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Year: 2023

Version: Accepted version (Final draft)

Please cite the original version:

Karvonen, A., Klemme, I., Räihä, V., & Hyvärinen, P. (2023). Enriched rearing environment enhances fitness-related traits of salmonid fishes facing multiple biological interactions. Canadian Journal of Fisheries and Aquatic Sciences, 80(11). https://doi.org/10.1139/cjfas-2023-0083

Enriched rearing environment enhances fitness-related traits of salmonid fishes facing multiple biological interactions

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Abstract

To improve stocking success in threatened populations, captive-reared animals are often familiarized to natural environment. However, whether such actions improve the ability to cope with multiple biological interactions, such as competition, predation and parasitism that impose contradicting pressures on decision-making, is not well understood. Here, we explored short-term (4 weeks) and long-term (10 months) effects of enriched rearing on fitness-related traits of Atlantic salmon (*Salmo salar*). Salmon with different backgrounds of enrichment and parasite infection (eye fluke *Diplostomum pseudospathaceum*) were released to semi-natural ponds and monitored for activity, growth and predation susceptibility. Fish from enriched rearing showed lower short-term activity and higher short-term growth, suggesting that they coped better with novel conditions. However, predation susceptibility, and longer-term growth and survival, were unaffected by rearing treatment. Importantly, parasitism did not remove the positive effect of enrichment on growth, although the infection decreased both short-term and long-term growth and survival. These results suggest that enriched rearing can enhance fitness-related traits, such as growth, of stocked fish particularly during the critical early days, which can have important implications for stock enhancement activities.

Keywords: Aquaculture, Competitive ability, *Esox lucius*, Fish behavior, Predator avoidance

Introduction

Natural animal populations are increasingly threatened by overharvest, habitat loss and fragmentation, leading to spatial isolation and reduced genetic diversity (Sanchez-Bayo and Wyckhuys 2019; Tilman et al. 2017). To maintain viable and/or harvestable populations, they are often supplemented with captive-raised individuals. This is commonly practiced, for example, in many species of salmonid fishes (Brown and Day 2002; Einum and Fleming 2001; Valiquette et al. 2014), which, like many other fish species, have become endangered as a consequence of overfishing and habitat destruction (Arthington et al. 2016). However, such stocking actions typically have low success rates (ICES 2012) as conditions in captivity differ drastically from those in the wild (Hyvärinen and Rodewald 2013; Jepsen et al. 2000; Kekäläinen et al. 2008).

The ability of organisms to cope with biological interactions is a central component of fitness. For example, competition with conspecifics can influence growth, condition and reproductive success (Nunn et al. 2012; Ward et al. 2006), while predation results in mortality and therefore imposes the ultimate fitness cost. Numerous counter-adaptations, such as resource acquisition through territoriality (Lindström and Pampoulie 2005; Ord 2021) or avoidance of predators through decreased activity and avoidance of risky areas (Ferrari et al. 2015; Godin 1997; Lind and Cresswell 2005), have evolved to reduce these negative fitness effects. Importantly, such fitness-related behaviors may not be innate alone but can include adjustment and learning based on earlier experience. This can improve the individual performance under competition and predation (Brown et al. 2008; Brown and Dreier 2002; Brown and Chivers 2005).

Parasitism can also influence the ability to cope with competitors or predators through energy depletion and pathology, and alterations in host behaviors such as foraging, sociality and activity (Gopko et al. 2015; Hernandez and Sukhdeo 2008; Klein 2003; Lafferty and Shaw 2013; Seppälä et al. 2008). For example, three-spined sticklebacks (*Gasterosteus aculeatus*) infected with energetically demanding cestode larvae become fearless of predators, but thereby can also outcompete uninfected conspecifics for food (Milinski 1985). Thus, multiple simultaneous biological interactions faced by an individual can impose contradicting pressures on decision-making and result in sub-optimal behaviors regarding one or several overlapping demands. Effects of parasitism can also be amplified in captive environments, where animals often experience high infection rates and can have higher susceptibility to infections compared to wild conspecifics (Buchmann 2022; Karvonen et al. 2016).

To reduce the harmful effects of captivity, the rearing environment can be enriched to better resemble natural conditions and to promote experience gain (Reading et al. 2013). In fish hatcheries, this can be done by equipping rearing tanks with different types of structures, such as stones, covers and shelters (Karvonen et al. 2016; Näslund et al. 2013). Studies of enriched rearing on fish in laboratory and aquaculture conditions have demonstrated improved stress tolerance (Näslund et al. 2013; Rosengren et al. 2017), brain function and learning capability (Arechavala-Lopez et al. 2020; Salvanes et al. 2013; Strand et al. 2010), growth (Brunet et al. 2022; Crank et al. 2019), anti-predatory behaviors (Roberts et al. 2011; Salvanes and Braithwaite 2005), foraging skills (Brown et al. 2003; Rodewald et al. 2011), survival during disease epidemics (Karvonen et al. 2016; Räihä et al. 2019), and survival during migration (Hyvärinen and Rodewald 2013). Recently, it has also been suggested that positive effects of enrichment in parent fish could pass on to their offspring (Berbel et al. 2020). However, how

environmental enrichment influences the ability to cope with multiple biological interactions in conditions relevant for post-release survival of fish is still largely unknown.

Here, we investigated short-term and long-term effects of environmental enrichment on activity, growth and survival of Atlantic salmon (*Salmo salar*). We first reared the fish either in standard tanks with no enrichments or in enriched tanks with natural stones and covers to simulate natural river conditions (Räihä et al. 2019). We also exposed half of the fish to the eye fluke *Diplostomum pseudospathaceum*, a common parasite of freshwater and brackish water fish in nature (Seppälä et al. 2011; Valtonen et al. 1997) as well as in aquaculture (Field and Irwin 1994; Karvonen et al. 2006). The parasite reduces the eyesight of fish by causing cataracts, which results in reduced foraging ability and growth (Crowden and Broom 1980; Karvonen and Seppälä 2008) and impaired avoidance of aerial predators (Seppälä et al. 2005). We then released the fish to semi-natural streams, simulating stocking of a large group of hatchery-raised fish to the wild. Over a period of four weeks, we followed their activity and growth, and survival under piscivorous predation. This corresponded to the early post-stocking period, which is commonly considered most critical for hatchery-raised salmonids in the wild (Jepsen et al. 1998; Kekäläinen et al. 2008). Subsequently, we focused on longer-term effects of enrichment and parasitism by monitoring growth and survival of the same fish in the same tanks over the next 10 months, through winter and until the next summer. We predicted that enriched rearing would improve the performance of fish by increasing growth and survival compared to fish raised in standard conditions. We also predicted that the effects of enrichment would be most evident right after the introduction and level off thereafter as the fish, including those raised in standard conditions, become familiarized with the natural environment. Furthermore, we predicted that the parasite infection established during rearing would reduce or remove the beneficial effects of enrichment.

Material and methods

Rearing of fish

The study was conducted at Kainuu Fisheries Research Station (KFRS, www.kfrs.fi, 64.404°N, 27.516°E) during September 2017-July 2018. The station is a flow-through facility, taking water from the depth of 7 m of the nearby Lake Kivesjärvi. The water temperature in the facility follows the natural temperature of the lake and all rearing units and experimental tanks receive similar water supply.

