https://doi.org/10.17221/100/2025-VETMED

Susceptibility of different rainbow trout (*Oncorhynchus mykiss*) genetic lines to infectious disease

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Citation: Papezikova I, Vaibarova V, Pojezdal L, Matejickova K, Blahova J, Mendel J, Maresova E, Mikulikova I, Palikova M, Toulova I (2026): Susceptibility of different rainbow trout (*Oncorhynchus mykiss*) genetic lines to infectious disease. Vet Med-Czech 71, 310–321.

Abstract: This study assessed the resistance of three rainbow trout (*Oncorhynchus mykiss*) genetic lines to three pathogens, the bacterium *Flavobacterium psychrophilum* and the parasites *Ichthyophthirius multifiliis* and *Tetracapsuloides bryosalmonae*, by monitoring mortality, immune parameters (leukocyte count and phagocytic activity), and pathogen load. Two of the fish lines tested carried quantitative trait loci associated with resistance to the selected pathogens. Unexpectedly, the line with declared genetic resistance exhibited the highest mortality rate following *F. psychrophilum* infection, suggesting the influence of additional factors such as differences in strain virulence or interactions between genetic background and environmental conditions. Following *I. multifiliis* infection, the resistant line initially exhibited a higher parasite load but later achieved more efficient parasite clearance. Overall, the findings suggest that disease resistance in trout is a complex trait influenced by interactions between genotype, pathogen and environment. Consequently, resistant lines must always be tested under specific conditions of the target aquaculture facility before implementation.

Keywords: genetic resistance; *Ichthyophthirius multifiliis;* proliferative kidney disease; rainbow trout fry syndrome; white spot disease

Funded through Project QK21010030 "Globalisation, modern technology and climate change as sources of new opportunities and threats for salmonid fish breeding management", NAZV QK22010369 (both supported by the Ministry of Agriculture of the Czech Republic), and Project PROFISH CZ.02.1.01/0.0/0.0/16_019/0000869 "Sustainable production of healthy fish under various aquaculture systems", supported by the Ministry of Education, Youth and Sports of the Czech Republic.

https://doi.org/10.17221/100/2025-VETMED

In intensive aquaculture, infectious diseases represent one of the major threats to fish health and production. Traditionally, disease control has relied primarily on chemical treatments, including antibiotics and chemotherapeutics. However, these approaches face increasing limitations due to emerging pathogen resistance, the limited number of approved therapeutics and vaccines, stringent European Union (EU) regulations on antibiotic use, and the growing demand for environmentally sustainable practices.

As a result, aquaculture is progressively shifting toward alternative preventive strategies, with the rearing of genetically resistant fish seen as a promising approach. Currently, genetically resistant fish lines are being developed using a combination of traditional selective breeding and marker-assisted selection. Quantitative trait loci (QTLs) have now been identified for a wide range of economically and evolutionarily important traits, including resistance to diseases and parasites (reviewed in Mendel et al. 2018). The rearing of resistant fish not only minimises the need for chemical intervention but also contributes to the maintenance of ecological balance within aquaculture systems, and reduces economic losses from mass mortalities. When combined with optimised management practices, biosecurity measures and improved nutrition, selective breeding for disease resistance offers a modern, sustainable approach to fish production.

Rainbow trout (*Oncorhynchus mykiss*) is widely cultivated in fish farms around the world. Several diseases represent major challenges to its production. One of the most serious is rainbow trout fry syndrome (RTFS), a bacterial infection caused by *Flavobacterium psychrophilum*, which predominantly affects fry and early juveniles and often results in high mortality rates in hatcheries. The disease is associated with systemic infection, clinical signs typically including lethargy, exophthalmia, erosion of the caudal peduncle or caudal fin, skin ulcerations, increased skin pigmentation and organ haemorrhaging (Barnes and Brown 2011; Loch and Faisal 2015). Ichthyophthiriasis, caused by the parasitic ciliate *Ichthyophthirius multifiliis*, is one of the most common parasitic diseases of freshwater fish. The disease can spread rapidly, particularly in intensive culture systems, and can cause severe respiratory distress, osmoregulatory dysfunction and mortality if not promptly managed (Buchmann et al. 2001; Matthews 2005). Proliferative kidney disease (PKD) is a chronic disease caused by the myxozoan parasite *Tetracapsuloides bryosalmonae*. It affects both wild and cultured salmonids, leading to kidney swelling, anaemia and immune suppression (Palikova et al. 2017). Outbreaks are exacerbated by elevated water temperatures and suboptimal environmental conditions (Bettge et al. 2009).

Studying genetically resistant rainbow trout lines is particularly valuable given the challenges involved in controlling the above-mentioned diseases. Previous genetic studies have demonstrated that resistance to RTFS has a multilocus architecture, with several QTLs identified across multiple chromosomes (Fraslin et al. 2018; Liu et al. 2015; Mathiessen et al. 2023; Pouil et al.2025). Likewise, two highly significant QTLs located on chromosomes 16 and 17 have been associated with resistance to ichthyophthiriasis (Jaafar et al. 2020; Buchmann et al. 2022). Genetically resistant lines have previously been successfully tested, showing significantly higher survival after controlled pathogen exposure compared with non-resistant fish (Marancik et al. 2015; Mathiessen et al. 2023). Although resistance-associated QTLs have been identified, the underlying biological mechanisms that contribute to disease resistance are still unclear.

The main aim of this study was to assess the disease resistance of three commercially available rainbow trout genetic lines. Two of these lines were developed using marker-assisted selection and marketed as possessing enhanced resistance to RFTS and RTFS combined with ichthyophthiriasis (aquasearch.dk/qtls). Resistance to RTFS and ichthyophthiriasis was tested under controlled laboratory conditions, while resistance to PKD was evaluated in a field experiment due to the challenges associated with performing controlled experimental infection with *T. bryosalmonae*. All three lines were also monitored throughout the entire production cycle in the trout farm.

