Enriched rearing environment and wild genetic background can enhance survival and disease resistance of salmonid fishes during parasite epidemics

Anssi Karvonen\textsuperscript{1*}, Mariella Aalto-Araneda\textsuperscript{2}, Anna-Maija Virtala\textsuperscript{2}, Raine Kortet\textsuperscript{3}, Perttu Koski\textsuperscript{4} and Pekka Hyvärinen\textsuperscript{5}

\textsuperscript{1}Department of Biological and Environmental Science, University of Jyväskylä, PO Box 35, FI-40014 Jyväskylä, Finland; \textsuperscript{2}Faculty of Veterinary Medicine, University of Helsinki, PO Box 66, FI-00014 Helsinki, Finland; \textsuperscript{3}Department of Biology, University of Eastern Finland, PO Box 111, FI-80101 Joensuu, Finland; \textsuperscript{4}Finnish Food Safety Authority Evira, Elektronikatie 3, 90590 Oulu, Finland; and \textsuperscript{5}Natural Resources Institute Finland (Luke), Natural Resources and Bioproduction, Manamansalonkatu 90, 88300 Paltamo, Finland

Summary
1. The importance and volume of aquaculture is increasing world-wide. Rearing practices play a key role in determining growth rate, survival and disease resistance in aquaculture fishes. Recent evidence suggests that in comparison with a standard stimulus-poor rearing environment, an enriched or variable rearing environment has significant positive effects on several traits underlying growth and well-being of fish. However, the effect of enriched rearing on one of the most important threats for aquaculture development, occurrence of parasitic infections, remains unknown.

2. We used surveillance data of experimental salmonid populations of wild and hatchery origin under semi-natural parasite exposure to explore effects of enriched rearing on outbreaks of important aquaculture pathogens and associated fish mortalities in production-scale fish densities. We also conducted controlled parasite exposures to investigate if enriched rearing reduces susceptibility of fish to infection in comparison with standard rearing conditions.

3. We found evidence of enriched rearing influencing survival and disease resistance of aquaculture fish during parasite epidemics. Essentially, populations from enriched rearing had a higher survival rate, lower parasite occurrence and greater resistance to most infections compared to fish held in standard rearing conditions. Similarly, fish of wild genetic background had lower mortality during some of the epidemics compared to fish of hatchery origin. However, we also demonstrate significant variation in these patterns and in some cases a tendency for opposite effects of enriched rearing and genetic background depending on the fish species and nature of the epidemic.

4. Synthesis and applications. Our results suggest that parasitic infections and epidemics can be managed through enriched rearing conditions. This may have important implications for economically and ecologically sustainable parasite and disease prevention strategies in aquaculture.

Key-words: aquaculture, Atlantic salmon, disease prevention, domestication, epidemiology, parasite, rearing methods, \textit{Salmo salar}, virulence

Introduction
The importance and volume of aquaculture continue to grow world-wide (FAO 2005-2015). Food production is becoming increasingly dependent on farmed fish due to the high demand of fish products and depletion of wild fish stocks in marine and freshwater ecosystems. Aquaculture is also important for maintaining endangered fish populations. In particular, many salmonid fishes are highly endangered throughout the world and regularly maintained through hatchery-based breeding programmes.
and stocking of hatchery-raised fish (Brown & Day 2002). One major threat to aquaculture is the vulnerability of intensive farming units to parasitic infections. Aquaculture facilities typically provide favourable conditions for disease spread (Valtonen & Koskivaara 1994; Buchmann & Bresciani 1997; Pulkkinen et al. 2010) as disease-causing agents thrive in high host densities that facilitate parasite transmission and persistence. Infections often result in morbidity and mortality that cause economic losses (e.g. Wagner et al. 2002; Pulkkinen et al. 2010). Also, treating infections with drugs and chemicals is expensive and may result in environmental pollution and development of drug resistance (Cabello 2006; Martinez 2009). Thus, finding economically and ecologically sustainable methods to control pathogens in aquaculture is essential.