Five Atlantic salmon (hereafter "salmon") populations (originating from rivers Neva, Simo, Ii, Kemi and Tornio) were used. Fertilized eggs, originating from hatchery brood fish (20-42 males and 20-93 females per population) of the Natural Resources Institute Finland, were brought to KFRS in March 2016. Eggs hatched in May and 1250 fish were placed in each of the 20 tanks of 3.2 m^2. Environmental enrichment was carried out using the prodecure described in Räihä et al. (2019), with two standard and two enriched tanks for each fish population. Enrichments included gravel and shelter structures (Fig. 1), as well as changes in water flow direction, scaled to the number and size of the fish (hereafter "enriched fish"; Supplementary material; Table S1). More specifically, enriched tanks were initially equipped with three piles of 30-60 mm gravel. At the juvenile-smolt stage, three shelters were added above the gravel piles and one larger shelter on bricks in each tank (Fig. 1). Moreover, the direction of the water flow was changed between clockwise to counterclockwise 1-2 times per week (Table S1). In contrast, fish in standard tanks were reared without structural enrichments and changes in the direction of water flow (hereafter "standard fish"). In February 2017, 640

149 fish, selected evenly from all populations and rearing tanks in a random manner, were
150 individually tagged by injection of PIT-tags (12 × 2 mm, 0.1 g, half duplex; Biomark Inc.) into
151 the body cavity under anaesthesia (40 mg l^{-1} benzocaine). Populations were then pooled in two
152 replicate 3.2 m^2 tanks per rearing treatment. The remaining unmarked fish were used in other
153 experiments. All fish were fed with automated feeders and commercial fish feed (Veronesi
154 VITA 0.2/0.5 and Inicio Plus G 0.4).
155
156 *Parasite exposure*
157
158 Half of the salmon (n=320) were exposed to *D. pseudospathaceum* in July 2017. Parasite larvae
159 (cercariae) originated from 10 *Lymnaea stagnalis* snails (first intermediate host of the parasite)
160 collected from Lake Vuojärvi (62° 24' 54" N, 25° 56' 14" E) in Central Finland. Note that there
161 is no detectable population genetic structure in *D. pseudospathaceum* across a large
162 geographical scale in Finland (Louhi et al. 2010), which is why the parasite origin was unlikely
163 to affect the infection success in the fish populations. The snails were stored individually in
164 lake water at 4 ºC and fed with lettuce in excess. Prior to the exposure, snails were taken to
165 room temperature and allowed to release cercariae for three hours in 1.5 dl of lake water each,
166 after which the solutions from all snails were combined. Cercarial density in the solution was
167 estimated from ten 1 ml samples and no cercariae older than six hours were used, ensuring their
168 infectivity (Karvonen et al. 2003). The fish were exposed in four containers (two for standard
169 fish and two for enriched fish), each with 50 randomly selected fish equally from all
170 populations and 40 L of water (15.4 °C). The exposure dose was 350 cercariae per fish and the
171 exposure lasted for 30 min during which the water was continuously aerated with air pumps.
172 The unexposed control fish were sham exposed at the same time in four similar containers
173 receiving lake water without parasites. The infection procedure was repeated the same way for

174 the remaining fish by adjusting the amount of water in the containers according to the number
175 of fish.
176
177 After the exposure, all fish were returned to their home tanks. Note that *D. pseudospathaceum*
178 does not transmit directly between fish, allowing maintenance of exposed and unexposed fish
179 in the same tanks. Fifty-two days after the exposure, when the parasites were fully developed
180 and caused cataracts, fish length (155 ± 0.75 mm (mean ± SE)) and weight (37.4 ± 0.49 g) were
181 measured under anesthesia (40 mg L^{-1} benzocaine). Standard fish were slightly larger (157.1 ±
182 1.0 mm) than enriched fish (152.8 ± 1.1 mm) and uninfected fish (156.7 ± 1.1 mm) slightly
183 larger than infected fish (153.1 ± 1.0 mm). Infection of *D. pseudospathaceum* in the eye lenses
184 of exposed fish was confirmed and the coverage of parasite-induced cataracts was determined
185 for all fish at a scale of 0-100% of the lens area with 10% increments using slit-lamp
186 microscopy (Kowa SL-15) (Karvonen et al. 2004). All unexposed fish were also examined and
187 confirmed to be free of *D. pseudospathaceum* infection.
188
189 *Experiment 1: Short-term patterns in activity and susceptibility to predation*
190
191 The first experiment investigated the effects of environmental enrichment and parasitism on
192 activity, growth and survival of salmon during the first four weeks after stocking. Fish from
193 enriched and standard rearing (total n = 642 fish), half of which were infected with *D.*
194 *pseudospathaceum*, were introduced into eight round 50 m^2 semi-natural concrete outdoor
195 tanks. Each tank had a 15 m^2 stream section at the outer edge and a 35 m^2 pool section in the
196 middle (hereafter "stream" and "pool"; Fig. 2). The bottom of the stream was covered with
197 gravel (grain size 50-70 mm) and stones (200 mm) and had a water depth of 50 cm. The pool
198 was deeper (150 cm) and without stones or other structures. Water inflow was set to 3-6 L/s

(higher inflow in higher water temperatures), resulting in slow, but visible water current in the stream section. During the experiment, fish relied on unquantified numbers of natural invertebrate fauna established in the bottom of the tanks and supplemented by the incoming water to the stream (Fig. 2). The stream was isolated from the pool with a wall in one end and with a mesh (mesh size 45 mm) in the other. The mesh prevented the predators (see below) from entering the stream but allowed salmon to pass. Thus, the stream was considered as habitat with higher resources (invertebrate fauna, see above), but it also provided a refuge from predation in tanks with predators present. Tanks were equipped with two PIT antennas on both sides of the mesh with a recording frequency of nine times per second (Fig. 2). The antennas recorded individual PIT tags of the passing fish, monitoring all fish movements between stream and pool.

Wild northern pike (*Esox lucius*), a natural predator of juvenile salmonids (Hyvärinen and Vehanen 2004; Kekäläinen et al. 2008) were used as predators. They had been originally caught from Lake Oulujärvi (64.276°N, 27.205°E) and were maintained at KFRS before the study. Three pike, each weighing 1.5-3 kg, were placed to the pool section of each of four randomly selected tanks, while the other four tanks remained as predator-free controls. To ensure sufficient feed intake, pike were fed with dead roach (*Rutilus rutilus*) and smelt (*Osmerus eperlanus*) once a week during the experiment. Eighty salmon, four from each of the population-rearing-infection combination (i.e. 5 populations × 2 rearing treatments × 2 infection treatments × 4 fish = 80 fish), taken evenly and randomly from the rearing tanks, were then introduced to the stream section of each tank (total n = 642 fish; two of the tanks had 83 and 79 fish due to a counting error, the remaining six tanks had 80 fish). A removable smaller mesh separated the fish from the pool before the start of experiment. Four days after the introduction, the antennas were activated, and the smaller mesh was removed allowing the fish

to enter the pool. The experiment was terminated after 30 days, when the predators were removed, and all remaining experimental fish caught. Salmon from predator tanks were euthanized with an overdose (200 mg L^{-1}) of benzocaine anesthetic, and their length and weight were measured. Fish from tanks without predators were measured under anesthesia (40 mg L^{-1} benzocaine) and subsequently used in Experiment 2.