MATERIAL AND METHODS

Experimental fish

All fish used in the following experiments were obtained as juveniles from a Czech commercial rainbow trout farm, where they had been stocked as eyed eggs purchased from AquaSearch Ova ApS (Denmark). During the laboratory trials

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(Experiments 1 and 2), the fish were kept in 100-l glass aquaria containing 80 l of water, with a total system volume (including filtration and tubing) of approximately 96 l. Each aquarium was equipped with an independent recirculating system and maintained under a 12 h light/12 h dark photoperiod. The tanks were supplied with dechlorinated, carbon-filtered tap water, which was maintained at a temperature of 13 °C, a pH of 7.0 ± 0.4 and a dissolved oxygen concentration >5.5 $mg.l^{-1}$. The water temperature was monitored continuously. Dissolved oxygen concentration and pH were measured daily, while other water chemistry parameters, including ammonia (below 0.2 mg/l), nitrite (below 1 mg/l) and nitrate concentrations (below 30 mg/l), were measured twice per week. Fish health was assessed visually by direct observation of clinical signs and behavioural changes at least twice a day. The fish were fed twice daily with a commercial pelleted diet (Inicio Plus, 0.5 mm; Biomar Group, Brande, Denmark) at 2% of their body weight.

Three trout lines were tested: Fresh, which had no QTLs associated with resistance traits; QTL RTFS, which had been selectively bred for genetic resistance to *F. psychrophilum*; and QTL RTFS + Ich, selectively bred for combined genetic resistance to *F. psychrophilum* and *I. multifiliis* (for clarity, the fish lines are referred to as Fresh, Flavo and Flavo + Ich, respectively, throughout the text). In Experiment 3, the Flavo + Ich line was not used as suitable fish of the required age and size were unavailable at the time of the trial.

Genetic variability analysis

For genotyping, pectoral fin samples were collected from 25 individuals of each line and fixed in 96% ethanol. Genomic DNA was then isolated from the samples using the DEP-25 extraction kit (Top-Bio, Prague, Czech Republic). Microsatellite loci were amplified through PCR using the STR Multiplex OMident10 kit (Institute of Vertebrate Biology, Czech Academy of Sciences; S7iFish Software v3.1). Using the Qiagen multiplex PCR Kit (Qiagen, Hilden, Germany), a total of ten loci were amplified in two multiplex reactions. Fragment analysis was performed on an ABI PRISM 3130 Genetic Analyser (Applied Biosystems, Foster City, USA) using the GeneSca™ 600 LIZ® internal size standard (Life Technologies, Carlsbad, USA). Alleles were scored in Peak Scanner v1.0 and checked for potential genotyping errors using Micro-Checker v2.2.3 (Brookfield 1996). Subsequently, a principal component analysis was conducted in three-dimensional space (3D PCA) using Genetix v4.05.2 (Belkhir et al. 2004).

Experiment 1: Resistance to *Flavobacterium psychrophilum*

Before the experiment began, the fish were examined for the presence of *F. psychrophilum* using nested PCR using modified methods of Wiklund et al. (2000) and Baliarda et al. (2002). Negative (no template DNA) and positive (DNA of *F. psychrophilum* strain ATCC 49418) controls were included in each reaction.

EXPERIMENTAL DESIGN

Two *F. psychrophilum* strains isolated from clinical outbreaks in the Czech Republic were selected for the experiment: 560/7 (mPCR type 2), isolated from the brain of a diseased rainbow trout with symptoms of RTFS in 2024, and 6739/7 (mPCR type 2), isolated from the spleen of a diseased rainbow trout in 2018. The strains were revived from the glycerol cryostock and cultivated for 72 h at 15 °C and 160 rpm in TYES broth, which contained 0.4% tryptone (Tryptone; Oxoid, Basingstoke, UK), 0.04% yeast extract (Yeast Extract; Oxoid, Basingstoke, UK), 0.02% $CaCl_2 \cdot 2H_2O$ (Sigma-Aldrich, St. Louis, MO, USA), 0.05% $MgSO_4 \times 7H_2O$ (Merck KGaA, Darmstadt, Germany) and 0.05% glucose (Erba Lachema, Brno, Czech Republic). The culture was mixed well to obtain a homogeneous suspension, after which a 7.5 ml aliquot was transferred to 1 500 ml of TYES broth and cultivated for 48 h at 15 °C and 160 rpm. The culture, which had a final concentration of 10^8 CFU/ml, was then diluted in dechlorinated tap water for use in the bath challenge (see below).

For the experiment, juvenile fish (2.49 ± 0.52 g and 56 ± 1.4 mm) were divided into six experimental groups (30 fish per treatment). After a 21-day acclimatisation period, the fish were exposed to the two different strains of *F. psychrophilum* using the bath challenge method, with three groups (Fresh, Flavo and Flavo + Ich) infected with strain 560/7 (serotype Th) and three (Fresh, Flavo and Flavo + Ich) with strain 6739/7 (also serotype Th)

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through exposure for two hours to aerated tap water containing 10^7 CFU/ml of the bacteria.

Mortality was monitored throughout the experiment and expressed as cumulative mortality rate. All fish that died during the experiment underwent post-mortem examination. Swabs were collected from the spleen, kidney and brain and cultured at 17 °C for five days on Tryptone Yeast Extract Salts (TYES) agar, as described in Vaibarova et al. (2024). Bacterial colonies were identified using PCR (Cepeda and Santos 2000) and matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS), using a MALDI Biotyper v3.0 system (Bruker Daltonics, Coventry, UK). The experiment was repeated twice.

The experiment finished 28 days after experimental infection, at which point ten fish were sampled from each experimental group. At the end of the experiment, the fish in the Fresh group had a weight and length of 4.01 ± 0.9 g and 70.5 ± 5.5 mm, respectively; in the Flavo group, 2.59 ± 0.73 g and 60.5 ± 5.9 mm; and in the Flavo + Ich group, 3.33 ± 0.86 g and 67.8 ± 5.9 mm. Blood samples were obtained by puncturing caudal vessels using heparinised insulin syringes. Following blood collection, the fish were euthanised with a blow to the head. All fish underwent pathoanatomical examination, during which swabs from the spleen were inoculated onto TYES agar and cultivated at 17 °C for five days, as described above.

