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Rearing background and exposure environment together explain higher survival of aquaculture fish during a bacterial outbreak

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ABSTRACT

1. Parasitic diseases represent one of the greatest challenges for aquaculture worldwide and there is an increasing emphasis on ecological solutions to prevent infections. One proposed solution is enriched rearing, where traditional stimulus-poor rearing tanks are equipped with different types of structures to increase habitat complexity. Such spatial enrichment is

known to increase survival of fish during parasite epidemics, but the underlying mechanisms are still unclear.

2. We studied whether enriched rearing affected infection of an important fish pathogen *Flavobacterium columnare* in young Atlantic salmon (*Salmo salar*) and sea-migrating brown trout (*Salmo trutta*). First, we used natural bacterial exposures and multiple fish populations in a common garden experiment to address the role of host genetic background in effects of enriched rearing. Second, fish from standard and enriched rearing were experimentally exposed to controlled bacterial doses in standard and enriched environments in a full factorial design to explore the relative roles of rearing background and environment of exposure on survival of fish.

3. Enriched rearing significantly increased survival of fish during the natural bacterial outbreak. This effect was also fairly consistent and observed in eight of the ten fish populations. In the controlled exposure, fish exposed in enriched environment had higher survival regardless of their rearing background, suggesting a stronger impact of the environment on the disease progression. Additionally, the survival in the enriched environment was highest among the fish of enriched rearing background, supporting the idea of their higher resistance.

4. *Synthesis and applications.* Our result suggests that the enhanced survival of fish in enriched rearing results from a combined effect of the environment and improved fish condition, and to a lesser degree from host genetic background. This has important implications for when and how environmental enrichment should be applied. Overall, these results indicate that environmental enrichment has the potential to improve survival of fish during parasitic epidemics and thus reduce use of antibiotics in aquaculture.

Keywords: Antibiotics, Atlantic salmon, Aquaculture, Brown trout, Disease epidemiology, Enriched rearing, *Flavobacterium columnare*, Genetic variation

INTRODUCTION

Parasites and parasitic diseases can significantly impact natural host populations, best illustrated by devastating outcomes of diseases invading novel host populations (e.g. van Riper, van Riper, Goff & Laird 1986). While natural disease outbreaks are often less severe and many more pass undetected, severe epidemics are common in production environments (e.g. Scholz, 1999; Calnek 2001; Gilchrist et al., 2006; Pulkkinen et al., 2010). This is primarily because of elevated pathogen transmission rates in high densities of susceptible hosts. For example, causative agents of the Marek's disease (a herpesvirus; Calnek, 2001), salmonellosis (different species of the bacterium *Salmonella*; Boyen et al., 2008), African swine fever (a DNA virus; Costard et al., 2009) and the columnaris disease (the bacterium *Flavobacterium columnare*; Declercq, Haesebrouck, Van den Broeck, Bossier, & Decostere, 2013), to mention a few, are commonly found infecting production animals in agriculture and aquaculture. Consequently, infections reduce economic profitability of the industry by impairing growth, and increasing morbidity and mortality of hosts. Infections are prevented and treated with chemicals, antibiotics and vaccinations (Brown & Gratzek, 1980; McEven & Fedorka-Cray, 2002), which is expensive and can result in secondary problems such as increased pathogen resistance to drugs (Levy & Marshall, 2004; Martinez, 2009; Durso & Cook 2014) and spill-over of chemical residues into the environment (Guardabassi,

Dalsgaard & Raffatellu, 2000; Sorum, 2006; Martinez, 2009). Thus, ecologically sustainable methods for preventing diseases in production environments are constantly being explored.