Rearing methods and conditions play a key role in determining growth rate and physical condition of farmed fish (Pickering 1993; Conte 2004; Huntingford et al. 2006). Factors such as poor water quality and inadequate fish density can markedly influence welfare of fish (Huntingford et al. 2006). Tanks typically used in large-scale fish production provide simplified, low-stimulus environments with steady conditions of water supply. Recent studies have shown, however, that simple enrichment of the rearing environment, such as adding physical structures into the tanks, decreases the metabolic rate and stress levels in fish (Millidine, Armstrong & Metcalfe 2006; Näsland et al. 2013) and enhances necessary survival skills (Braithwaite & Salvanes 2005), foraging abilities (Rodewald, Hyvärinen & Hirvonen 2011) and survival of fish after introduction into the wild (Hyvärinen & Rodewald 2013). While some neutral or negative effects of enriched rearing have been reported (Brockmark et al. 2007; Brockmark, Adriaenssens & Johnsson 2010), the current evidence strongly highlights positive effects of environmental enrichment on fish physiology and behaviour. If enriched rearing had the potential to improve physical condition and disease resistance of fish, for instance through improved immune function or lowered stress levels, or decrease the level of exposure to infective stages of parasites, it could reduce the incidence of parasitic infections in aquaculture. Thus, enriched rearing could also reduce the costs related to parasitic infections in fish production. Alternatively, in some cases enrichment might also increase the probability of infection, for instance if additional physical structures in the tanks acted as surfaces for parasite growth and multiplication. Currently, however, there are no published data on the effects of enriched rearing on occurrence, infectivity and virulence of parasitic diseases in production-scale aquaculture.

Physical factors of the rearing environment, such as water temperature, host density and water current dynamics, are known to affect disease transmission in aquaculture (Valtonen & Koskivaara 1994; Bodenstein et al. 2000; Suomalainen, Tiirila & Valtonen 2005; Karvonen et al. 2010). However, less attention has been given to genetic factors potentially affecting disease resistance of the host. Captive-bred fish are commonly less fit compared to offspring of wild parents because of domestication that leads to gradual loss of genetically controlled characteristics essential for reproduction in the wild (Araki, Cooper & Blouin 2007). This process can be rapid and take place within just two generations (Araki, Cooper & Blouin 2007), and poses a problem for stocking and restoration programmes that aim to maintain and supplement endangered fish populations. If domestication also alters host susceptibility to infections, disease dynamics can change, which may influence the survival of fish during hatchery-rearing as well as after their release into the wild. This could also have important interactions with enrichment of the rearing environment, for instance if domesticated fish were less capable of responding to effects of enrichment during parasite epidemics.

In this study, we explored the occurrence of disease outbreaks, associated mortalities and parasite resistance of salmonid fishes reared in standard and enriched aquaculture conditions. In a semi-natural infection design, we followed cohorts of Atlantic salmon of two different genetic origins, hatchery-reared and wild, in standard and enriched rearing conditions for 2 years and recorded mortality of fish during outbreaks of different infectious diseases. To simulate environmental variation in the wild, we applied an enriched rearing protocol to production-scale fish densities by combining structural enrichment (increased complexity and shelters) in the tanks with irregular changes in water current dynamics (i.e. water level, velocity and direction) (Hyvärinen & Rodewald 2013). Secondly, we experimentally exposed landlocked Atlantic salmon and brown trout to controlled doses of a macroparasite to study the effect of enriched rearing on host susceptibility while keeping the level of exposure constant. We demonstrate significant effects of enriched rearing on mortality and resistance of fish with infections of most disease-causing agents being less-virulent and prevalent among the fish in enriched rearing. We also show considerable variation in the disease patterns depending on the genetic background and species of fish, and nature of the disease outbreak.

**Materials and methods**

The study was conducted in the Kainuu Fisheries Research Station (KFRS) (www.kfrs.fi, 64.404428° N 27.5169603 E) of the Finnish Game and Fisheries Research Institute (2015 onwards Natural Resources Institute Finland (Luke)) in 2009–2013. Two experiments were designed to test the effect of enriched vs. standard rearing on survival and parasite resistance of salmonid fishes of different species and genetic backgrounds against common disease-causing agents.