Heparinised blood (Heparin Léčiva; Zentiva, Prague, Czech Republic) was used to assess white blood cell counts and evaluate phagocyte activity. White blood cells were counted manually using a haemocytometer, as described in Svobodova et al. (2012). Phagocyte activity was measured via luminol-enhanced chemiluminescence, as described by Papezikova et al. (2016). The results were expressed as peak values and integrals of the resulting kinetic curves, and peak-time (the time point at which the chemiluminescence signal reached its maximum value).

Experiment 2: Resistance to *Ichthyophthirius multifiliis*

Before the experiment began, skin and gill scrapings were taken from all fish and examined under a Nikon YS2-H microscope (Nikon, Japan; ×40 magnification) to confirm they were free of ectoparasites. The fish were also screened for *I. multifiliis* using a quantitative PCR assay targeting the 18S SSU rDNA gene of *I. multifiliis*, in accordance with the methodology of Jousson et al. (2005), employing the QuantiTect SYBR Green PCR Kit (Qiagen, Hilden, Germany).

EXPERIMENTAL DESIGN

The naturally infected fish were originally obtained from a commercial fish farm with a confirmed *I. multifiliis* population, but without the presence of other ectoparasites. *I. multifiliis* was subsequently maintained in the laboratory through serial passage to naïve rainbow trout, which were then used to infect all three experimental groups. For Experiment 2, juvenile fish (2.78 ± 0.71 g and 60.5 ± 7.4 mm) were allocated into three experimental groups (Fresh, Flavo, Flavo + Ich; two tanks for each group with 16 fish in each tank). After a 21-day acclimation period, the fish were exposed to *I. multifiliis* through cohabitation with infected rainbow trout for a 24-hour period. The infection was done in a single aquarium divided into four compartments using perforated partitions to allow for parasite dissemination without mixing of the experimental groups.

The fish were sampled 7 and 14 days after infection, with six fish taken from each tank at each time point. At the end of the experiment (14 days after infection), the fish in the Fresh group had a weight and length of 5.48 ± 0.45 g and 78.5 ± 4.7 mm, respectively; in the Flavo group, 4.11 ± 1.17 g and 74.0 ± 6.5 mm; and in the Flavo + Ich group, 4.89 ± 0.59 g and 70.0 ± 3.3 mm.

Blood samples were then taken by puncturing the caudal vessels using heparinised insulin syringes (Heparin Léčiva; Zentiva, Prague, Czech Republic). Following blood collection, the fish were euthanised with a blow to the head.

Skin smears were taken from the entire left side of the fish's body, excluding the head, using the blunt edge of a scalpel. For microscopic examination of the gills, epithelial tissue from four gill arches on the left side of the body was collected, considering the small size of the fish.

The skin and gill smears were examined under a microscope Olympus BX51, Japan (×40 magnification), and *I. multifiliis* trophonts were counted and summed for ten fields of view. The blood samples were used for total leukocyte counts and

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assessment of phagocytic activity, following the methods described previously. The experiment was repeated twice.

Experiment 3: Resistance to *Tetracapsuloides bryosalmonae*

In this experiment, resistance was assessed in trout from the Fresh (non-resistant; average weight 11.7 ± 3.7 g, average length 105.2 ± 10.7 mm; n = 20) and Flavo lines (resistant to RTFS; average weight 14.3 ± 3.5 g, average length 112.9 ± 8.67 mm; n = 20). Before the experiment started, fish from both lines (n = 10) were screened for ecto- and endoparasites by microscopic examination. In addition, qPCR was performed on caudal kidney samples, as described by Bettge et al. (2009), to confirm that fish were negative for PKD.

EXPERIMENTAL DESIGN

The fish were transferred to a facility where PKD occurs annually and is endemic. To ensure equal exposure to *T. bryosalmonae* spores, both groups were housed together in a single flow-through tank. Over the course of the six-week experiment, water temperature was measured twice daily and the fish were monitored each day for behavioural changes and mortalities. After six weeks, the fish were euthanised with a blow to the head and dissected. During dissection, the skin, gills, eyes and internal organs were examined under a microscope to control for parasitic infection. The degree of kidney and spleen swelling was evaluated and indexed as 0 (no enlargement), 1 (slight enlargement), 2 (moderate enlargement) or 3 (severe enlargement). Samples of caudal kidney were taken and placed in 70% ethanol for subsequent PCR analysis to assess *T. bryosalmonae* load. Additionally, a sample of caudal fin tissue was collected from each fish for genetic variability analysis to confirm group identity (i.e., to distinguish between the two genetic lines), as described above.

All experiments were performed in compliance with the Czech law on protection of animals against cruelty, as approved by the Ethical Committee of the University of Veterinary Sciences in Brno, Czech Republic (Permit No. MZe 2407, č. j. MZE-11060/2023-13143 and MSMT-17863/2022-3, IGA VETUNI 107/2022/FVL).

Statistical analysis

All statistical analyses were undertaken using the Unistat for Excel statistical software package (v6.5). As a first step, all datasets were assessed for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene's test. Where both assumptions were met, differences between the means of the three groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's HSD test for multiple comparisons, while an unpaired t-test was applied for comparisons between two groups. If the data did not meet assumptions of normality and/or homogeneity of variance, differences between the three groups were assessed using Kruskal–Wallis ANOVA, with Dunn's test employed for post-hoc multiple comparisons, with the Mann-Whitney U test used for two-group comparisons. In all cases, statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Genetic variability analysis

All lines analysed displayed clear genetic distinctiveness, each forming a separate cluster (Figure 1). The Principal Component Analysis (PCA) across populations explains almost 100% of the variability with just two axes, indicating that QTL selection has produced extremely pure lines. The Flavo line is the most homogeneous and at the same time the most distinct from the others, suggesting that some alleles may have become fixed. Fresh, as the founding line, shows the highest genetic variation in the individual-based PCA, indicating that it possesses the broadest genetic base. Flavo and Flavo + Ich have diverged from Fresh under distinct selection pressures clearly illustrated by the PCA, where both separate from Fresh along different axes.