An outbreak of a parasitic disease is typically a consequence of interactions between the pathogen, the host and the environment (Schmid-Hempel, 2011). Often, both host resistance and parasite infectivity show significant genetic variation, and specific genotype by genotype ($G_{Host} \times G_{Parasite}$) interactions may determine the overall infection success and virulence (e.g. Carius, Little & Ebert 2001; Grech, Watt & Read, 2006). However, such interactions can also be shaped by the environment ($G_{Host} \times G_{Parasite} \times E_{nvironment}$), which could be an important component maintaining genetic polymorphism in host and parasite populations (Lazzaro & Little, 2009). Indeed, there is a growing body of literature supporting the role of environmental factors in shaping host and parasite performance (Laine, 2008; Seppälä & Jokela, 2010; Bryner & Rigling, 2011; Van den Wyngaert, Vanholsbeeck, Spaak & Ibelings, 2014), which is fundamentally important in determining the outcome of an infection. Such interactions are important also from an applied perspective, as specific interactions between hosts, parasites and their environment not only determine the severity of a disease in production environments, but also the magnitude of preventative measures necessary for eradication of an infection. However, these interactions have received relatively little attention in intensive farming environments.

Recent evidence suggests that increasing the variability of a standard stimulus-poor rearing environment to resemble more natural environment (referred to as enriched rearing) can affect many traits of animal physiology and behavior, as well as improve disease resistance (De Jonge, Bokkers, Schouten, & Helmond, 1995; Jones, McAdie, McCorquodale & Keeling, 2002; Bolhuis, Parmentier, Schouten, Schrama, & Wiegant, 2003; Bolhuis

Schouten, de Leeuw, Schrama, & Wiegant, 2004). For example in piglets, enriched rearing has been shown to reduce aggression (De Jonge et al., 1995). Studies on poultry also suggest that feather pecking behavior can be reduced by adding enrichments into the rearing environment (Jones et al., 2002). With fish, increasing heterogeneity of rearing tanks with different types of structures has been shown to enhance foraging, exploratory and learning abilities (Strand et al., 2010; Rodewald, Hyvärinen & Hirvonen, 2011; Makino, Masuda & Tanaka, 2015), as well as reduce stress and improve recovery from stress (Näslund et al., 2013). Moreover, use of enriched rearing techniques can facilitate shelter-seeking and anti-predatory behaviors of fish, as well as survival of stocked hatchery-reared fish in the wild (Salvanes & Braithwaite, 2005; Hyvärinen & Rodewald, 2013).

Recent evidence also suggests that enriched rearing can enhance survival of aquaculture fish under parasite infection (Karvonen et al., 2016). More specifically, these results indicate that simple structures within a rearing tank can significantly improve fish survival in some host-parasite combinations, with the effects being less clear in others. However, how the genetic background of the host associates with responses to enriched rearing, and what are the mechanisms underlying the higher fish survival in these environments are still largely unknown. For example, host genotype by environment ($G_{Host}\times E_{nvironment}$) interactions could reveal to what extent the variation in the effects of rearing environment is explained by host genetic background. This could have important implications for the evolution of diseases in captive host populations, but also for specific rearing practices and disease-treatment protocols. Second, mechanistically, the higher survival of fish in enriched rearing could result from enhanced resistance of fish or from lower rate of establishment and spread of a disease within the enriched tanks. Separating

between the relative importance of host resistance and disease progression is essential when considering when and how environmental enrichment should be applied.

The goal of this study was to investigate how host genetic background and rearing/exposure environment shapes fish survival during columnaris disease epidemics, caused by the fish pathogen *Flavobacterium columnare*. This bacterium is currently considered one of the most important fish pathogens in aquaculture worldwide (Shoemaker, Olivares-Fuster, Arias, & Klesius, 2008; Declercq, et al., 2013). Columnaris disease causes epidermal lesions on fish (Suomalainen, Kunttu, Valtonen, Hirvelä-Koski, & Tiirola, 2006), and leads to high mortality and economic losses if not treated with antibiotics (Pulkkinen et al., 2010; Declercq et al., 2013). We used both semi-natural and experimental bacterial exposures on a number of populations of Atlantic salmon (*Salmo salar*) and sea-migrating brown trout (*Salmo trutta*) raised in standard and enriched conditions in moderate production-scale densities. Both species are important aquaculture salmonids for commercial and preservation purposes, as well as in recreational fisheries (Almodóvar & Nicola, 2004; ICES, 2007). First, we compared disease dynamics among the fish species and populations in different rearing conditions to tackle the effect of host genetic background in responses to the rearing environment. The fish were naturally exposed to the bacterium via incoming water in their home tanks in a common garden experimental setup. Second, we experimentally exposed two of the brown trout populations with standard and enriched rearing background to *F. columnare* in standard and enriched conditions in a full factorial design. Here, the aim was to compare the relative importance of the rearing background and the environment of exposure to the disease for the survival of fish. Our results suggest that the exposure environment has a stronger effect on the disease-derived mortality

irrespective of the host genetic background, while mortality is influenced to some extent also by host rearing background and resistance.