**EXPERIMENT 1: SEMI-NATURAL EXPOSURE OF ATLANTIC SALMON**

Disease occurrence in a cohort of Atlantic salmon *Salmo salar* L. was monitored under standard and enriched rearing conditions during two consecutive years. The fish originated from the River Tornionjoki, north-western Finland, and were bred from two
genetic backgrounds: hatchery-reared and wild-caught parents. Offspring of the hatchery-reared parents were produced in autumn 2008 from 68 first-generation hatchery males and 68 second-generation females by crossing two to three males with each female. The offspring of the wild-caught parents were produced using 27 females and 12 males captured from the River Tornionjoki, and crossing two males with each female and each male with four to five females.

The initial number of fish in each parent background was 40 000, totalling 80 000 fish. After hatching in spring 2009, the fish were maintained in indoor and outdoor tanks from 30 May 2009 to 31 August 2010, and surveyed for mortality and parasite prevalence and abundance during parasite outbreaks in summer 2009 and 2010. Preliminary trials of enriched rearing (introduction of gravel into the tanks) started during the yolk sac phase of the fish with half of both offspring in May 2009. During this time, fish mortality (not disease-related) did not differ between standard and enriched rearing (4569 and 4700 fish, respectively). The full set-up of enrichment (changes in water inflow, introduction of gravel and shelters) was initiated on 30 May 2009 when the fish were divided into 16 indoor round fibreglass tanks (diameter approx. 2 m, area 3.2 m²). The tanks included four replicates of each rearing method and parent fish combination (i.e. standard–hatchery, standard–wild, enriched–hatchery, enriched–wild). The number of fish in each tank was initially 4025, corresponding to moderate fish densities in commercial aquaculture. The fish were transferred to new tanks of similar size and rearing treatments on 15 March 2010, and decreased to 2500 fish per tank following routine reduction in density as they grew larger. On 7 July 2010, the fish were transferred to outdoor round concrete tanks (diameter approx. 8 m, area 50 m²) with corresponding treatment combinations, and the number of fish in each tank was 2095. The fish remained in these tanks until the end of the experiment.

Fish were maintained according to normal aquaculture protocols, the only exception being differences in the conditions between the standard and enriched rearing tanks. Standard rearing treatment included tank conditions with stable inflow of water, water volume and direction of water circulation. Fish were fed with regular sized commercial pellets adjusted to the requirements of the fish. In enriched rearing, the water inflow, volume and direction were changed irregularly (Table S1, Supporting Information), and the fish were fed with pellets of two sizes. In addition, the enriched tanks were supplied with small gravel and shelters made of different materials (Table S1, Fig. 1). Water for each indoor tank (taken from the basin of the lake) and outdoor tank (combination of basin and surface water) came from the Lake Kivesjärvi, situated upstream of the facility. The amount of incoming water was set according to the rearing treatment in each tank and water temperature corresponded to natural fluctuations in the lake. This ensured that all tanks became exposed to same physical conditions and composition of parasite infective stages present in the water. After the transfer of fish to outdoor tanks, however, half of the tanks, including two tanks of each rearing method–parent fish combination, were accidentally given only surface water from the lake for a period of 8 days. Consequently, the water temperature rose above 20 °C and an outbreak of Flavobacterium columnare occurred in those tanks. Temperature remained between 16 and 18 °C in the other eight tanks where an outbreak of Ichthyophthirius multifiliis was later detected (see Results).

Fig. 1. Standard (left) and enriched (right) rearing tanks during (a) start feeding stage of the fish (approx. 1 month old), (b) in juvenile stage (one summer old) and (c) in smolt stage (1 year old) used in the two experiments. Enrichment included introduction of gravel and shelters into the smaller 3-2-m² tanks (a & b) or shelters and stones in the larger 50-m² tanks (c). In addition, irregular changes of water inflow, volume and direction were applied in the enriched tanks.
A total of three disease-causing agents were detected: the flagellate *Ichthyobodo necator* in indoor tanks in 2009 and the ciliate *Ichthyophthirius multifiliis* and the bacterium *Flavobacterium columnare* in outdoor tanks in 2010 (see Results). *I. necator* (also known as *Costia necatrix*) is a common ectoparasite of aquaculture fish particularly in early days when fish are learning to feed (Rintamäki-Kinnunen & Valtonen 1997). The parasite can also occur in cold water (Robertson 1979) and typically causes mortality if untreated with formalin baths. The life cycle of the ciliate *I. multifiliis* differs from other protozoans as it includes multiplication in tank structures outside the fish host (Valtonen & Keränen 1981; Dickerson 2006), which gives rise to infective stages of the parasite. Infections are common during summer water temperatures (>15 °C) and can result in high mortality (Valtonen & Keränen 1981; Valtonen & Koskivaara 1994; Rintamäki-Kinnunen & Valtonen 1997). *Flavobacterium columnare* is an opportunistic pathogen which infects a wide range of wild and farmed fish species (Declercq et al. 2013). Infections are common in warm water and can lead to significant losses in aquaculture conditions (Pulkkinen et al. 2010; Declercq et al. 2013). The disease, called columnaris disease, is treated with antibiotics.