Experiment 1: Resistance to *Flavobacterium psychrophilum*

Mortality began five days after experimental infection with *F. psychrophilum*, which is consistent with the acute course of RTFS observed in previous experimental salmonid challenges (Marancik et al. 2014; Macchia et al. 2022). All mortalities exhibited

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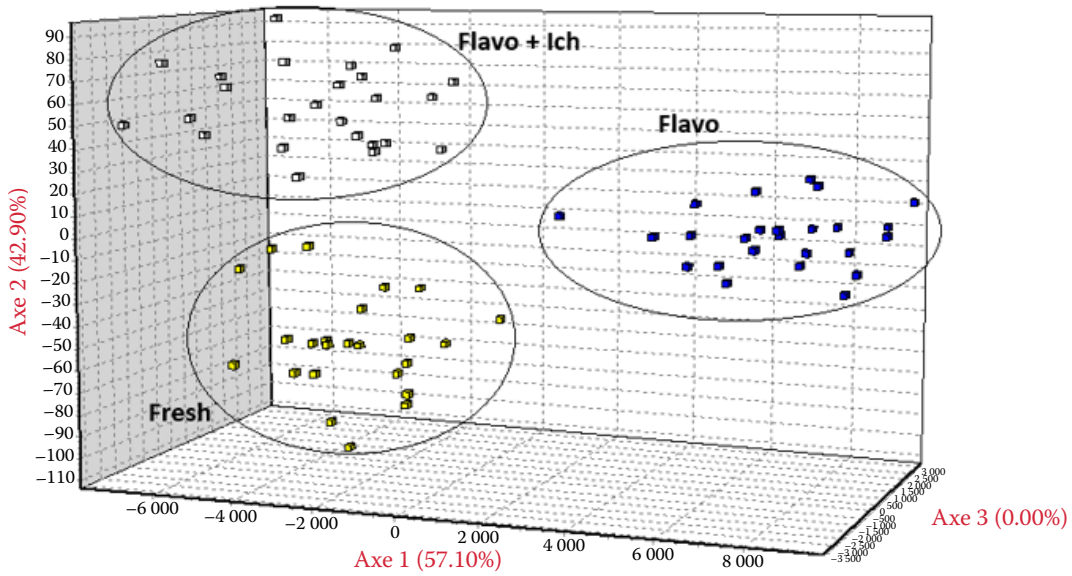

Figure 1. 3D principal component analysis (PCA) of the examined lines illustrating their genetic differentiation
Each point in the plot represents an individual. Fresh: line without genetic resistance, Flavo: line selectively bred for genetic resistance to *F. psychrophilum*, Flavo + Ich: line selectively bred for genetic resistance to both *F. psychrophilum* and *I. multifiliis*

massive systemic infection with *F. psychrophilum*. While most fish showed no distinct patho-anatomical changes, some exhibited small skin haemorrhages and deep, round, yellow-pigmented ulcers that penetrated the muscle tissue. At the end of the experiment, there were no visible patho-anatomical changes in surviving fish, and spleen swabs proved negative for *F. psychrophilum*.

Cumulative mortality varied considerably depending on the *F. psychrophilum* strain used, with strain 560/7 proving markedly more virulent than strain 6739/7. This agrees with earlier findings, which showed that *F. psychrophilum* isolates varied greatly in pathogenicity, even when derived from similar host species or geographic origins (Macchia et al. 2022). Unexpectedly, the highest mortality rate was observed in the Flavo line, which carries known QTLs associated with resistance to RTFS. In contrast, the Flavo + Ich line, which was developed for dual resistance to RTFS and ichthyophthiriasis, exhibited higher survival rates when challenged by both bacterial types. The Fresh line, which lacks any known resistance QTLs, exhibited the lowest cumulative mortality to both *F. psychrophilum* strains, with similar trends in each case (Figure 2A,B).

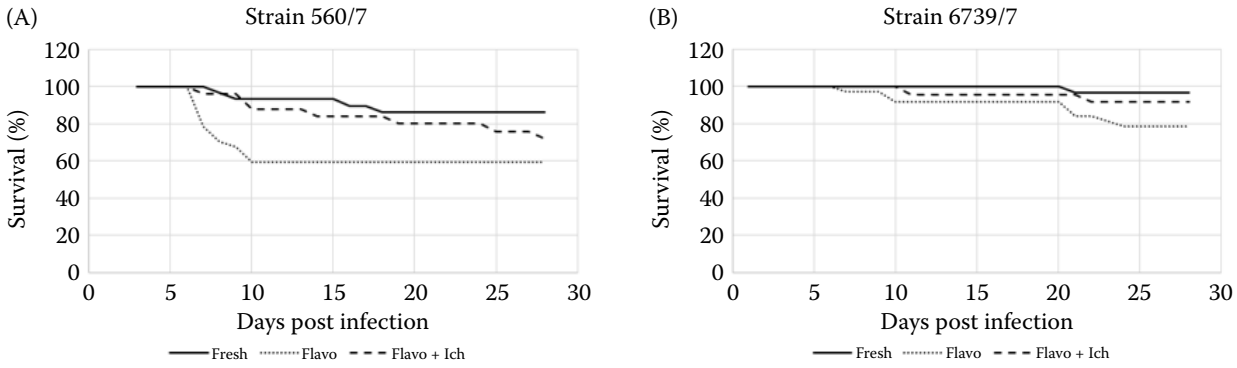

Figure 2. Survival of rainbow trout of three lineages after 28-day infection trial with *F. psychrophilum*
(A) Challenge with strain 560/7; (B) Challenge with strain 6739/7. Fresh: line without genetic resistance, Flavo: line selectively bred for genetic resistance to *F. psychrophilum*, Flavo + Ich: line selectively bred for genetic resistance to both *F. psychrophilum* and *I. multifiliis*