MATERIALS AND METHODS

Two experiments were designed to study the effects of enriched rearing and exposure environment on disease dynamics in different populations of Atlantic salmon and sea-migrating brown trout (hereafter 'salmon' and 'trout'). The first experiment included a semi-natural exposure of fish to the bacterium *Flavobacterium columnare* in their rearing tanks. The second experiment consisted of controlled exposures of fish to known doses of the bacterium. The experiments were conducted in Kainuu Fisheries Research Station (KFRS) of Natural Resources Institute Finland (Luke) at Paltamo (www.kfrs.fi, 64.404°N, 27.516°E) in 2016–2017. The station is a flow-through facility, taking water from a nearby lake Kivesjärvi (from the depth of 7 m). Water temperature in the facility follows the natural temperature of the lake and all rearing units receive similar water supply. Therefore, the physical properties and parasite exposure in the incoming water is similar for all rearing units throughout the year.

Experiment 1: Semi-natural exposure

In the first experiment, five trout and five salmon populations were used. The populations originate initially from different, geographically isolated rivers (minimum distance = 32 km, average = 493.1 km; Table S1) and are genetically differentiated (Koljonen, Tähtinen, Säisä &

Koskiniemi, 2002; Koljonen, Gross & Koskiniemi, 2014). The number of parent fish to produce the offspring varied between 40 and 189 per population and the breeding design used crosses of one to five males with one to five females. The fish were brough in to the facility at eye-egg stage and were divided in 3.2 m^2 tanks, 1250 fish in each. There were two standard and two enriched tanks for each population, totalling 40 tanks. Treatments with different fish populations and rearing methods were randomized across the tanks. The enrichment followed the procedure described in Karvonen et al. (2016), but excluding fluctuations in water level and in the amount of incoming water. Enrichments included gravel and shelter structures, as well as changes in water flow direction, and they were scaled according to the initial number of fish in the tanks (1250) and subsequently modified as the fish grew larger during the experiment (Fig. S1, Table S2). No fish were added to the tanks during the experiment. Fish in standard tanks were raised without enrichments, but the water level was raised similarly to the enriched tanks as the fish grew larger. All fish were fed with automated feeders and commercial fish feed (Veronesi VITA 0.2/0.5 and Inicio Plus G 0.4). All the tanks were cleaned daily and the number of dead fish was recorded.

A natural *F. columnare* outbreak was observed in the tanks in 17-31 August 2016 (15 days). Exposure was considered equal for all the tanks because of the common water source. Dead fish were counted and removed from the tanks daily, compiling a daily mortality data for each tank. On day 10 of the epidemic, an antibiotic course (Orimycine mixed in fish feed) was initiated in the tanks to eradicate the disease. Water temperature was 14.3-16.6 °C during the epidemic.

Experiment 2: Experimental infection

Based on their high susceptibility to the disease in the first experiment, two trout populations, Isojoki and Lestijoki, were selected for the second experiment in 2017. The number of parent fish to produce the offspring varied between 173 and 306 per population and the breeding design used crosses of one male to one female. The fish were reared in the two rearing conditions, standard and enriched, as described above (Table S3), each population-rearing combination with three replicate rearing tanks. Fish density was initially 1950 fish per tank, corresponding to moderate production scale density similarly to the first experiment. In July 23, fish (mean length ± SE = 43.7 ± 0.06 mm) from both populations and rearing treatments were transferred to 40 tanks (0.4 m^2) for the bacterial exposure, each tank with 60 l of water and 100 fish. Half of the exposure tanks were enriched with small gravel (grain size 40–60 mm) that had not been in contact with lake water prior to the experiment, while the other half remained standard without gravel (Fig. 1). Fish from each population were divided so that they were exposed either in environment resembling their original rearing environment (i.e. standard-standard and enriched-enriched) or in a novel environment (i.e. standard-enriched and enriched-standard), totalling eight different combinations. Each combination had five replicates, four of which received bacterial exposure while one tank served as an unexposed control. The 100 fish for each exposure tank were combined evenly from the three replicate rearing tanks. The exposure started in July 24 when 200 ml of fresh, overnight-grown bacterial culture was added into each tank to reach infection dose of 4.5×10^6 colony forming units per ml in each tank. In control tanks, 200 ml of Shieh broth without bacteria was added. The fish were exposed to the bacteria in