All tanks were followed daily for mortality and the number of dead fish were recorded (Table S2). During abnormally high mortality, samples of fish with symptoms were analysed in KFRS or dead fish were recorded (Table S2). During abnormally high mortality, samples of fish with symptoms were analysed in KFRS or dead fish were recorded (Table S2). During abnormally high mortality, samples of fish with symptoms were analysed in KFRS or dead fish were recorded (Table S2). All tanks were followed daily for mortality and the number of dead fish were recorded (Table S2). During abnormally high mortality, samples of fish with symptoms were analysed in KFRS or dead fish were recorded (Table S2).

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**EXPERIMENT 2: EXPERIMENTAL EXPOSURE OF LANDLOCKED ATLANTIC SALMON AND BROWN TROUT**

Experiment 2 investigated the effect of enriched rearing on resistance of landlocked Atlantic salmon *Salmo salar m. sebago* L. and brown trout *Salmo trutta m. lucastris* L. against controlled parasite exposure. Landlocked salmon originated from the River Pielsjoki, Saimaa watercourse. Offspring were produced in autumn 2011 from first-generation hatchery parents (116 males and 116 females) by crossing one male with one female. Brown trout originated from River Varisjoki, Oulujoki watercourse, and the offspring were produced in autumn 2011 from third- and fourth-generation hatchery parents (six males and six females) crossing one male with one female. Both species were reared in standard and enriched conditions with two replicate tanks of each rearing treatment in salmon and three replicate tanks in trout. The rearing methods were similar as in Experiment 1, but the enrichment for landlocked salmon was already started from the eye egg stage on 2 March 2012 and for brown trout right after fertilization in 14 October 2011.

The experimental exposure was conducted using the trematode *Diplodectes pseudophasaeceum* (Niewiadomska), the common eye fluke of several freshwater fish species. Infections are also commonly observed in aquaculture fish (Field & Irwin 1994; Buchmann & Bresciani 1997; Karvonen et al. 2006). The parasite life cycle includes a fish-eating avian definitive host, and snail and fish intermediate hosts. Asexual reproduction in the snail produces large numbers of free-swimming cercariae larvae which infect the fish. In the fish, each cercaria develops into metacercaria in the eye lenses. Infection in the eye can lead to severe effects such as cataract formation, reduced feeding efficiency and growth, as well as increased susceptibility of fish to predation (reviewed in Karvonen 2012). Parasite cercariae for the experimental exposures were obtained from eight naturally infected *Lymnaea stagnalis* (L.) snails collected from Lake Vuojärvi, Central Finland. Note that there is no detectable genetic structure in the parasite population over a large geographical scale in Finland (Louhi et al. 2010) and therefore the parasite origin was unlikely to influence cercarial infectivity. Snails were brought to laboratory and allowed to release cercariae for 4 h in 1.5 dL of water (20 °C) after which the suspension from the snails was combined representing a mixture of a minimum of eight different parasite genotypes. The cercarial density was estimated by taking ten 1-mL samples from the well-mixed suspension.