Observations from the PIT antennas were recorded using TIRIS data logger program (Citius Solutions Ltd, Kajaani, Finland) and transformed to one second resolution with the PIT Data program (www.pitdata.net). The antenna-specific ASCII data were further analyzed using AV Bio-Statistics v 5.2. (Alioravainen et al. 2018; Vainikka et al. 2012). The following variables were determined for each fish: 1) entry (yes/no) into pool, i.e. predator side in four of the tanks, 2) latency to the first entry into pool (hours from beginning of the experiment), 3) number of changes between stream and pool, 4) % time spent in pool, 5) % change in body length (Δ length), and 6) survival (yes/no, for fish that entered the pool in predator tanks). Change in weight was not used in the analysis as most of the fish lost some weight during the experiment, which is normal in hatchery-raised fish after a transition from high-energy aquaculture pellets to natural feed (Vehanen et al. 2009). Thus, Δ length provided a more reliable estimate of growth (hereafter "growth"). At the end of the experiment, all pike and the bottom of all tanks were scanned for PIT-tags with handheld readers to record fish consumed by predators. In the predator tanks, no tags were recovered from the bottom of the streams, suggesting that tags found in pools originated from depredated fish and had been excreted by pike. No tags were recovered from fish that died in the control tanks. There was no mortality among the pike during the experiment.

248 Data were analyzed using generalized linear mixed models (GLMM) with restricted pseudo-
249 likelihood estimation, Satterthwaite's computation of degrees of freedom (*df*), and the
250 following error distributions: Gaussian (growth), negative binomial (number of changes
251 between stream and pool sections), gamma (latency to the first entry into pool), beta [% cataract
252 coverage (mean of right and left eye) and % time spent in pool], and binomial (probability of
253 entering pool and survival). Rearing treatment (standard/enriched), predator presence
254 (present/absent), parasitism (infected/uninfected), and all their interactions were used as fixed
255 factors. Experimental tank and fish population were included as random factors (note that
256 different fish populations were used here to introduce natural heterogeneity and subsequently
257 broaden the generality of the results, not to focus on possible population-specific differences
258 *per se*). Survival analyses only included data from tanks with predators. When testing for
259 differences in cataract coverage between enriched and standard fish, and the effect of cataract
260 coverage on growth, rearing treatment was used as fixed factor, and experimental tank and
261 population as random factors. Length in the beginning of the experiment was used as a
262 covariate in all analyses, except those on growth. Because of slight differences in length
263 between the fish in the rearing and infection treatments in the beginning of the experiment (see
264 above), models were also fitted without the covariate, but this did not alter the main results.
265 Further, to explore how the spatial behavior (number of changes between pool and stream, time
266 spent in the pool) influenced growth in tanks without predators, separate GLMMs were fitted
267 by including these behavioral variables as continuous factors in the full model described above.
268

269 *Experiment 2: Long-term patterns in growth and survival*

270

271 The second experiment explored growth and survival of the salmon in winter (October 2017-
272 March 2018) and in the following spring-summer (March-July 2018). Fish from the tanks

without predators in the first experiment (77-79 per tank depending on the survival during Experiment 1) were released to four stream-pool tanks similar to Experiment 1 in October 2017. As no predators were used in this experiment, the wall and mesh separating the pool and the stream were removed to allow free movement for the fish. In March 2018, all fish were caught and measured for length under anesthesia, after which they were returned to the tanks. The experiment was terminated in July 2018, when all fish were euthanized with an overdose of benzocaine anesthetic and measured for length.

Growth was determined separately for the experimental periods October 2017-March 2018 and March-July 2018. Data were analyzed using repeated-measures GLMMs with either growth (Gaussian error distribution) or survival (binomial error distribution) as response variable, rearing treatment, parasitism and experimental period as fixed factors, and experimental tank and fish population as random factors. For survival, length in the beginning of the experiment was also used as a covariate. Additionally, repeated-measures GLMMs, including only the infected individuals, were fitted to study the effect of cataract coverage on growth and survival. All analyses were conducted using SAS 9.4.

All experimental protocols were approved by the Regional State Administrative Agency of Southern Finland (license number ESAVI/5183/04.10.07/2017) and all experiments were performed in accordance with relevant guidelines and regulations.

Results

Experiment 1

In total, 548/640 fish were detected at least once at the stream-side antenna, and 303 of these passed the pool-side antenna and entered the pool. Enriched fish had a lower probability of entering the pool, longer latency to the first entry and lower number of changes between stream and pool compared to standard fish (Fig. 3, Table 1), all indicating lower activity. The presence of predators did not influence any of these variables (Fig. 3, Table 1). However, predator presence decreased the time spent in the pool for both enriched and standard fish (Fig. 3, Table 1).

Enriched fish had higher growth during the experiment, but growth was not influenced by the presence of predators (Fig. 4, Table 1). Among tanks without predators, a higher number of changes between stream and pool associated with lower growth (GLMM, $F_{1, 201.2} = 11.82$, $p < 0.001$). Moreover, fish that spent more time in the pool had lower growth, but this effect was found only in uninfected fish (GLMM, time in pool × infection interaction: $F_{1, 303.1} = 11.66$, $p < 0.001$).

The survival of fish was 97.8 % in the control tanks and 72.5 % in the predator tanks. Within predator tanks, enrichment did not influence the survival of those fish that entered the pool [48/78 (61.5 %) of standard fish and 37/64 (57.8 %) of enriched fish were preyed upon] (Table 1). Rearing treatment had also no effect on survival when including all fish in the analysis, i.e. also those that did not enter the pool (GLMM: $F_{1,308} = 1.53$, $p = 0.216$).

There were no significant interactions between rearing, predator presence and parasitism in any of the tests, suggesting that parasitism did not alter the effects of enrichment and predation (Table 1). Parasitism increased the probability of entering the pool and the number of changes between stream and pool but had no impact on the latency to the first entry of pool (Fig. 3,

Table 1). The infection also decreased growth (Table 1) and more so among fish with the highest cataract coverage (Fig. S1, Table S2). Moreover, parasitism had no overall effect on the survival of those fish that entered pool in the predator tanks (Table 1), but among the infected fish, increasing coverage of parasitic cataracts decreased the probability of survival (Table S2). Enriched and standard fish did not differ in parasite-induced cataract coverage (GLMM: $F_{1, 303} = 0.01$, $p = 0.911$).

Experiment 2

In the over-winter experiment, fish growth differed between the time points so that the fish had higher growth in the latter part of the experiment in March-July compared to the preceding winter period in October-March (Fig. 4, Table 2). In both periods, growth was lower among infected fish compared to uninfected, but did not differ between enriched and standard fish (Fig. 4, Table 2). Higher coverage of parasitic cataracts also associated with lower growth in both periods (Fig. S2, Table S2).

Average survival was high during October-March (308/314 survived), but significantly lower during March-July (276/308 survived, Table 2). Rearing treatment did not influence survival, but there was a marginally significant interaction between parasitism and time (Table 2). Further analysis separately on the time periods indicated that infected fish had lower survival than uninfected fish during March-July (GLMM: $F_{1, 296} = 7.17$, $p = 0.008$). Among the infected fish, survival was negatively associated with cataract coverage, but only during March-July (significant cataract × time interaction, Table S2).

