Author(s): Karvonen, Anssi; Klemme, Ines; Räihä, Ville; Hyvärinen, Pekka

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1 Enriched rearing environment enhances fitness-related traits of salmonid fishes

2 facing multiple biological interactions

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4 Anssi Karvonen[1*], Ines Klemme[1], Ville Räihä[1], Pekka Hyvärinen[2]

5

6 [1]University of Jyvaskyla, Department of Biological and Environmental Science, P.O. Box 35,

7 FI-40014 University of Jyvaskyla, Finland

8 [2]Natural Resources Institute Finland (Luke), Aquatic population dynamics, Manamansalontie

9 90, 88300 Paltamo, Finland

10

11

12 *Corresponding author: Anssi Karvonen, University of Jyvaskyla, Department of Biological

13 and Environmental Science, P.O. Box 35, FI-40014 University of Jyvaskyla, Finland.

14 Email: anssi.t.karvonen@jyu.fi. Tel. +358408053882

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26 **Abstract**

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28 To improve stocking success in threatened populations, captive-reared animals are often

29 familiarized to natural environment. However, whether such actions improve the ability to cope

30 with multiple biological interactions, such as competition, predation and parasitism that impose

31 contradicting pressures on decision-making, is not well understood. Here, we explored short-

32 term (4 weeks) and long-term (10 months) effects of enriched rearing on fitness-related traits

33 of Atlantic salmon (*Salmo salar*). Salmon with different backgrounds of enrichment and

34 parasite infection (eye fluke *Diplostomum pseudospathaceum*) were released to semi-natural

35 ponds and monitored for activity, growth and predation susceptibility. Fish from enriched

36 rearing showed lower short-term activity and higher short-term growth, suggesting that they

37 coped better with novel conditions. However, predation susceptibility, and longer-term growth

38 and survival, were unaffected by rearing treatment. Importantly, parasitism did not remove the

39 positive effect of enrichment on growth, although the infection decreased both short-term and

40 long-term growth and survival. These results suggest that enriched rearing can enhance fitness-

41 related traits, such as growth, of stocked fish particularly during the critical early days, which

42 can have important implications for stock enhancement activities.

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51 **Introduction**

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53 Natural animal populations are increasingly threatened by overharvest, habitat loss and

54 fragmentation, leading to spatial isolation and reduced genetic diversity (Sanchez-Bayo and

55 Wyckhuys 2019; Tilman et al. 2017). To maintain viable and/or harvestable populations, they

56 are often supplemented with captive-raised individuals. This is commonly practiced, for

57 example, in many species of salmonid fishes (Brown and Day 2002; Einum and Fleming 2001;

58 Valiquette et al. 2014), which, like many other fish species, have become endangered as a

59 consequence of overfishing and habitat destruction (Arthington et al. 2016). However, such

60 stocking actions typically have low success rates (ICES 2012) as conditions in captivity differ

61 drastically from those in the wild (Hyvärinen and Rodewald 2013; Jepsen et al. 2000;

62 Kekäläinen et al. 2008).

63

64 The ability of organisms to cope with biological interactions is a central component of fitness.

65 For example, competition with conspecifics can influence growth, condition and reproductive

66 success (Nunn et al. 2012; Ward et al. 2006), while predation results in mortality and therefore

67 imposes the ultimate fitness cost. Numerous counter-adaptations, such as resource acquisition

68 through territoriality (Lindström and Pampoulie 2005; Ord 2021) or avoidance of predators

69 through decreased activity and avoidance of risky areas (Ferrari et al. 2015; Godin 1997; Lind

70 and Cresswell 2005), have evolved to reduce these negative fitness effects. Importantly, such

71 fitness-related behaviors may not be innate alone but can include adjustment and learning based

72 on earlier experience. This can improve the individual performance under competition and

73 predation (Brown et al. 2008; Brown and Dreier 2002; Brown and Chivers 2005).

74

75   Parasitism can also influence the ability to cope with competitors or predators through energy

76   depletion and pathology, and alterations in host behaviors such as foraging, sociality and

77   activity (Gopko et al. 2015; Hernandez and Sukhdeo 2008; Klein 2003; Lafferty and Shaw

78   2013; Seppälä et al. 2008). For example, three-spined sticklebacks (*Gasterosteus aculeatus*)

79   infected with energetically demanding cestode larvae become fearless of predators, but thereby

80   can also outcompete uninfected conspecifics for food (Milinski 1985). Thus, multiple

81   simultaneous biological interactions faced by an individual can impose contradicting pressures

82   on decision-making and result in sub-optimal behaviors regarding one or several overlapping

83   demands. Effects of parasitism can also be amplified in captive environments, where animals

84   often experience high infection rates and can have higher susceptibility to infections compared

85   to wild conspecifics (Buchmann 2022; Karvonen et al. 2016).