40 l of water for 1 hour, after which the incoming water was turned on and the water level raised back to 60 l.

Flavobacterium columnare strain B351 used in the experimental exposures was originally isolated from outlet water of a fish farm (Sundberg et al., 2016), and has been stored as pure culture frozen in -80 °C in a stock containing 10 % glycerol and 10 % fetal calf serum. The strain has been used in several previous studies and shown to be virulent (e.g. Sundberg et al., 2016; Sundberg & Karvonen, 2018). Before the experiment, the strain was revived overnight, inoculated in modified Shieh broth (Song et al., 1988) and grown at 25.0 °C with constant agitation (100 rpm). After 23 h, the bacterial culture was enriched and overnight-grown culture was used for the exposure.

The condition of the fish was monitored every six hours for the first 40 hours of the experiment and every four hours afterwards, and fatally moribund fish suffering from signs of *F. columnare* infection (bleaching of the dorsal skin, loss of normal swimming buoyancy and mobility) were removed from the experiment, euthanized and measured for length (mm). All fish surviving the experiment (including control fish) were euthanized in benzocaine solution in the end of the experiment at 140 h post-exposure and measured for length. To verify *F. columnare* infection, bacterial samples were taken from the first five moribund fish per tank and additional five samples per tank from the fish that survived the experiment. Bacterial cultures were taken on Shieh agar plates supplemented with tobramycin (1 µg ml^{-1}, Decostere, Haesebrouck, & Devriese, 1997). After incubation of 48 h in room temperature, appearance of yellow colonies with rhizoid morphology typical for *F. columnare* indicated positive bacterial infection. The experiments were performed with

permission from Regional State Administrative Agency of Southern Finland (ESAVI/5183/04.10.07/2017).

Statistical analyses

Survival differences between the fish species, populations and rearing methods were analyzed with generalized linear mixed models (GLMM with binomial probability distribution). In Experiment 1, survival differences were first compared between the fish species using a model with rearing method and fish species as fixed factors to explore their interactions and tank nested under rearing method × fish species interaction as a random factor to account for possible tank effects. When comparing survival differences between the populations, data were split between the fish species and the model used rearing method and fish population as fixed factors and rearing tank as random factor as described above. In Experiment 2, the model included rearing environment (standard/enriched), exposure environment (standard/enriched) and fish population as fixed factors. Exposure tank was used as a random factor as described above and length of individual fish as a covariate. Pairwise comparisons on the effects of individual factors used the same approach. All analyses were performed in R 3.3.2.

RESULTS

Experiment 1: semi-natural bacterial exposure

In this experiment, replicated salmon and trout populations were kept in standard and enriched rearing tanks, and monitored for survival during a naturally occurring *F. columnare* infection. Enriched rearing significantly improved the survival of fish during the *F. columnare* outbreak. Although there was high variation in the overall level of mortality between the fish populations within each fish species (Fig. 2, Table 1), the positive effect of enriched rearing was observed in eight out of ten populations, suggesting consistency in this pattern. In trout, populations Iso, Lesti and Inga showed higher mortality compared to Ii and Musta. Great majority of fish mortalities (96.6 %) in these three populations occurred in standard tanks, whereas mortality in enriched tanks was very low. This resulted in significant interaction in the GLMM between rearing treatment and trout population (Fig. 2, Table 1). In salmon, there was no such interaction and mortality was lower in enriched rearing in all populations. However, the overall level of mortality did not differ statistically among the salmon populations, or between the fish species, presumably because of the high variation among populations and individual tanks. Number of fish in the rearing tanks differed between the species but not between the rearing treatments prior to infection (ANOVA: $F_{1, 36} = 7.705$, $p = 0.009$ (species); $F_{1, 36} = 1.421$, $p = 0.241$ (treatment); $F_{1, 36} = 0.473$, $p = 0.496$ (species×treatment)).