Both fish species were exposed to the parasite first in 2012 when the fish were one summer old (average length and weight = 52.4 ± 0.4 mm and 2.0 ± 0.04 g (salmon); 57.2 ± 0.4 mm and 2.8 ± 0.06 g (trout)) and again a year later in 2013 (130.9 ± 1.9 mm and 22.0 ± 0.9 g (salmon); 148.9 ± 1.6 mm and 38.4 ± 1.2 g (trout)), using different sets of fish, snails and parasite genotypes each time. In 2012, different parasite doses were used for salmon (80 cercariae per fish) and trout (250 cercariae per fish) because of greater susceptibility of small salmon to acute mortality following cercarial infection (see Karvonen 2012). In 2013, when the fish were larger, both species were exposed to a dose of 300 cercariae per fish. Fish were taken haphazardly from all treatment tanks so that the number of replicates was 100, 20 and 20 for salmon in 2012 and 2013, respectively, and 20 for trout in both years. Exposures took place in containers holding an individual fish and 0.5 L (2012) or 1 L (2013) of water (higher volume was used in 2013 because of larger fish size to ensure sufficient concentration of oxygen). Water temperature was 18.2 °C in 2012 and 15.5 °C in 2013. Exposure time was 30 min. After the exposure, fish were maintained in cages (all species–treatment–tank–combinations separately), in a single tank for 48 h to allow parasite establishment. Fish were then euthanized using MS-222, measured individually for length and weight, and dissected for the number of parasite metacercariae in the eye lenses. The experiments were carried out with permission from Finnish Regional State Administrative Agency (licences ESLH-2008-04178/Ym-23 and ESAVI/2458/04.10.03/2011).

**STATISTICS**

Survival data from Experiment 1 (*I. necator* outbreak from 30 May to 31 August 2009, 94 days; and *I. multifiliis* and *F. columnare* outbreaks from 7 July to 31 August 2010, 56 days) were analysed using generalized linear mixed models (GLMM with binomial probability distribution) with rearing treatment
(standard, enriched) and parent background (hatchery, wild) as fixed factors, and tank nested under rearing x parent interaction as a random factor. Infections of *I. multifiliis* and *F. columnare* were analysed separately from a total of eight tanks in each case because of differences in disease occurrence. Overall, the average fish size was slightly larger in standard rearing (average length ± SE = 137.3 ± 1.3 mm) compared to enriched rearing (127.1 ± 1.5 mm), and in fish of wild origin (135.8 ± 1.6 mm) compared to hatchery origin (129.7 ± 1.3 mm) in 2010 when the fish were sampled for occurrence of *I. multifiliis*. However, since these differences were small and the size distributions overlapped considerably between the treatments (Fig. S1), the size differences were considered negligible for disease progression and fish mortality in these tanks.

Differences in prevalence of *I. multifiliis* in the fish samples were analysed using stepwise logistic regression analysis using rearing treatment and parent background as categorical covariates. Presence/absence of previous *F. columnare* infection (found in half of the tanks) was also included as a factor. Fish condition factor, calculated as $K = \text{weight}/\text{length}^3 \times 100$ (Bolger & Connolly 1989), was used as a continuous covariate. Differences in *I. multifiliis* abundance (categorized as 0–2) were analysed using multinomial logistic regression with rearing treatment as a factor and fish condition factor as a covariate.

Data on abundance of *Diplodonomum* in Experiment 2 were analysed using GLM with negative binomial probability distribution and log link function. Data from salmon and trout were analysed separately as different exposure doses were used in 2012. Rearing treatment (standard, enriched), year (primarily 2010), infection begun approximately 2 weeks after the fish were transferred to outdoor tanks and ranged between 1.4 and 6.6% (mean 3.3 ± 0.6%) depending on the tank. Mortality was significantly higher among fish in standard rearing while the genetic origin of the fish had no effect on mortality (Fig. 3, Table 1). With *Flavobacterium*, the mortality in the tanks was higher compared to *Ichthyophthirius*, ranging between 5.3 and 17.5% (mean 10.6 ± 1.7%) and increasing substantially about 1 week after the fish transfer. Unexpectedly, the mortality was higher among the fish of wild origin and tended to be higher among the fish in enriched rearing (Fig. 2, Table 1). *Ichthyophthirius* was also later detected in the tanks with the previous *F. columnare* infection. However, subsequent mortality associated with *I. multifiliis* in these tanks (mean number ± SE of dead fish = 0.18 ± 0.03 per tank per day) was significantly lower compared to the tanks without *F. columnare* infection history (1.46 ± 0.25 per tank per day) ($t$-test on log-transformed data: $t = 9.635$, d.f. = 14, $P < 0.001$).