86

87   To reduce the harmful effects of captivity, the rearing environment can be enriched to better

88   resemble natural conditions and to promote experience gain (Reading et al. 2013). In fish

89   hatcheries, this can be done by equipping rearing tanks with different types of structures, such

90   as stones, covers and shelters (Karvonen et al. 2016; Näslund et al. 2013). Studies of enriched

91   rearing on fish in laboratory and aquaculture conditions have demonstrated improved stress

92   tolerance (Näslund et al. 2013; Rosengren et al. 2017), brain function and learning capability

93   (Arechavala-Lopez et al. 2020; Salvanes et al. 2013; Strand et al. 2010), growth (Brunet et al.

94   2022; Crank et al. 2019), anti-predatory behaviors (Roberts et al. 2011; Salvanes and

95   Braithwaite 2005), foraging skills (Brown et al. 2003; Rodewald et al. 2011), survival during

96   disease epidemics (Karvonen et al. 2016; Räihä et al. 2019), and survival during migration

97   (Hyvärinen and Rodewald 2013). Recently, it has also been suggested that positive effects of

98   enrichment in parent fish could pass on to their offspring (Berbel et al. 2020). However, how

99   environmental enrichment influences the ability to cope with multiple biological interactions

100 in conditions relevant for post-release survival of fish is still largely unknown.

101

102 Here, we investigated short-term and long-term effects of environmental enrichment on

103 activity, growth and survival of Atlantic salmon (*Salmo salar*). We first reared the fish either

104 in standard tanks with no enrichments or in enriched tanks with natural stones and covers to

105 simulate natural river conditions (Räihä et al. 2019). We also exposed half of the fish to the

106 eye fluke *Diplostomum pseudospathaceum*, a common parasite of freshwater and brackish

107 water fish in nature (Seppälä et al. 2011; Valtonen et al. 1997) as well as in aquaculture (Field

108 and Irwin 1994; Karvonen et al. 2006). The parasite reduces the eyesight of fish by causing

109 cataracts, which results in reduced foraging ability and growth (Crowden and Broom 1980;

110 Karvonen and Seppälä 2008) and impaired avoidance of aerial predators (Seppälä et al. 2005).

111 We then released the fish to semi-natural streams, simulating stocking of a large group of

112 hatchery-raised fish to the wild. Over a period of four weeks, we followed their activity and

113 growth, and survival under piscivorous predation. This corresponded to the early post-stocking

114 period, which is commonly considered most critical for hatchery-raised salmonids in the wild

115 (Jepsen et al. 1998; Kekäläinen et al. 2008). Subsequently, we focused on longer-term effects

116 of enrichment and parasitism by monitoring growth and survival of the same fish in the same

117 tanks over the next 10 months, through winter and until the next summer. We predicted that

118 enriched rearing would improve the performance of fish by increasing growth and survival

119 compared to fish raised in standard conditions. We also predicted that the effects of enrichment

120 would be most evident right after the introduction and level off thereafter as the fish, including

121 those raised in standard conditions, become familiarized with the natural environment.

122 Furthermore, we predicted that the parasite infection established during rearing would reduce

123 or remove the beneficial effects of enrichment.

124

Material and methods

126

Rearing of fish

128

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.

134

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 °C 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 $\pm$ SE)) and weight (37.4 ± 0.49 g) were

181   measured under anesthesia (40 mg L^{-1} benzocaine). Standard fish were slightly larger ($157.1 \pm$

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

199 (higher inflow in higher water temperatures), resulting in slow, but visible water current in the

200 stream section. During the experiment, fish relied on unquantified numbers of natural

201 invertebrate fauna established in the bottom of the tanks and supplemented by the incoming

202 water to the stream (Fig. 2). The stream was isolated from the pool with a wall in one end and

203 with a mesh (mesh size 45 mm) in the other. The mesh prevented the predators (see below)

204 from entering the stream but allowed salmon to pass. Thus, the stream was considered as habitat

205 with higher resources (invertebrate fauna, see above), but it also provided a refuge from

206 predation in tanks with predators present. Tanks were equipped with two PIT antennas on both

207 sides of the mesh with a recording frequency of nine times per second (Fig. 2). The antennas

208 recorded individual PIT tags of the passing fish, monitoring all fish movements between stream

209 and pool.