Experiment 2: Experimental exposure

In this experiment, two trout populations, originating either from standard or enriched rearing tanks, were exposed to *F. columnare* in standard and enriched experimental tanks (Fig. 1) in a full factorial design. There was a significant interaction effect on survival of fish between the rearing background and the exposure environment (Fig. 3, Table 2). Pairwise comparisons individually for the effects of rearing and exposure environments indicated that survival of fish was higher in enriched exposure environment regardless of the rearing background (χ^2= 22.750, $p < 0.001$; Fig 3.), whereas the effect of the rearing background was not significant (χ^2= 0.298, $p = 0.585$; fish populations combined). Further pairwise comparisons indicated that fish from standard rearing had higher survival when exposed in enriched conditions compared to standard conditions (χ^2= 3.893, $p = 0.049$), whereas fish from enriched rearing had lower survival when exposed in standard conditions compared to enriched conditions (χ^2= 28.640, $p < 0.001$). Effect of fish length was significant essentially because smaller fish had higher probability of dying during the experiment.

Fish from enriched rearing also had higher survival than fish from standard rearing when these groups were exposed in enriched conditions (χ^2= 7.367, $p < 0.01$; rearing treatment), but this depended on the population (χ^2= 5.384, $p = 0.020$; population*rearing). The significant interaction term was due to unexpected decrease in survival of enriched Lestijoki fish in enriched exposure conditions during the last 12h of the experiment (Fig. 3). Overall, these results indicate that although fish from both standard and enriched rearing had higher survival in enriched exposure environment, the latter group still survived better, suggesting a combinatory effect of enriched rearing and exposure environment on survival. All exposed tanks were positive for *F. columnare*.

Among the controls, fish mortality was low and detected in five out of eight tanks, with an average overall mortality of 2.63 ± 1.39 % (SE). All bacterial samples taken from the controls were negative, indicating that these fish died of other causes. Treatment with the lowest mortality (enriched-enriched) did not differ from the control tanks in the Isojoki population (Mann-Whitney U = 14.5, p = 0.056), but was different in the Lestijoki population (U = 16.0, p = 0.029).

DISCUSSION

Infectious diseases represent one of the greatest challenges for aquaculture development by inhibiting growth, and increasing morbidity and mortality of cultured animals (Murray & Peeler 2005; Lafferty et al., 2015). This results in reduced ecological sustainability and economic profitability of the industry. While it is generally well established that specific host-pathogen interactions can affect the outcome of infection (Carius et al., 2001; Grech et al., 2006; Wolinska & King, 2009), the role of environmental factors in shaping disease dynamics in production units is still poorly understood. Here, we explored the effect of enriched rearing environment (Hyvärinen & Rodewald, 2013; Karvonen et al., 2016) on epidemiology of an aquaculture disease. First, we used different host genetic backgrounds (fish species and populations) raised in standard and enriched environments to investigate the magnitude of variation in host responses to a semi-natural exposure to the bacterium *F. columnare*. Second, we experimentally exposed fish from the different rearing backgrounds to *F. columnare* in standard and enriched environments to investigate the relative importance of the rearing background and the exposure environment for the survival of fish. We found that enriched rearing significantly increased the survival of fish during bacterial epidemic and this pattern was fairly consistent among

the fish populations. The controlled bacterial exposure indicated that survival was highest in the enriched exposure environment independently of the rearing background, suggesting stronger influence of the exposure environment (Fig. 3). However, fish with an enriched rearing background nevertheless showed the highest survival in the enriched exposure environment, which supports the idea that enriched rearing also improved their resistance. These results indicate that introduction of simple spatial variation in the tanks with natural materials (e.g. gravel) has a significant role in attenuating disease progression and associated mortality in different fish species and populations.