Discussion

348

349 Biological interactions, such as those between conspecifics or predators and prey, are important
350 determinants of fitness (Both and Visser 2000; Harris and Siefferman 2014; Munday 2001).
351 Coping with them can be innate or learned, and further shaped by other interactions, such as
352 parasitism. Here, we explored the short-term and longer-term effects of environmental
353 enrichment (implemented through enriched rearing) and parasitism (infection of the eye fluke
354 *D. pseudospathaceum*) on activity, growth and survival of Atlantic salmon. By introducing
355 young salmonids into semi-natural streams, we found that enriched fish showed lower activity
356 and higher growth compared to standard fish during the first weeks after release, suggesting
357 that they coped better with the introduction to a novel environment. However, these differences
358 disappeared as the fish became familiarized with the environment during the next few months.
359 We also observed that parasitism and associated eye cataracts reduced both short-term and
360 long-term growth and survival of the fish. Importantly, the effects of environmental enrichment
361 on activity and growth were still evident, regardless of the overall negative effects of the
362 infection, suggesting beneficial effects of enrichment for the fish during the critical first weeks
363 after stocking.

364

365 Higher growth in enriched fish (see also Brunet et al. (2022); Crank et al. (2019)) could be
366 caused by factors such as enhanced competitive ability, lower metabolism and/or higher
367 tolerance to stress. Enriched fish could be competitively superior to standard fish, which is
368 supported by results from small mammals, where enrichment improved feeding success (Díez-
369 León et al. 2013; Monosevitz 1970), and from birds and fish, where enrichment enhanced
370 territorial behavior (Berejikian et al. 2001; Leone and Estevez 2008). Indeed, competitive
371 ability is an important fitness-component of territorial animals such as juvenile salmonids
372 (Jonsson and Jonsson 2011). Although we did not detect differences in time spent in pool

(habitat presumably with lower resources) between enriched and standard fish, it is possible that enriched individuals were able to secure the best feeding grounds within the streams regardless of their slightly smaller initial size. Second, enriched fish showed lower activity, which could be related to securing territories (see above), but could also suggest lower overall energy expenditure and/or metabolic rate (Millidine et al. 2006), resulting in positive effects on growth. It is also possible that standard fish showed higher activity due to boldness and explorative behaviors, which can be promoted by a traditional rearing environment (Sundström et al. 2004). Enrichment can also decrease boldness and risk-taking behaviors in salmonids (Roberts et al. 2011), although several studies have shown also the opposite of increased boldness, aggression and exploratory behavior (Berejikian et al. 2001; Braithwaite and Salvanes 2005; Brunet et al. 2022; Lee and Berejikian 2008). Third, enriched individuals may also be more flexible in adjusting their behaviors (Braithwaite and Salvanes 2005; Campbell et al. 2013; Salvanes et al. 2013; Strand et al. 2010), which can facilitate recovery from stress after introduction. This can be related to earlier familiarization or habituation to river-like conditions, thus reducing fear, anxiety and random movements in a novel environment (Lee et al. 2019). Overall, all these factors, alone or in concert, could underlie the differences in activity and growth between enriched and standard salmon.

The presence of piscine predators in the pool section of the experimental tanks increased the occupancy of the stream section, but did not influence the other activity measures, indicating that the fish did not detect predator presence prior entering the pool. However, there was no difference in susceptibility to predation between enriched and standard fish. In general, predators can have strong regulatory effects particularly among young, inexperienced individuals (Ferrari et al. 2015). With pike, this is emphasized in predation on newly stocked fish (Hyvärinen and Vehanen 2004; Kekäläinen et al. 2008). Earlier studies using visual or

chemical predation cues, in some cases in connection with enriched rearing, have indicated that predator-conditioned fish show more efficient anti-predator behaviors (e.g. Berejikian et al. (2003); Berejikian et al. (1999); Roberts et al. (2011), see also Tetzlaff et al. (2019) for a review on animal translocations). It is possible that the enrichment used here did not sufficiently "train" the fish against live predators in an open environment such as the pool, although we assumed it to enhance shelter seeking behaviors (use of stones) in general. The latter idea is indirectly supported by the lower activity observed among enriched fish (see above). Unlike in nature, our setup also did not allow predators to enter the streams, where the salmon had more opportunities to hide. Thus, possible beneficial effects of this type of enriched rearing on susceptibility to predation needs to be explored further.

Parasite infection in a central sensory organ did not remove the beneficial effects of enrichment, despite the overall negative effects of the infection (Crowden and Broom 1980; Karvonen and Seppälä 2008). In general, parasite infections can be acquired in nature after release and/or, like simulated here, during rearing prior to release. The fluke *D. pseudospathaceum* is commonly found in aquaculture (Field and Irwin 1994; Karvonen et al. 2006) as well as in fresh water and coastal brackish water areas (Seppälä et al. 2011; Valtonen et al. 1997). Cataracts caused by the infection impair the vision of fish, which was seen here as negative effect on growth, likely because of a reduction in foraging efficiency [reviewed in Karvonen (2012)]. Also, the positive effect of time spent in the primary feeding habitat, the stream, on growth was not observed in infected fish, suggesting that these individuals benefited less from the available resources. Interestingly, however, enriched fish had lower activity and higher growth than standard fish regardless of the infection and similar coverage of cataracts. This suggests that enrichment mitigated the negative effects of the infection, possibly through

422 mechanisms related to competitive ability, metabolism or stress tolerance discussed above. The
423 detailed mechanisms, however, require further work.
424
425 The positive effect of enrichment on growth was no longer evident after the fish had over-
426 wintered in the tanks. This is reasonable as the effects of enrichment are likely to even out
427 between enriched and standard fish (see also Bergendahl et al. (2016)), if they all experience
428 more heterogeneous environment long enough in the wild, or like here, in semi-natural tanks.
429 However, the fact that enriched fish did better than standard fish during the first weeks can give
430 them an advantage right after introduction, which is typically most critical for survival (Jepsen
431 et al. 1998; Kekäläinen et al. 2008). Regardless of the loss of the effect of enrichment, the
432 negative effects of *D. pseudospathaceum* infection remained and were seen as lower growth
433 and survival of infected fish. These effects also intensified with increasing coverage of
434 cataracts. This is also logical as the cataracts increase with parasite numbers and infected fish
435 cannot get rid of the infections in the eye lenses, although some degree of recovery from
436 cataracts can take place in cold water (Klemme et al. 2021).
437
438 Several parasite taxa, including *D. pseudospathaceum*, alter the phenotype of their hosts
439 [reviewed in Moore (2002)]. Fish infected with *D. pseudospathaceum* show impaired shelter-
440 seeking behavior, camouflage coloration, escape response, and group cohesion [reviewed in
441 Karvonen (2012)], which can all increase their susceptibility to predation. We found that
442 susceptibility to a piscine predator was not affected by the infection *per se*, but among infected
443 fish, those with the highest cataract coverages had the lowest survival. Earlier laboratory trials
444 with non-native fish hosts have demonstrated higher susceptibility of fish infected with *D.*
445 *pseudospathaceum* to simulated avian predation (next host in the life cycle), but not to piscine
446 predators (dead-end hosts for the parasite) (Gopko et al. 2017; Seppälä et al. 2005, 2006). The

present data suggest that higher cataract coverage (and higher infection intensities that cause them, see Karvonen et al. (2004)) can increase the susceptibility of natural fish hosts also to live piscine predation. These effects are also likely to be long-lasting (see above) and can have important implications for the survival of stocked fish depending on their infection history during farming and susceptibility to further infections in the wild.

