

Family group	Time at risk	Incidence rate	Number of fish	Number of mortalities	Survival time		
					25%	50%	75%
Susceptible	1434	0.0732	114	105	7	10	14
Resistant	3501	0.0060	108	21			
Total	4935	0.0255	222		9	19	

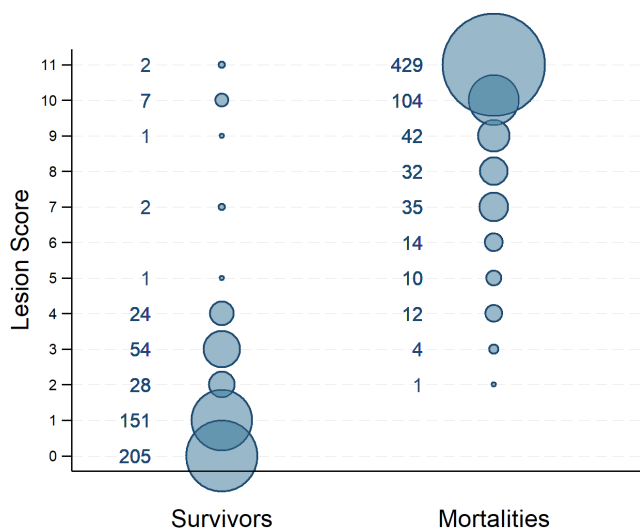
variability between tanks. It may have resulted in a small amount of variation in mortality, but would not be expected to contribute to a visible difference in survival by tank (Table 1). There was noted slight variability in temperature profiles of tanks C–E compared to tanks A and B (Figure 2). The staggered experimental design was required for logistical purposes to process moribund and dead fish but also introduced a timing gap as tanks A and B were infected later than tanks C–E. This timing gap also necessitated preparation of a different spore batch to complete the later infections in tanks A and B. The spore preparation methods were standardized but may have introduced some variation in resulting infection.

The 11-point *Saprolegnia* scoring system was developed by considering the distribution patterns of *Saprolegnia* cutaneous lesions previously described in salmonids (Aller-Gancedo & Fregeneda-Grandes, 2019; Ciepliński et al., 2018) and reflected the severity of infection. The estimated heritabilities using the *Saprolegnia* score were very similar for both survival and mortality with high negative genetic and phenotypic correlations (Table 5). This indicates that the assessment of the external appearance of an infected fish with *Saprolegnia* is a reliable predictor of death. Therefore, this scoring system could be useful in studies where repeated non-lethal assessments are needed, such as determining the efficacy of novel

therapeutics (Pottinger & Day, 1999). However, more assessments are warranted to investigate whether the scoring system is equally predictive throughout the infection course when fish are repeatedly assessed as the lesions develop.

The hazard of dying due to *Saprolegnia* infection was 1509% higher in susceptible families compared with resistant families. This level of variability indicates a significant opportunity for the Atlantic salmon farming sector to select for *Saprolegnia* resistance to combat the disease and reduce use of chemical antifungal bath treatments. The magnitude of variability measured in between-family response to *Saprolegnia* infection should be noted as it indicates that the genetic composition, or rather overall genetic variability, plays an important role in a study. This could be of major importance when conducting efficacy studies for novel treatments or vaccination.

Heritability for disease resistance in salmonids has been demonstrated for many viral (Boison et al., 2019; Gonen et al., 2015; Moen, 2010), bacterial (Evenhuis et al., 2015; Henryon et al., 2005; Purcell et al., 2014; Silverstein et al., 2009; Yáñez et al., 2013), parasitic (Wynne et al., 2008) and fungal (Nilsson, 1992) diseases. Nilsson (1992) estimated the first published heritability data for *Saprolegnia* in Arctic char using sire and dam models of  $0.34 \pm 0.14$  and  $0.10 \pm 0.08$ , respectively. There were 92 full-sib families included (36 sires, 32 dams) in this natural outbreak of multiple sites. The data provided a snapshot as the specific cause of death was



**FIGURE 5** Scatter plot with weighted markers showing the frequency of reporting *Saprolegnia* within the 11-point lesion scores in survivors and mortalities

not determined and those individuals recorded as alive were not confirmed as such. In the present study, we completed a controlled challenge and, hence, were able to reduce environmental impacts that cannot be controlled in naturally occurring outbreaks. Our estimated heritabilities using an animal model were within the same range at  $\sim 0.25$  for all three traits evaluated (i.e., survival, mortality and score).