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The results demonstrate that interactions between fish genotypes, pathogen characteristics and environmental conditions are highly complex, potentially yielding non-intuitive or even contradictory outcomes. The findings also suggest that other genetic or physiological factors in the Fresh line, not yet uncharacterised, could confer tolerance or resistance to these *F. psychrophilum* isolates. Conversely, the increased mortality in the Flavo could indicate potential trade-offs associated with resistance loci. These trade-offs could include metabolic costs or altered immune regulation, which reduce performance under certain infection conditions (Robinson et al. 2023). PCA revealed strong homogeneity within the Flavo line, suggesting that the selected haplotypes along with QTL-based resistance may not be optimal for local conditions.

While no significant differences were observed in white blood cell count between the experimental groups, significant differences were observed in phagocytic activity between the groups infected with strain 6739/7 (Table 1). Highest phagocytic activity (intensity of reaction to an activator, reflected by a peak in the kinetic curve) was observed for the Flavo line, with the peak differing significantly from the Flavo + Ich line. A similar trend was observed for groups infected with strain 560/7, though the differences were not significant, most likely due to high inter-individual variability. Differences were also noted in the onset rate of the phagocyte response, with Fresh line fish infected with strain 6739/7 showing a significantly delayed onset compared to the Flavo and Flavo + Ich lines (Table 1). This delay could indicate slower initial immune recognition or response mobilisation. However, it did not result in higher mortality, suggesting that factors beyond the timing of phagocyte activation, such as local immune efficiency or humoral defence, may also play a compensatory role in this line's unexpected resistance.

The Flavo group, which displayed highest phagocytic activity, also exhibited the highest mortality rate following challenge with *F. psychrophilum*. In healthy fish that have not been exposed to pathogens, high phagocytic activity generally indicates a greater innate ability to defend against infection. This response may also indicate an immunological adaptation that improves disease resistance and overall health status, e.g., following vaccination or administration of immunostimulants (Siwicki et al. 1994; Huang et al. 2014). During infection, however, strongly elevated phagocytic activity may suggest a dysregulated immune response, triggered by either a high infectious load or a stress stimulus. Excessive activation of phagocytes can contribute to tissue damage through the release of reactive oxygen species and inflammatory mediators, potentially exacerbating disease severity. Overactivation of the immune system does not necessarily lead to effective pathogen elimination. Instead, it may impose a physiological burden on the organism and contribute to overall systemic overload (Soliman and Barreda 2023).

Table 1. Leukocyte counts and phagocytic activity of the whole blood phagocytes in fish infected with two strains of *F. psychrophilum* (560/7 and 6739/7)

Parameter	*F. psychrophilum* strain/fish lineage					
	560/7 Fresh	560/7 Flavo	560/7 Flavo + Ich	6739/7 Fresh	6739/7 Flavo	6739/7 Flavo + Ich
Leukocytes (G/l)	39.3 ± 12.2	40.1 ± 12.8	42.8 ± 18.9	42.9 ± 11.5	51.0 ± 26.9	37.5 ± 11.7
Phagocyte activity – integral/1 000 RLU·min	35.6 ± 34.2	51.8 ± 35.4	43.8 ± 49.0	23.4 ± 9.1	37.2 ± 26.8	19.2 ± 23.7
Phagocyte activity – peak (RLU)	415.7 ± 243.7	823.1 ± 556.8	736.9 ± 828.6	383 ± 155^{ab}	594.1 ± 414.8^{a}	183.3 ± 150.2^{b}
Phagocyte activity – peak-time (min)	**48.7 ± 19.5**	49.4 ± 15.4	49.3 ± 13.9	**70.8 ± 4.8^{a}**	45.0 ± 18.6^{b}	43.0 ±13.9^{b}

^{a,b}Statistically significant differences among groups are marked with different letters. Statistically significant differences between groups infected with strains 560/7 and 6739/7 respectively, are marked in bold. Data represent means ± standard deviations. Fresh: line without genetic resistance, Flavo: line selectively bred for genetic resistance to *F. psychrophilum*, Flavo + Ich: line selectively bred for genetic resistance to both *F. psychrophilum* and *I. multifiliis*

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Experiment 2: Resistance to *Ichthyophthirius multifiliis*

Seven days after infection, *I. multifiliis* trophonts were detected in all fish groups, with the number of trophonts present on gills markedly higher than on skin (Table 2). At this time point, the number of trophonts on skin was significantly higher in the Flavo + Ich group than the other two groups (Table 2), suggesting an increased initial susceptibility or reduced capacity to limit early parasite establishment. A similar trend was also observed on the gills, with the Flavo + Ich group harbouring significantly more trophonts than the Flavo line. These results suggest that the Flavo + Ich line may have a delayed or less effective early immune response to *I. multifiliis*.

By the second sampling (14 days post-infection), there had been a marked change in trophont dynamics, with the highest number of parasites now found on the skin of the Flavo line fish, while trophont counts on the gills were similar across all three groups. A comparison of the two post-infection intervals revealed that, 14 days after infection, a significantly higher number of trophonts were present in the Fresh and Flavo groups (Table 2). Conversely, the Flavo + Ich group had significantly fewer trophonts on the skin than at 7 days post-infection, while the intensity of gill infection remained stable. This suggests that, over time, the Flavo + Ich line may clear parasites from the skin more effectively, potentially reflecting activation of protective immune mechanisms between days 7 and 14. Buchmann et al. (2022) compared two lines of rainbow trout: one carrying two QTLs associated with resistance to *I. multifiliis* and one without. The QTL line exhibited significantly lower parasite loads 16 days post-infection and showed slightly delayed mortality compared to the non-QTL line. Unlike the aforementioned work, no mortality was observed in our study, suggesting that although the infection intensity was substantial, it did not surpass the fish's physiological tolerance. This may indicate adequate immune function or a less virulent parasite strain. To date, at least five serotypes of *I. multifiliis* have been identified that differ in virulence, and this likely contributes to the substantial variation in infection dynamics reported across different studies (Swennes et al. 2006).