To conclude, our results suggest that enrichment of the rearing environment of aquaculture fish can improve their performance after stocking, resulting, for example, in higher growth. These effects were evident regardless of exposure of fish to common biological interactions such as predation and parasitism. Overall, interactions between enrichment, parasitism and predation are important not only from ecological and evolutionary perspectives but have also important applied implications. Many natural salmonid populations are currently endangered. Aquaculture fish stocked to support these populations typically face a range of predators and parasites in the wild. Habituation through enriched rearing could potentially improve survival and fitness of fish and, consequently, the success and economic viability of these actions.

Acknowledgements

We thank staff of the KFRS and Anssi Vainikka from University of Eastern Finland for technical assistance.

Competing Interest

The authors declare that there are no competing interests.

Funding Statement

This study was supported by the Academy of Finland (grants #263864, #292736, #310632 for AK) and by European Maritime and Fisheries Fund (#43521) for PH.

Data availability

All data will be made available in JYX Digital Repository of the University of Jyvaskyla upon acceptance.

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739 Table 1. Results of GLMM analyses on the variables measured in Atlantic salmon in
740 Experiment 1. Rearing treatment, predator presence, parasitism and their interactions were
741 entered as fixed factors and experimental tank and fish population as random factors. Fish body
742 length before the experiment was used as a covariate in all tests, except on growth. Survival
743 under piscine predation was analyzed using only data from tanks with predators, thus excluding
744 the factor "predator presence". All models are full models, but non-significant interactions are
745 not shown ($p > 0.119$ for all).

Variable	**Factor**	**df 1**	**df 2**	**F**	**p**
Probability of entering pool	Rearing	1	633.0	13.24	<0.001
(N = 642 salmon)	Predator presence	1	6.0	0.64	0.455
	Parasitism	1	633.0	4.74	0.030
Latency to first entry of pool	Rearing	1	287.6	4.50	0.035
(N = 302 salmon)	Predator presence	1	6.1	0.01	0.908
	Parasitism	1	287.4	0.01	0.927
Number of changes between stream and pool	Rearing	1	633.0	5.76	0.017
(N = 642 salmon)	Predator presence	1	5.9	2.08	0.201
	Parasitism	1	633.0	8.49	0.004
Proportional time spent in pool	Rearing	1	527.4	2.25	0.133
(N = 544 salmon)	Predator presence	1	9.0	42.58	<0.001
	Parasitism	1	527.6	3.65	0.057
Growth	Rearing	1	528.6	12.72	<0.001
(N = 544 salmon)	Predator presence	1	6.9	2.29	0.175
	Parasitism	1	528.1	48.52	<0.001

Survival (fish that entered the pool)	Rearing	1	130.0	0.14	0.708
(N = 142 salmon)	Parasitism	1	130.0	<0.01	0.962

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767 Table 2. Results of repeated-measures GLMM analyses on differences in growth and survival
768 of Atlantic salmon in Experiment 2. Rearing treatment, parasitism and time (October 2017 -
769 March 2018 and March - July 2018) were entered as fixed factors, and experimental tank and
770 fish population as random factors. For survival, body length before the experiment was
771 included as a covariate. All models are full models, but non-significant interactions with $p >$
772 0.1 are not shown ($p > 0.106$ for all). For survival, the model did not converge with the rearing
773 × parasitism interaction and this term was excluded from the final model. N = 290 salmon × 2
774 time points = 580 observations for growth, N = 311 salmon × 2 time points = 622 observations
775 for survival.

Variable	**Source**	**df 1**	**df 2**	**F**	**p**
Growth	Rearing (R)	1	565.5	2.19	0.140
(N = 580 observations)	Parasitism (P)	1	565.4	30.72	<0.001
	Time (T)	1	565	148.25	<0.001
	R × P	1	565.8	3.54	0.061
Survival	Rearing (R)	1	616	1.79	0.182
(N = 622 observations)	Parasitism (P)	1	616	0.23	0.634
	Time (T)	1	616	11.70	<0.001
	P × T	1	616	3.65	0.056

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783 **Figure legends**

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785 Figure 1. Standard (left) and enriched (right) rearing tanks during (a) start feeding stage and
786 (b) juvenile-smolt stage of Atlantic salmon used in the two experiments. Enrichment included
787 introduction of (a) gravel piles, and (b) gravel piles and shelters. In addition, direction of the
788 water inflow was changed between clockwise and counterclockwise in the enriched tanks.
789 Standard tanks had no structures or changes in direction of water inflow.

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791 Figure 2. Schematic overview of the experimental setup in 50 m^2 outdoor tanks (panel a),
792 antennas between stream and pool sections (panel b) and an overall view of the experimental
793 tank (panel c). Pool and stream were separated with a wall at one end (solid black line in panel
794 a) and with antennas detecting the passing PIT-tagged fish at the other (panel b). The antenna
795 system consisted of two antennas, one on the stream and the other on the pool side, installed
796 over a white plastic channel (panel a). The arrow indicates the location and direction of the
797 water inflow, the thick dashed line the mesh, the thin dashed lines the PIT-antennas and the
798 thin solid grey lines the plywood walls of the antenna channel. Outflow was in the middle of
799 the pool. Predators (three pike) were introduced to the pool in four of the eight tanks and
800 Atlantic salmon to the stream of all tanks before the start of the Experiment 1. For experiment
801 2 (without predators, four tanks), the wall and the mesh were removed to allow free movement
802 of the fish.

803

804 Figure 3. Predicted mean probability of entering the pool section of a tank (a), latency to first
805 entry to pool section (b), number of changes between stream and pool sections (c), and
806 proportion of time spent in the pool (d) (±SE) in Atlantic salmon in different combinations of