During the natural infection, enriched rearing had highly significant effect on disease epidemiology and associated mortality. For example, the three most susceptible trout populations showed, on average, 37 times higher mortality in standard rearing compared to enriched tanks. The effect of rearing environment was also somewhat consistent across the fish species and populations with the positive effect of enrichment detected in eight out of ten salmonid populations. These results suggest that fairly simple structures in a rearing tank can dramatically change epidemiology of a disease (see also Karvonen et al., 2016). Such a strong effect is surprising particularly in case of an opportunistic pathogen like *F. columnare* that can effectively transmit between fish (Welker, Shoemaker, Arias & Klesius, 2005), which suggests effective inhibitory mechanisms of bacterial colonization or transmission operating within the enriched tanks (see below). Two trout populations with the lowest overall mortalities, however, showed no difference between the treatments. This suggests that there might be a threshold in disease exposure or severity above which the positive effect of enriched rearing becomes evident. However, data from salmon do not support this suggestion, although it is possible that the onset phase of the disease differs

between these two fish species. Indeed, salmon mortality was very low compared to trout. Yet, we found no or only weak signatures of fish species or population affecting the disease-related mortality in both experiments, which may reflect generalist nature of the bacterium or low level of variation in resistance to infection among the hosts. It is also possible that these results are partly influenced by the characteristics of the bacterial strain, although differences in performance of *F. columnare* strains in different host backgrounds is currently unknown.

Mechanisms underlying the positive effects of enriched rearing are generally poorly known (Karvonen et al., 2016). For example, studies reporting lower disease-related mortality of fish under enriched conditions (Becker, Kirkland, Heath, Heath & Dixon, 2014; Karvonen et al., 2016), have not investigated detailed role of epidemiological or fish condition-related factors in disease progression. Our results from the experimental exposure suggest that the higher survival was partly owing to resistance-related factors of fish, but for the larger part to the properties of the exposure environment itself. This is intriguing as it suggests that simple physical characteristics of the enrichment (in this case gravel, note that shelters were not used in the exposure tanks) can significantly reduce disease progression. There are several potential non-exclusive mechanisms to explain this. First, additional structures could allow development of beneficial microbiome (Dehler, Secombes & Martin, 2017; Webster, Consuegra, Hitchings & de Leaniz 2018), which could inhibit *F. columnare*. Second, gravel can provide shelter and maintain some distance between fish individuals compared to standard tanks (Fig. 1), which could potentially influence disease epidemiology despite all exposed fish presumably were positive for the bacterium (Suomalainen, Tiirola & Valtonen, 2005). Interestingly, these potential

mechanisms could have been different between the experiments. For example, the rearing tanks could have housed well-established microbiomes on the gravel (gravel in the exposure tanks had not been in contact with lake water prior to the experiment), while their higher fish density compared to exposure tanks allowed only a small proportion of individuals occupying shelters at a time, thus increasing direct contacts between the fish elsewhere in the tank. It is clear that these aspects need further experimental work and detailed microbiological analyses of the rearing environments.

We also found evidence of improved resistance of enriched fish to the bacterium compared to standard fish in enriched exposure conditions. This may be related to physiological resistance operating through the immune system, or to lower stress levels which can directly or indirectly influence many physiological traits of fish (Bonga, 1997), including the immune system (Yin, Lam & Sin, 1995; Ortuño & Esteban, 2001). For example, stress-induced elevated ventilation rate (Mikheev, Pasternak, Valtonen & Taskinen, 2014) could have enhanced bacterial establishment through gills (Suomalainen et al., 2006) among the standard fish. The effect of stress is also supported by the higher mortality of enriched fish exposed in standard tanks. This result was similar in both trout populations, which strongly suggests that fish originating from enriched environment suffer more from change of environment. The result is also somewhat consistent with our previous results showing faster progression of *F. columnare* infection among enriched fish soon after transfer (Karvonen et al., 2016). While in that study the receiving tank was also enriched (different types and sizes of enrichments), disease dynamics may act unpredictably depending on the specific interactions between the fish, pathogen and the environment.