Significant differences in phagocytic activity were only recorded in peak-time values. At 7 days post-infection, a significantly shorter peak-time (i.e., a faster reaction to the activator) was observed in the Flavo + Ich group compared to the other two groups. At 14 days post-infection, the peak-time value was significantly higher than at 7 days post-infection (Table 3). While there was no significant difference in leukocyte counts between groups, a comparison of the two post-infection intervals showed a decrease in leukocyte number in all groups at 14 days post-infection. An 18.7% decrease was observed in the Fresh group and an 18.4% decrease in the Flavo + Ich group, both of which were non-significant. In contrast, the Flavo group showed a significant decrease of 52.7%. Absolute leukocyte counts are provided in Table 3. A decrease in leukocyte counts in rainbow trout following infection with *I. multifiliis* was also reported by Abdel-Hafez et al. (2014) in rainbow trout and by Witeska et al. (2010) in common carp (*Cyprinus carpio*). Witeska et al. (2010) also demonstrated an association between reduced leu-

Table 2. Numbers of *I. multifiliis* trophonts on skin and gills 7th and 14th day post infection (p. i.)

Time post infection	Tissue	Line		
		Fresh	Flavo	Flavo + Ich
7 days p. i.	skin	1.17 ± 0.75[ab]	1.0 ± 1.26[a]	7.33 ± 6.19[b]
	gills	13.33 ± 8.71[ab]	9.0 ± 5.06[a]	33.17 ± 21.25[b]
14 days p. i.	skin	1.0 ± 1.1	**3.83 ± 2.64**	**1.33 ± 2.8**
	gills	**29.33 ± 11.93**	**30.83 ± 14.63**	28.33 ± 24.06

[a,b]Statistically different values among lineages are marked with different letters. Data represent numbers of trophonts in ten fields of view (40× magnification) – means and standard deviations. For 14 days post infection, statistically significant differences from the 7-day interval are marked in bold. Fresh: line without genetic resistance, Flavo: line selectively bred for genetic resistance to *F. psychrophilum*, Flavo + Ich: line selectively bred for genetic resistance to both *F. psychrophilum* and *I. multifiliis*

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Table 3. Leukocyte counts and phagocytic activity of the whole blood phagocytes 7th and 14th day post infection (p. i.)

Parameter	Time post infection	Line		
		Fresh	Flavo	Flavo + Ich
Leukocytes (G/l)	7 days p. i.	45.5 ± 13.4	48.2 ± 12.3	33.7 ± 3.9
	14 days p. i.	37.0 ± 15.1	**22.8 ± 10.7**	27.5 ± 7.7
Phagocyte activity – integral/1 000 (RLU·min)	7 days p. i.	29.2 ± 17.8	44.6 ± 33.0	20.8 ± 13.5
	14 days p. i.	64.4 ± 54.7	69.4 ± 35.4	33.9 ± 26.5
Phagocyte activity – peak (RLU)	7 days p. i.	453 ± 275	691 ± 498	458 ± 317
	14 days p. i.	997 ± 876	1 013 ± 520	495 ± 331
Phagocyte activity – peak-time (min)	7 days p. i.	64.5 ± 11.2[a]	56.5 ± 13.3[a]	39.5 ± 6.1[b]
	14 days p. i.	64.0 ± 10.5	58.0 ± 13.4	**81.5 ± 29.4**

[a,b]Statistically different values among lineages are marked with different letters. Data represent means ± standard deviations. For 14 days post infection, statistically significant differences from the 7-day interval are marked in bold. Fresh: line without genetic resistance, Flavo: line selectively bred for genetic resistance to *F. psychrophilum*, Flavo + Ich: line selectively bred for genetic resistance to both *F. psychrophilum* and *I. multifiliis*

kocyte counts and disease severity and poor outcomes. In fish that survived the infection, however, leukocyte numbers tended to recover.

Experiment 3: Resistance to *Tetracapsuloides bryosalmonae*

Throughout the six-week experiment, no mortalities, clinical symptoms or behavioural abnormalities were observed in the experimental fish. Examination at the end of the exposure period revealed the presence of *T. bryosalmonae* DNA in all the tested fish. Most fish also exhibited varying degrees of kidney and spleen swelling, which is typical of chronic PKD pathology (Feist and Longshaw 2006; Palikova et al. 2017). No significant differences were found between the experimental groups regarding either degree of organ swelling or *T. bryosalmonae* load (Table 4). An absence of visible clinical symptoms, mortality, or behavioural abnormalities is consistent with previous reports that mortality induced by PKD is temperature-dependent and generally low when water temperatures remain below approximately 15 °C (Bettge et al. 2009). The temperature profile during this trial (15–17 °C in the first week, then mostly below 15 °C; Figure 3) most likely limited parasite proliferation and host pathology, resulting in mild to moderate infection outcomes. Bettge et al. (2009) demonstrated that the time course of kidney swelling development was similar at 12 °C as it was at 18 °C. However, mortality was very low, and the degree of kidney swelling was much reduced. The absence of differences in *T. bryosalmonae* load and patho-anatomical changes between the lines examined in this study indicates that genetic resistance of the Flavo line to flavobacteriosis does not provide cross-protection against PKD. This suggests that environmental factors, particularly water temperature, play a dominant role in determining infection severity.