807 rearing treatment (enriched/standard) and *Diplostomum pseudospathaceum* infection
808 (infected/uninfected) in tanks with and without predators in Experiment 1.
809
810 Figure 4. Predicted mean proportional change in length (±SE) in Atlantic salmon in different
811 combinations of rearing treatment (enriched/standard) and *Diplostomum pseudospathaceum*
812 infection (infected/uninfected). In experiment 1, tests were made in tanks with and without
813 predators during August-September 2017 (panel a). In experiment 2, longer-term effects were
814 explored in tanks without predators, first over-winter during October 2017-March 2018 and
815 then in the next spring-summer during March-July 2018 (panel b).
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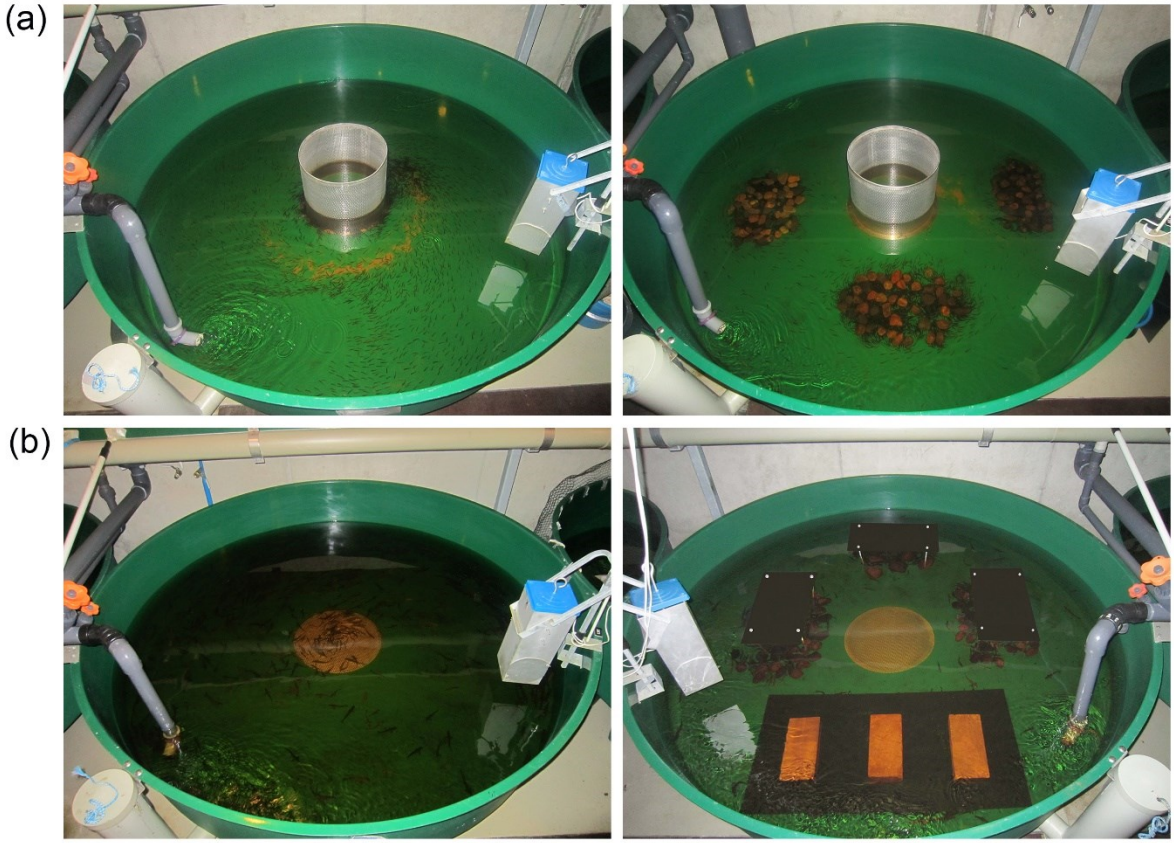