Nilsson (1992) also recorded fish weights at the time of marking (April), with mortality beginning to occur in July and reported a genetic correlation for weight with mortality as  $-0.50$  and phenotypic correlation as  $.00$ . Results suggested a negative genetic correlation between weight at tagging and mortality, although there was no phenotypic correlation. Our data also suggested a slight negative genetic and phenotypic correlation between weight prior to challenge and increased mortality (Table 5). However, weight correlations were not strong, suggesting that selection for improved resistance to *Saprolegnia* would not have a large negative effect on freshwater growth. Nonetheless, since growth is one of the main traits in the breeding goal for Atlantic salmon breeders, the correlation between freshwater growth and infection, and saltwater growth and infection, should be assessed and the effect of including *Saprolegnia* resistance in the breeding goal should be evaluated on a population basis.

The survival analysis of weight indicated that there is a slight negative correlation between higher weight in Atlantic salmon and increased hazard to *Saprolegnia* infection such that the hazard increased by 7.8% for every 10g increase in fish weight. The survival analysis of sex indicated that sex did not correlate with the hazard of dying from infection. This could be a result of life stage of the Atlantic salmon used or Atlantic salmon as a species. With saprolegniasis in wild brown trout, Richards and Pickering (1978) noted that mature male salmonid fish prior to spawning will have higher incidence of infection than in females. This difference was expected to be correlated with physiological changes associated with sexual maturity (Pickering, 1977; Richards & Pickering, 1978, 1979).

There are many opportunities to continue this research further as the fish farming industry continues to develop methods to mitigate the impact from *Saprolegnia*. Future challenges should be completed on additional year classes of Atlantic salmon to ensure that our reported heritability holds and to further characterize the genetic variability of this trait. The present challenge model involved a controlled infection with no contemplation to rescue the enrolled fish after a set period of days post-infection. This approach addressed our desire to determine whether *Saprolegnia* resistance was

	Start weight	Survival	Mortality	Score
Start weight	<b>0.548</b> ( $\pm 0.079$ )	$-0.260$ ( $\pm 0.140$ )	$-0.272$ ( $\pm 0.144$ )	$0.293$ ( $\pm 0.141$ )
Survival	$-0.109$ ( $\pm 0.036$ )	<b>0.282</b> ( $\pm 0.058$ )	$0.975$ ( $\pm 0.011$ )	$-0.959$ ( $\pm 0.019$ )
Mortality	$-0.116$ ( $\pm 0.035$ )	$0.945$ ( $\pm 0.003$ )	<b>0.246</b> ( $\pm 0.055$ )	$-0.985$ ( $\pm 0.011$ )
Score	$0.152$ ( $\pm 0.035$ )	$-0.873$ ( $\pm 0.007$ )	$-0.922$ ( $\pm 0.005$ )	<b>0.247</b> ( $\pm 0.054$ )

**TABLE 5** Heritability (on diagonal) with genetic (above diagonal) and phenotypic (below diagonal) correlations between a start weight prior to a *Saprolegnia parasitica* infection, Survival (days to mortality), Mortality (dead/alive) and Score (11-point *Saprolegnia* score)

Bold (on the diagonal) are the heritabilities. Heritabilities are often shown in bold when the genetic and phenotypic correlations are included to allow for quick differentiation.



a heritable trait in Atlantic salmon but is not entirely indicative of industry production methods. To this end, completing a controlled infection followed by a set bath treatment regimen reflecting industry practices might be of interest to determine whether similar family performance would result.

In conclusion, we demonstrated a successful adaptation of the Ami-Momi method to conduct a controlled and scalable challenge model for *Saprolegnia* infections. A *Saprolegnia* 11-point scoring method was developed and shown to be a reliable predictor for mortality that could help future non-lethal assessment of severity of infection. Our results revealed that resistance to *Saprolegnia* infection on individual Atlantic salmon is a heritable trait that could be selected for to mitigate economic impact on hatchery operations and improve fish welfare.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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