There was a significant difference in the number of dead fish between the standard and enriched rearing treatments in the early days of the *Flavobacterium* outbreak, that is the first 7 days after the transfer: mortality

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**Results**

**EXPERIMENT 1: MORTALITY AND INFECTION OF ATLANTIC SALMON DURING DISEASE OUTBREAKS**

The overall mortality of the one-summer-old salmon during the *Ichthyobodo necator* outbreak in the indoor tanks in 2009 was 4.5–16.0% (mean ± SE = 9.2 ± 0.9%) depending on the tank. Mortality was significantly higher among fish in standard rearing compared to enriched rearing irrespective of the parent background. Mortality was also higher among the fish of hatchery origin compared to wild origin (Fig. 2, Table 1).

In 2010, mortality associated with *Ichthyophthirius* infection began approximately 2 weeks after the fish were transferred to outdoor tanks and ranged between 1.4 and 6.6% (mean 3.3 ± 0.6%) depending on the tank. Mortality was significantly higher among fish in standard rearing while the genetic origin of the fish had no effect on mortality (Fig. 3, Table 1). With *Flavobacterium*, the mortality in the tanks was higher compared to *Ichthyophthirius*, ranging between 5.3 and 17.5% (mean 10.6 ± 1.7%) and increasing substantially about 1 week after the fish transfer. Unexpectedly, the mortality was higher among the fish of wild origin and tended to be higher among the fish in enriched rearing (Fig. 3, Table 1). *Ichthyophthirius* was also later detected in the tanks with the previous *F. columnare* infection. However, subsequent mortality associated with *I. multifiliis* in these tanks (mean number ± SE of dead fish = 0.18 ± 0.03 per tank per day) was significantly lower compared to the tanks without *F. columnare* infection history (1.46 ± 0.25 per tank per day) ($t$-test on log-transformed data: $t = 9.635$, d.f. = 14, $P < 0.001$).

There was a significant difference in the number of dead fish between the standard and enriched rearing treatments in the early days of the *Flavobacterium* outbreak, that is the first 7 days after the transfer: mortality
from the disease was higher in the enriched rearing tanks (mean number of dead fish per tank: 26.5 ± 7.2 and 2.0 ± 0.7, in enriched and standard rearing, respectively; *t*-test on log-transformed data: *t* = 5.728, d.f. = 6, *P* = 0.001). This suggests that the outbreak began earlier in the enriched rearing tanks. No such pattern, however, was observed in *Ichthyophthirius* during the first 2 weeks after the transfer including the first days of the outbreak (*t*-test on log-transformed data: *t* = 0.072, d.f. = 6, *P* = 0.945).

*Ichthyophthirius* prevalence was lower in enriched rearing compared to standard rearing in August during the highest parasite occurrence (Table 2, Table S3). The pattern was observed both in hatchery and wild backgrounds. Fish of wild background also showed lower *Ichthyophthirius* prevalence compared to hatchery background, but this was likely caused by the 1-week interval in sampling. Similarly, parasite abundance was lower in enriched rearing among fish of hatchery origin in August (multinomial logistic regression: *P* = 0.001), particularly in the highest abundance category (*P* = 0.001; Table 2). No difference in prevalence of *Ichthyophthirius* was detected between the treatments in July when the parasites were fewer (Table 2, Table S3). *Flavobacterium* infection showed significant interactions in some cases with rearing treatment and parent background because of lower *Ichthyophthirius* prevalence in tanks with *F. columnare* history.

**EXPERIMENT 2: DIPLOSTOMUM INFECTION IN LANDLOCKED ATLANTIC SALMON AND BROWN TROUT**

There was a statistically significant interaction between the rearing treatment and year in salmon exposed to *Diplostomum* indicating that one-summer-old fish from standard rearing had significantly higher parasite abundance (36% difference) compared to fish from enriched rearing (Fig. 4, Table 3). This suggests greater resistance of these fish to infection. However, the main effect of enriched rearing was not significant as no such effect was detected in the same fish group 1 year later. No difference in parasite abundance between the treatments was detected in brown trout either in 2012 or 2013 (Fig. 4, Table 3).