Fig. 1

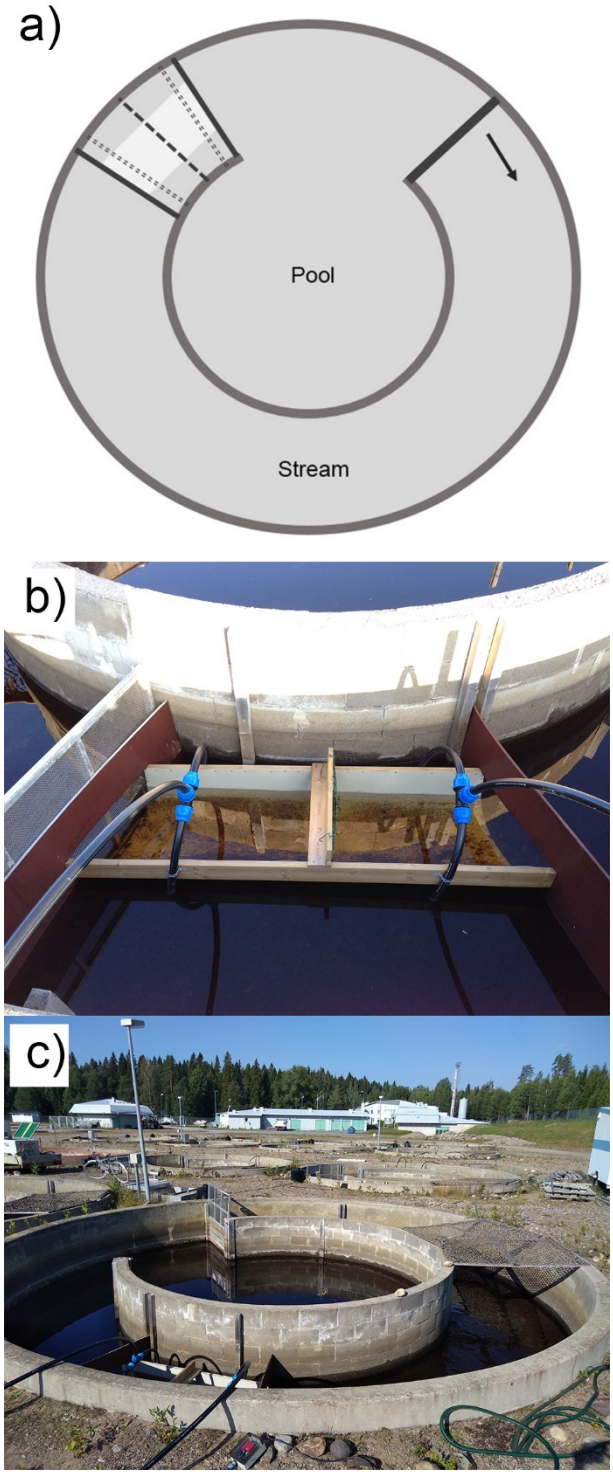

Fig. 2

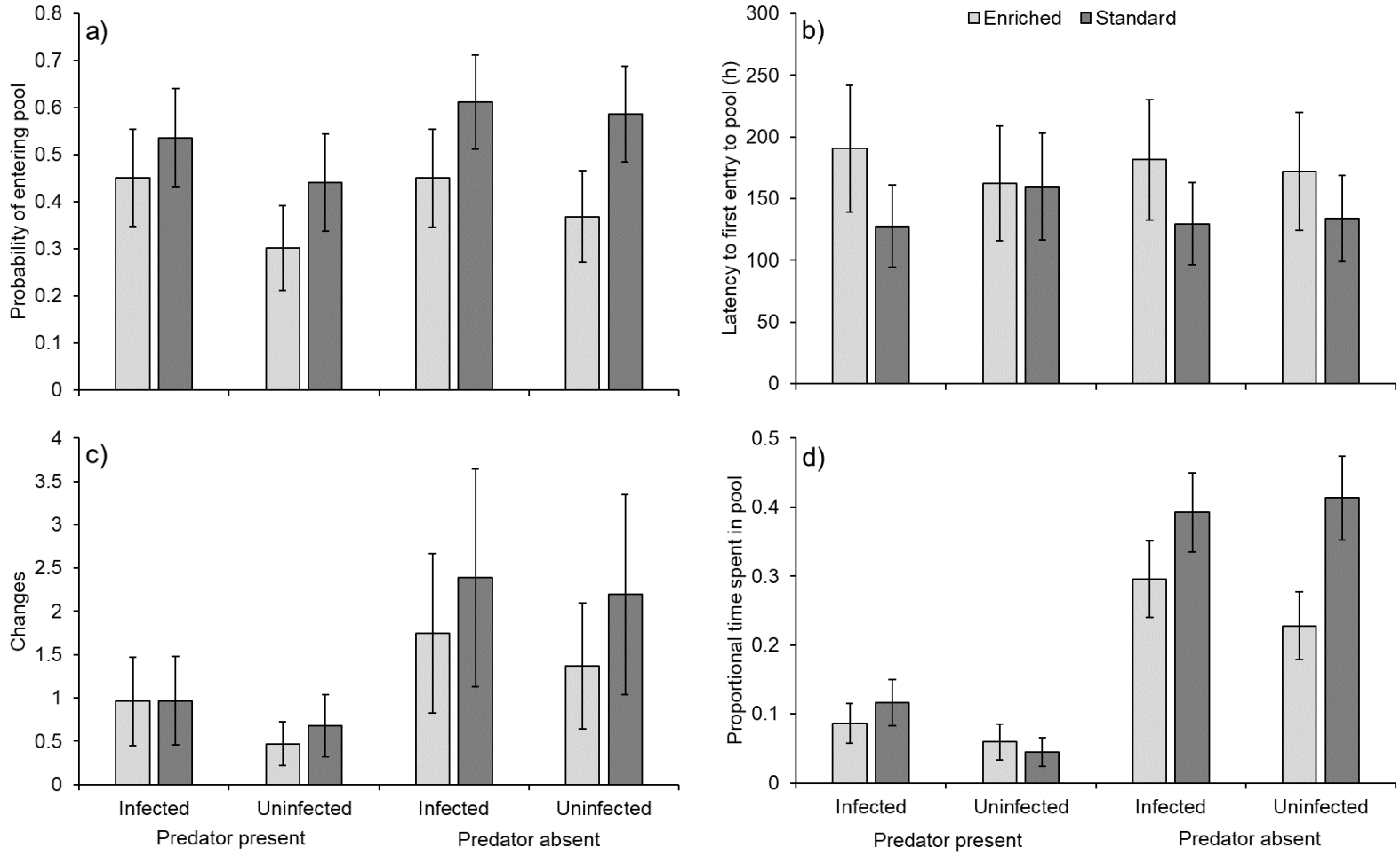
a)
Probability of entering pool
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
b)
Enriched
Standard
Latency to first entry to pool (h)
300
250
200
150
100
50
0
c)
Changes
4
3.5
3
2.5
2
1.5
1
0.5
0
d)
Proportional time spent in pool
0.5
0.4
0.3
0.2
0.1
0
Infected
Uninfected
Infected
Uninfected
Predator present
Predator absent

Fig. 3

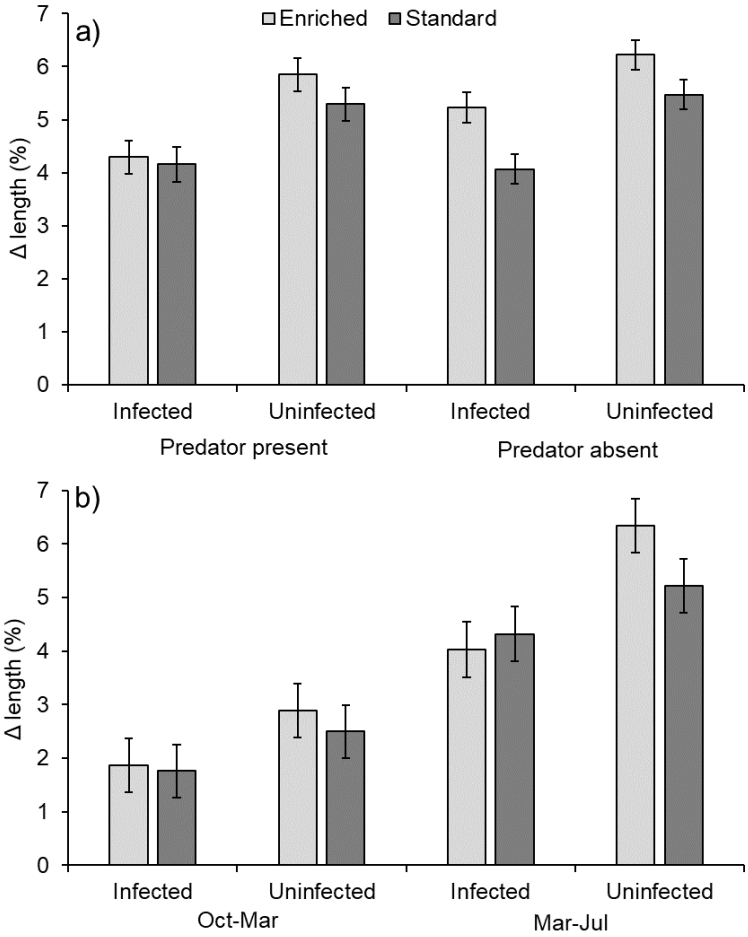
a)
Enriched
Standard
Δ length (%)
0
1
2
3
4
5
6
7
Infected
Uninfected
Infected
Uninfected
Predator present
Predator absent
b)
Δ length (%)
0
1
2
3
4
5
6
7
Infected
Uninfected
Infected
Uninfected
Oct-Mar
Mar-Jul

Fig. 4

Supplementary material: Karvonen et al. Enriched rearing environment enhances fitness-related traits of salmonid fishes facing multiple biological interactions

Table S1. Changes in the quantity and quality of enrichments (water flow direction and structures) in enriched rearing tanks of Atlantic salmon (*Salmo salar*) in 2017. Enriched tanks were equipped with gravel piles and three shelters of 25 x 50 cm polystyrene plates with metal legs and one shelter of 60 x 90 cm plywood board on bricks. Water flow direction was changed between clockwise and counterclockwise. Standard tanks remained without enrichments, but had the same adjustments to water depth and inflow (l/s) as the fish grew.

Time	**Water depth (cm)**	**Inflow (l/s)**	**Flow direction**	**Structures**
Mar 21-30	11	0.4	Clockwise	Eggs on a gravel bed
Mar 31	11	0.4	Clockwise	Gravel (30-60 mm) piles x 3
Jun 20	11	0.4	Changing 1-2 x per week	Gravel (30-60 mm) piles x 3
Jun 28	11	0.4	Changing 1-2 x per week	Gravel (30-60 mm) piles x 3, shelters x 4
Aug 15	15	0.5	Changing 1-2 x per week	Gravel (30-60 mm) piles x 3, shelters x 4
Aug 27	20	0.7	Changing 1-2 x per week	Gravel (30-60 mm) piles x 3, shelters x 4

Table S2. Results of GLMM (Experiment 1) and repeated-measures GLMM (Experiment 2) analyses on the effect of the coverage of parasitic cataracts caused by *Diplostomum pseudospathaceum* on growth and survival of Atlantic salmon (*Salmo salar*). Rearing treatment (enriched and standard) and time (October 2017 - March 2018 and March - July 2018) in Experiment 2 were used as fixed factors, cataract coverage as a covariate, and experimental tank and salmon population as random factors. All models are full models, but non-significant interactions with $p > 0.1$ are not shown ($p > 0.118$ for all). For survival, the model did not converge with the rearing × cataract coverage interaction in Experiment 1, or with the rearing × time interaction in Experiment 2. Thus, these terms were excluded from the final models. In experiment 2, N = 138 salmon × 2 time points = 276 observations for growth, N = 153 salmon × 2 time points = 306 observations for survival.