Table 4. Spleen and kidney swelling (indexed from 0 to 3) and *T. bryosalmonae* load in caudal kidney (Ct value measured by qPCR)

Line	Kidney swelling		Spleen swelling		*T. bryosalmonae* load	
	degree	% of fish	degree	% of fish	Ct value	% of fish
Fresh	1.5 ± 0.76	90	1.15 ± 1.04	70	19.0 ± 2.04	100
Flavo	1.7 ± 0.73	100	0.75 ± 0.72	65	18.6 ± 2.84	100

Data represent means ± standard deviations. No statistically significant differences were found between the experimental groups. Fresh: line without genetic resistance, Flavo: line selectively bred for genetic resistance to *F. psychrophilum*

https://doi.org/10.17221/100/2025-VETMED

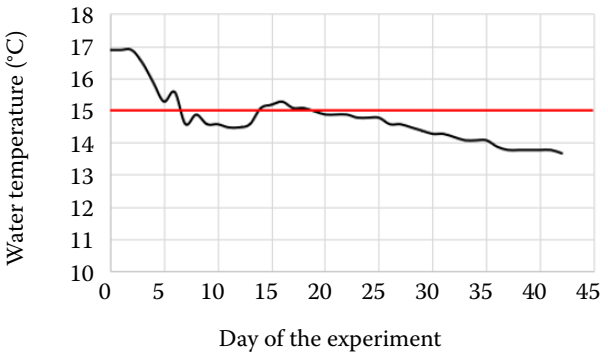

Figure 3. Water temperature during the PKD experiment
Water temperature of 15 °C, considered as critical for the development of clinical signs is marked with red line

The trout farm from which the juvenile fish for the experiments were collected had conducted long-term monitoring of survival in its cultured stocks. All lines were imported at the eyed-egg stage in excellent condition, with minimal losses prior to or following hatching. The hatched larvae were vigorous, and there were very few deformed individuals. However, the impact of genetic resistance on cumulative mortality was negligible. During the one-year monitoring period, the cumulative mortality rate in the Flavo line was around 1% higher than that for the Fresh line, whereas the Flavo + Ich line showed a mortality rate around 1.5% lower than the Fresh line.

Rainbow trout lines with declared resistance failed to exhibit the expected superior performance consistently compared to non-resistant fish. The results of the study indicate that the genetic resistance of fish to infectious diseases is not universal and may depend on the specific pathogen and strain, as well as the rearing conditions. Therefore, any resistant lines must be tested under the specific conditions of the target aquaculture facility before implementation.

Acknowledgment

The authors would also like to thank Dr. Kevin Roche for proofreading the text.

Conflict of interest

Aquasearch ova ApS, Denmark, is a commercial trout breeding company.

REFERENCES

Abdel-Hafez G, Lahnsteiner F, Mansour N, Licek E. Pathophysiology of Ichthyophthirius multifiliis infection in rainbow trout (oncorhynchus mykiss) and chub (Leuciscus cephalus). J Comp Pathol. 2014 Nov;151(4):394-9.

Baliarda A, Faure D, Urdaci MC. Development and application of a nested PCR to monitor brood stock salmonid ovarian fluid and spleen for detection of the fish pathogen Flavobacterium psychrophilum. J Appl Microbiol. 2002; 92(3):510-6.

Barnes ME, Brown ML. A review of Flavobacterium psychrophilum biology, clinical signs, and bacterial cold water disease prevention and treatment. Open Fish Sci J. 2011;4:40-8.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F. GENETIX 4.05, logiciel sous Windows pour la genetique des populations [GENETIX 4.05, Windows software for population genetics]. Montpellier: Laboratoire Genome, Populations, Interactions; 2004. French.

Bettge K, Segner H, Burki R, Schmidt-Posthaus H, Wahli T. Proliferative kidney disease (PKD) of rainbow trout: Temperature- and time-related changes of Tetracapsuloides bryosalmonae DNA in the kidney. Parasitology. 2009 May;136(6):615-25.

Brookfield JF. A simple new method for estimating null allele frequency from heterozygote deficiency. Mol Ecol. 1996 Jun;5(3):453-5.

Buchmann K, Nielsen T, Mathiessen H, Marana MH, Duan Y, Jorgensen LVG, Zuo S, Karami AM, Kania PW. Validation of two QTL associated with lower Ichthyophthirius multifiliis infection and delayed-time-to-death in rainbow trout. Aquac Rep. 2022;23:101078.

Buchmann K, Sigh J, Nielsen CV, Dalgaard M. Host responses against the fish parasitizing ciliate Ichthyophthirius multifiliis. Vet Parasitol. 2001 Sep 12;100(1-2): 105-16.

Cepeda C, Santos Y. Rapid and low-level toxicity PCR-based method for routine identification of Flavobacterium psychrophilum. Int Microbiol. 2000;3(4):235-8.

Feist SW, Longshaw M. Phylum Myxozoa. In: Woo PTK, editor. Fish diseases and disorders. 2nd ed. Vol. 1: Protozoan and metazoan infections. Wallingford: CABI; 2006. p. 230-96.

Fraslin C, Dechamp N, Bernard M, Krieg F, Hervet C, Guyomard R, Esquerre D, Barbieri J, Kuchly C, Duchaud E, Boudinot P, Rochat T, Bernardet JF, Quillet E. Quantitative trait loci for resistance to Flavobacterium psychrophilum in rainbow trout: Effect of the mode of infection and evidence of epistatic interactions. Genet Sel Evol. 2018 Nov 16;50(1):60.

https://doi.org/10.17221/100/2025-VETMED

Huang HY, Chen YC, Wang PC, Tsai MA, Yeh SC, Liang HJ, Chen SC. Efficacy of a formalin-inactivated vaccine against Streptococcus iniae infection in the farmed grouper Epinephelus coioides by intraperitoneal immunization. Vaccine. 2014 Dec 5;32(51):7014-20.

Jaafar R, Odegard J, Mathiessen H, Karami AM, Marana MH, von Gersdorff Jorgensen L, Zuo S, Nielsen T, Kania PW, Buchmann K. Quantitative trait loci (QTL) associated with resistance of rainbow trout Oncorhynchus mykiss against the parasitic ciliate Ichthyophthirius multifiliis. J Fish Dis. 2020 Dec;43(12):1591-602.