**Discussion**

Parasitic diseases represent one of the most important threats for aquaculture development world-wide and there is high demand for sustainable disease prevention methods. We tested effects of enriched rearing (Rodewald, Hyvärimo & Hirvonen 2011; Hyvärimo & Rodewald 2013; Näslund et al. 2013) on occurrence of important and widespread aquaculture pathogens and associated fish mortalities. In most cases we found lower parasite prevalence and abundance, and a higher survival rate of fish in enriched rearing during the outbreaks compared to standard conditions. Experimental exposure of fish to a trematode parasite also supported greater parasite resistance in enriched rearing although this effect was not consistent in all host backgrounds. This suggests that enriched rearing conditions can improve survival and disease resistance of aquaculture fishes. However, we also observed variation in mortality and parasite occurrence depending on the pathogen, fish species and fish age. Overall, this suggests that preventative effects of enriched rearing on infection occurrence may vary depending on the nature of the disease outbreak.

The exact mechanisms underlying lower parasite occurrence and host mortalities in enriched rearing are currently unclear, but our results support the role of both reduced host exposure and susceptibility. Fish survived
better in enriched rearing during *I. necator* and *I. multifilis* outbreaks while the pattern tended to be opposite in *F. columnare*. This probably reflects specific transmission mechanisms of each pathogen. The protozoans *I. necator* and *I. multifilis* release transmission stages to water from infected fish (*I. necator*) or from tank structures (*I. multifilis*). Thus, the higher water turnover in the enriched tanks could have reduced host exposure mechanically by removing infective stages from the tank (Bodensteiner et al. 2000). With *I. necator*, however, the higher survival rate of fish in enriched tanks was already observed before the introduction of water current dynamics, suggesting an influence of other elements of the enriched environment on parasite transmission or resistance of fish. The tendency for higher mortality in enriched tanks associated with *F. columnare* is nevertheless surprising and contrary to our expectation. The outbreak began slightly earlier in enriched rearing suggesting enhancement of the initial disease establishment. It is possible that the additional structures in enriched tanks could have favoured formation of a bacterial biofilm, which is associated with higher virulence in *F. columnare* (Cai, De La Fuente & Arias 2013). However, it is also possible that the early interception of the disease with antibiotics could have masked differences between the rearing treatments emerging later during the outbreak. Therefore, this result should be interpreted with caution.

Outbreaks of different diseases in aquaculture are typically consecutive, making it possible for previous outbreaks to influence disease progression during subsequent infections (Buchmann et al. 2001). For example, mortality of fish during *I. necator* infection in 2009 was significantly higher in standard rearing, which may have altered proportions of susceptible and resistant individuals in these tanks and influenced disease progression in the outbreaks in 2010. Moreover, tanks with a prior *F. columnare* outbreak had lower mortality from subsequent *I. multifilis* infection in 2010, possibly reflecting the effects of reduced fish density, activation of fish immune system, or previous antibiotic treatments. Overall, these results suggest that the effects of rearing methods on disease dynamics may be influenced by infection history within each tank.

In addition to a higher survival rate, enriched rearing also decreased the prevalence and abundance of *I. multifilis*. Interestingly, this effect was most evident during the highest parasite abundance in August when the infection was already being treated with formalin. This suggests improved treatment efficiency in enriched rearing. *Ichthyophthirius multifilis* can protect itself from the external environment by burrowing into the skin of fish (Dickerson 2006). Thus, any effects of the enriched rearing that

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Table 2. Prevalence (%) and categorical abundance of *Ichthyophthirius multifilis* in fish samples in Experiment 1 in 2010 in relation to parent background and rearing treatment (tanks with different *F. columnare* background are combined). Statistically significant differences between standard and enriched rearing treatments are marked * (see Results and Table S3 for details).

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Table 3. Results of GLM analyses on the number of *D. pseudopathaceum* in landlocked Atlantic salmon and brown trout from standard and enriched rearing following an experimental exposure to the parasite in Experiment 2. Rearing treatment (standard vs. enriched) and year (2012 vs. 2013) were used as factors and fish condition factor as a covariate.