Experiment	Factor	Source	df1	df2	F	p
1	Growth	Rearing	1	256.5	0.83	0.364
	(N = 265 salmon)	Cataract coverage	1	258.2	20.07	<0.001
	Survival	Rearing	1	70	1.02	0.316
	(N = 80 salmon)	Cataract coverage	1	70	6.76	0.011
2	Growth	Rearing (R)	1	264.9	2.46	0.118
	(N = 276 observations)	Cataract coverage (C)	1	264.6	13.24	<0.001
		Time (T)	1	264.1	30.59	<0.001
		R×T	1	264	3.42	0.066
	Survival	Rearing	1	299.9	0.82	0.367
	(N = 306 observations)	Cataract coverage	1	297.1	0.73	0.395
		Time	1	297.8	2.82	0.094
		C×T	1	297.4	6.51	0.011

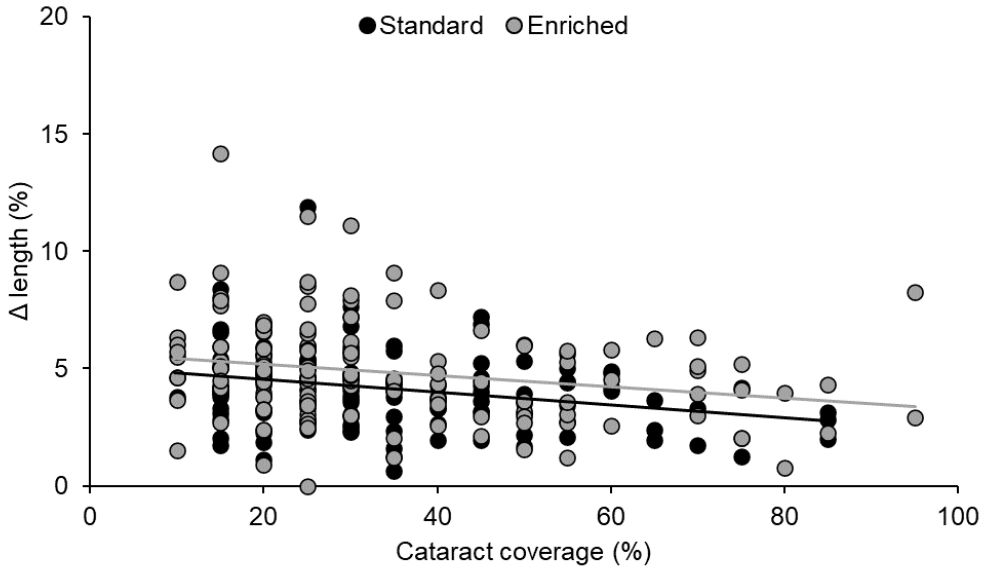

Figure S1. Relationship between eye cataract coverage caused by *Diplostomum pseudospathaceum*, and the percentage change in length in Atlantic salmon with standard and enriched rearing background in Experiment 1. The fitted lines are linear regressions to illustrate the direction of the relationships.

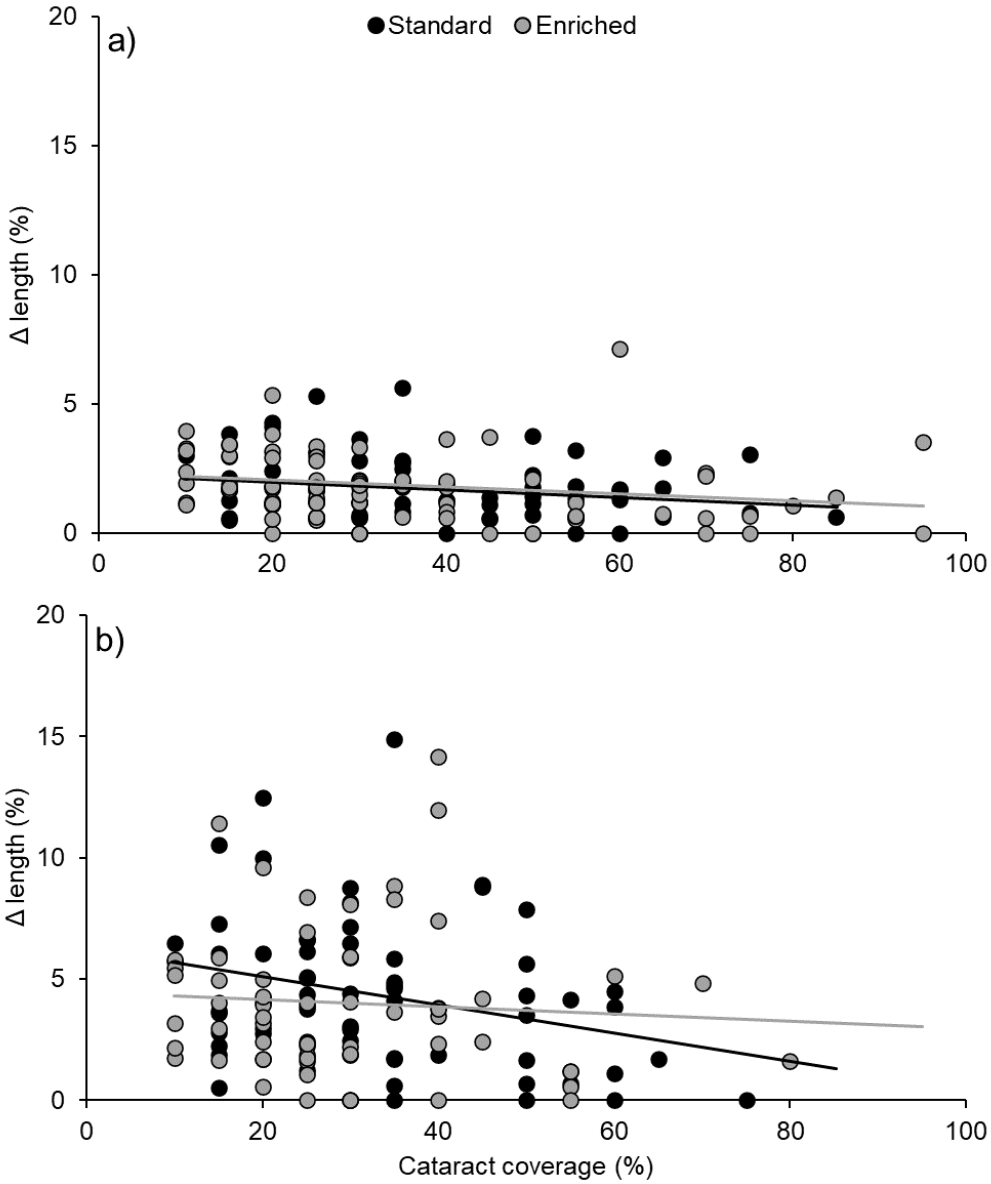

Figure S2. Relationships between eye cataract coverage caused by *Diplostomum pseudospathaceum*, and the percentage change in length in Atlantic salmon with standard and enriched rearing background in Experiment 2 in October 2017-March 2018 (panel a) and March-July 2018 (panel b). The fitted lines are linear regressions to illustrate the direction of the relationships.

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