Flavobacterium columnare is currently considered one of the most harmful pathogens in aquaculture and is commonly treated with antibiotics (Shoemaker et al., 2008; Peatman et al., 2013), which has in many cases resulted in emergence of antibiotic resistant flavobacterium strains (Akinbowale, Peng & Barton, 2006; Hesami et al., 2010). We showed that enriched environment significantly reduced mortality associated with *F. columnare*, both directly and indirectly through fish resistance. These are promising leads for further studies exploring what types of enrichments are most effective against pathogens, and when and how these should be applied during a course of an epidemic. For example, results from our second experiment (standard fish in enriched exposure environment) suggest that even a short-term application of enrichments could significantly reduce disease progression. On the other hand, fish from enriched rearing could also suffer from transport to standard conditions, which should be taken into consideration when designing the enrichment protocols. Overall, reduction in disease-related losses through enriched rearing could decrease the need and use of antibiotics, and consequently increase the sustainability of aquaculture. Enriched rearing could also benefit supportive stocking of fish as it enhances learning abilities, predator avoidance, and development of resistance against parasites and diseases; traits that are important in the wild. Thus, we strongly recommend aquaculture companies and operatives to undertake trials of enriched rearing.

AUTHORS' CONTRIBUTIONS

All authors conceived the ideas, designed methodology and collected the data; V.R. and A.K. analyzed the data; and V.R. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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DATA ACCESSIBILITY

Data available via the Dryad Digital Repository doi: 10.5061/dryad.nd637r5 (Räihä et al. 2019).

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Table 1. Results of GLMM analyses on survival differences between species, populations and rearing treatments of salmon and trout during *F. columnare* epidemic in 17-31 August 2016 in Experiment 1. Models used species, population and rearing treatment as fixed factors, and rearing tank as a random factor (see Table S4 for parameter estimates).

Species	Source	χ^2	df	P
Salmon & Trout	Species (S)	0.97	1	0.323
	Rearing method (R)	5.6	1	0.179
	S×R	2.46	1	0.117
Salmon	Population (P)	3.47	4	0.483
	Rearing method (R)	0.74	1	0.008
	P×R	1.21	4	0.875
Trout	Population (P)	13.86	4	0.008
	Rearing method (R)	0.74	1	0.39
	P×R	55.48	4	< 0.001

Table 2. Result of GLMM analysis on survival differences between different population-rearing method-exposure environment combinations of trout exposed to *F. columnare* in Experiment 2. Trout population, rearing method and exposure environment were used as fixed factors, exposure tank as a random factor, and fish length as a covariate (see Table S5 for parameter estimates).

Source	χ^2	df	P
Population (P)	0.038	1	0.84
Rearing (R)	2.12	1	0.15
Exposure (E)	3.66	1	0.056
Length	18.23	1	<0.001
P×R	0.303	1	0.56
P×E	0.2	1	0.65
R×E	8.9	1	0.003
P×R×E	1.68	1	0.2

FIGURE CAPTIONS

Figure 1. Standard (left panel) and enriched (right panel) exposure tanks in the second experiment. Gravel was added in the enriched tanks to increase spatial heterogeneity in resemblance to the rearing tanks.

Figure 2. Average mortality (% ± SE) among the different trout (top panel) and salmon (bottom panel) populations raised in standard and enriched rearing during *Flavobacterium columnare* outbreak in August 17-31 2016. Each bar indicates average from two replicate tanks. Note different y-axis scales in different panels.

Figure 3. Survival curves of two populations (Isojoki and Lestijoki) of trout (*Salmo trutta*) from different rearing background-exposure environment combinations (e.g. enriched-enriched refers to fish reared and exposed in enriched environment) exposed to *Flavobacterium columnare* in the second experiment in July 2017.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Distances between the rivers of origin for the fish populations used in the study.

Tables S2 & S3. Details of rearing conditions.

Tables S4 & S5. Parameter estimates for the GLMM tests.

Fig. S1. Enriched rearing tank.