<table>
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<tr>
<td>Landlocked Atlantic salmon</td>
<td>Rearing</td>
<td>1.931</td>
<td>1</td>
<td>0.165</td>
</tr>
<tr>
<td></td>
<td>Year</td>
<td>26.298</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Rearing × Year</td>
<td>3.897</td>
<td>1</td>
<td>0.048</td>
</tr>
<tr>
<td></td>
<td>Condition factor</td>
<td>11.845</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Brown trout</td>
<td>Rearing</td>
<td>0.828</td>
<td>1</td>
<td>0.363</td>
</tr>
<tr>
<td></td>
<td>Year</td>
<td>204.424</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Rearing × Year</td>
<td>0.061</td>
<td>1</td>
<td>0.805</td>
</tr>
<tr>
<td></td>
<td>Condition factor</td>
<td>0.242</td>
<td>1</td>
<td>0.623</td>
</tr>
</tbody>
</table>

make the parasites more vulnerable to treatments would result in lower infection. Fish also acquire immunity to *I. multifiliis* (Valtonen & Keränen 1981; Buchmann et al. 2001; Dickerson & Findly 2014), which may have caused differences in parasite occurrence between the treatments if the development of disease resistance was different. Details of such processes, however, are currently unknown. Experimental exposure of landlocked salmon in standardized conditions to the trematode *D. pseudopathaceum*, however, supports the idea of elevated host resistance in enriched rearing. Mechanistically, this can involve complex interactions between the rearing conditions and activation of different immune components that operate in fish against the infection (Chappell, Hardie & Secombes 1994). Interestingly, the effect was evident in the first summer but not 1 year later in the same fish groups, which may be related to age-dependent activation of specific immunity or development of the immune system. The difference in susceptibility between the rearing treatments early on is nevertheless important as younger fish are typically more susceptible to infections, including *Diplostomum* (Karvonen 2012). Thus, elevated parasite resistance early in life can be crucial for later survival. No such effect, however, was observed in brown trout, which suggests that effects of enriched rearing may be species-specific. Many more replicated species contrasts are needed to test this.

Disease occurrence and fish mortalities were also significantly associated with the genetic background of the fish. Offspring of wild fishes are generally considered superior to domesticated hatchery offspring (Araki, Cooper & Blouin 2007) and our results concur with this for the higher survival rate of wild origin fish during the *I. necator* outbreak. This effect was evident despite the short history of domestication of the parent fishes, but is in accordance with the earlier evidence (Araki, Cooper & Blouin 2007; Araki et al. 2008; Frankham 2008). Nevertheless, the effect of the rearing environment tended to surpass the parent background effect on survival. In outdoor tanks, however, the result was somewhat opposite as the wild fish showed lower survival during *F. columnare* and *I. multifiliis* outbreaks while in the latter case this was not statistically significant. This result is again surprising and suggests greater susceptibility of wild fish to infection. Overall, it seems that effects of genetic background on fish survival in aquaculture parasite epidemics are context-dependent, possibly influenced by interactions between tank type, fish age and specific transmission patterns of each parasite species.

To conclude, the ability of aquaculture fish to fight parasitic infections is one of the essential components of their well-being, concerning not only commercial fish production, but also endangered hatchery-maintained fish populations in terms of their survival post-release. Our results suggest that fairly simple enrichment of the aquaculture environment can significantly improve survival and resistance of fish under parasite infections in production-scale conditions. Mechanistically, this probably involves complex interactions between host physiology, patterns of pathogen transmission and environmental conditions. These, together with host genetic background, age and species, are likely to reflect the observed variation in infections by different pathogens. We encourage aquaculture companies to undertake trials of enriched rearing to determine under which circumstances parasitic infections can be controlled most cost-effectively. We are already aware of such ongoing trials, and the data from these semi-natural experiments will be important in determining whether enriched rearing can provide a decisive step towards sustainable disease prevention in aquaculture.

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**Data accessibility**

Data are archived in Dryad Digital Repository doi: 10.5061/dryad.bf206 (Karvonen et al. 2015).

**References**