Jousson O, Pretti C, Di Bello D, Cognetti-Varriale AM. Non-invasive detection and quantification of the parasitic ciliate Ichthyophthirius multifiliis by real-time PCR. Dis Aquat Organ. 2005 Jul 18;65(3):251-5.

Liu S, Vallejo RL, Palti Y, Gao G, Marancik DP, Hernandez AG, Wiens GD. Identification of single nucleotide polymorphism markers associated with bacterial cold water disease resistance and spleen size in rainbow trout. Front Genet. 2015 Sep 24;6:298.

Loch TP, Faisal M. Emerging flavobacterial infections in fish: A review. J Adv Res. 2015 May;6(3):283-300.

Macchia V, Inami M, Ramstad A, Grammes F, Reeve A, Moen T, Torgersen JS, Adams A, Desbois AP, Hoare R. Immersion challenge model for Flavobacterium psychrophilum infection of Atlantic salmon (Salmo salar L.) fry. J Fish Dis. 2022 Nov;45(11):1781-8.

Marancik D, Gao G, Paneru B, Ma H, Hernandez AG, Salem M, Yao J, Palti Y, Wiens GD. Whole-body transcriptome of selectively bred, resistant-, control-, and susceptible-line rainbow trout following experimental challenge with Flavobacterium psychrophilum. Front Genet. 2015 Jan 8;5:453.

Marancik DP, Leeds TD, Wiens GD. Histopathologic changes in disease-resistant-line and disease-susceptible-line juvenile rainbow trout experimentally infected with Flavobacterium psychrophilum. J Aquat Anim Health. 2014 Sep;26(3):181-9.

Mathiessen H, Duan Y, Marana MH, Zuo S, Karami AM, Jaafar RM, von Gersdorff Jorgensen L, Kania PW, Dalsgaard I, Madsen L, Nielsen T, Grammes F, Odegard J, Macchia V, Buchmann K. Validation of a QTL for Flavobacterium psychrophilum resistance in rainbow trout (Oncorhynchus mykiss). Aquac Rep. 2023;30:101573.

Matthews RA. Ichthyophthirius multifiliis Fouquet and ichthyophthiriosis in freshwater teleosts. Adv Parasitol. 2005;59:159-241.

Mendel J, Janova K, Palikova M. Genetically influenced resistance to stress and disease in salmonids in relation to present day breeding practice – A short review. Acta Vet Brno. 2018;87(1):35-45.

Palikova M, Papezikova I, Markova Z, Navratil S, Mares J, Mares L, Vojtek L, Hyrsl P, Jelinkova E, Schmidt-Posthaus H. Proliferative kidney disease in rainbow trout (Oncorhynchus mykiss) under intensive breeding conditions: Pathogenesis and haematological and immune parameters. Vet Parasitol. 2017 Apr 30;238:5-16.

Papezikova I, Mares J, Vojtek L, Hyrsl P, Markova Z, Simkova A, Bartonkova J, Navratil S, Palikova M. Seasonal changes in immune parameters of rainbow trout (Oncorhynchus mykiss), brook trout (Salvelinus fontinalis) and brook trout x Arctic charr hybrids (Salvelinus fontinalis x Salvelinus alpinus alpinus). Fish Shellfish Immunol. 2016 Oct;57:400-5.

Pouil S, Rigaudeau D, Lee BH, Segret E, Desgranges A, D'Ambrosio J, Francois Y, Boudinot P, Duchaud E, Phocas F, Rochat T. Remarkable heterogeneity revealed in the genetic architecture of resistance to a key bacterial pathogen in two commercial rainbow trout populations. BMC Genomics. 2025 Nov 28;27(1):49.

Robinson NA, Robledo D, Sveen L, Daniels RR, Krasnov A, Coates A, Jin YH, Barrett LT, Lillehammer M, Kettunen AH, Phillips BL, Dempster T, Doeschl-Wilson A, Samsing F, Difford G, Salisbury S, Gjerde B, Haugen JE, Burgerhout E, Dagnachew BS, Kurian D, Fast MD, Rye M, Salazar M, Bron JE, Monaghan SJ, Jacq C, Birkett M, Browman HI, Skiftesvik AB, Fields DM, Selander E, Bui S, Sonesson A, Skugor S, Ostbye TK, Houston RD. Applying genetic technologies to combat infectious diseases in aquaculture. Rev Aquac. 2023 Mar;15(2):491-535.

Siwicki AK, Anderson DP, Rumsey GL. Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. Vet Immunol Immunopathol. 1994 May;41(1-2):125-39.

Soliman AM, Barreda DR. The acute inflammatory response of teleost fish. Dev Comp Immunol. 2023 Sep;146:104731.

Svobodova Z, Pravda D, Modra H. Metody hematologickeho vysetrovani ryb [Methods of haematological examination of fish]. Edice metodik, vol. 122. Vodnany: Research Institute of Fish Culture and Hydrobiology; 2012. 38 p. Czech.

Swennes AG, Noe JG, Findly RC, Dickerson HW. Differences in virulence between two serotypes of Ichthyophthirius multifiliis. Dis Aquat Organ. 2006 Apr 6;69(2-3):227-32.

Vaibarova V, Kralova S, Palikova M, Schwarzerova J, Nejezchlebova J, Cejkova D, Cizek A. Genetic and phenotypic diversity of Flavobacterium psychrophilum isolates from Czech salmonid fish farms. BMC Microbiol. 2024 Sep 18;24(1):352.

https://doi.org/10.17221/100/2025-VETMED

Wiklund T, Madsen L, Bruun MS, Dalsgaard I. Detection of Flavobacterium psychrophilum from fish tissue and water samples by PCR amplification. J Appl Microbiol. 2000 Feb;88(2):299-307.

Witeska M, Kondera E, Lugowska K. The effects of ichthyophthiriasis on some haematological parameters in common carp. Turk J Vet Anim Sci. 2010;34(3):267-71.

Received: November 28, 2025
Accepted: April 30, 2026
Published online: August 23, 2026