210

211 Wild northern pike (*Esox lucius*), a natural predator of juvenile salmonids (Hyvärinen and

212 Vehanen 2004; Kekäläinen et al. 2008) were used as predators. They had been originally caught

213 from Lake Oulujärvi (64.276°N, 27.205°E) and were maintained at KFRS before the study.

214 Three pike, each weighing 1.5-3 kg, were placed to the pool section of each of four randomly

215 selected tanks, while the other four tanks remained as predator-free controls. To ensure

216 sufficient feed intake, pike were fed with dead roach (*Rutilus rutilus*) and smelt (*Osmerus*

217 *eperlanus*) once a week during the experiment. Eighty salmon, four from each of the

218 population-rearing-infection combination (i.e. 5 populations × 2 rearing treatments × 2

219 infection treatments × 4 fish = 80 fish), taken evenly and randomly from the rearing tanks, were

220 then introduced to the stream section of each tank (total n = 642 fish; two of the tanks had 83

221 and 79 fish due to a counting error, the remaining six tanks had 80 fish). A removable smaller

222 mesh separated the fish from the pool before the start of experiment. Four days after the

223 introduction, the antennas were activated, and the smaller mesh was removed allowing the fish

224 to enter the pool. The experiment was terminated after 30 days, when the predators were

225 removed, and all remaining experimental fish caught. Salmon from predator tanks were

226 euthanized with an overdose (200 mg L^{-1}) of benzocaine anesthetic, and their length and weight

227 were measured. Fish from tanks without predators were measured under anesthesia (40 mg L^{-1}

228 benzocaine) and subsequently used in Experiment 2.

229

230 Observations from the PIT antennas were recorded using TIRIS data logger program (Citius

231 Solutions Ltd, Kajaani, Finland) and transformed to one second resolution with the PIT Data

232 program (www.pitdata.net). The antenna-specific ASCII data were further analyzed using AV

233 Bio-Statistics v 5.2. (Alioravainen et al. 2018; Vainikka et al. 2012). The following variables

234 were determined for each fish: 1) entry (yes/no) into pool, i.e. predator side in four of the tanks,

235 2) latency to the first entry into pool (hours from beginning of the experiment), 3) number of

236 changes between stream and pool, 4) % time spent in pool, 5) % change in body length (Δ

237 length), and 6) survival (yes/no, for fish that entered the pool in predator tanks). Change in

238 weight was not used in the analysis as most of the fish lost some weight during the experiment,

239 which is normal in hatchery-raised fish after a transition from high-energy aquaculture pellets

240 to natural feed (Vehanen et al. 2009). Thus, Δ length provided a more reliable estimate of

241 growth (hereafter "growth"). At the end of the experiment, all pike and the bottom of all tanks

242 were scanned for PIT-tags with handheld readers to record fish consumed by predators. In the

243 predator tanks, no tags were recovered from the bottom of the streams, suggesting that tags

244 found in pools originated from depredated fish and had been excreted by pike. No tags were

245 recovered from fish that died in the control tanks. There was no mortality among the pike

246 during the experiment.

247

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

273   without predators in the first experiment (77-79 per tank depending on the survival during

274   Experiment 1) were released to four stream-pool tanks similar to Experiment 1 in October

275   2017. As no predators were used in this experiment, the wall and mesh separating the pool and

276   the stream were removed to allow free movement for the fish. In March 2018, all fish were

277   caught and measured for length under anesthesia, after which they were returned to the tanks.

278   The experiment was terminated in July 2018, when all fish were euthanized with an overdose

279   of benzocaine anesthetic and measured for length.

280

281   Growth was determined separately for the experimental periods October 2017-March 2018 and

282   March-July 2018. Data were analyzed using repeated-measures GLMMs with either growth

283   (Gaussian error distribution) or survival (binomial error distribution) as response variable,

284   rearing treatment, parasitism and experimental period as fixed factors, and experimental tank

285   and fish population as random factors. For survival, length in the beginning of the experiment

286   was also used as a covariate. Additionally, repeated-measures GLMMs, including only the

287   infected individuals, were fitted to study the effect of cataract coverage on growth and survival.

288   All analyses were conducted using SAS 9.4.

289

290   All experimental protocols were approved by the Regional State Administrative Agency of

291   Southern Finland (license number ESAVI/5183/04.10.07/2017) and all experiments were

292   performed in accordance with relevant guidelines and regulations.

293

294   **Results**

295

296   *Experiment 1*

297

298   In total, 548/640 fish were detected at least once at the stream-side antenna, and 303 of these

299   passed the pool-side antenna and entered the pool. Enriched fish had a lower probability of

300   entering the pool, longer latency to the first entry and lower number of changes between stream

301   and pool compared to standard fish (Fig. 3, Table 1), all indicating lower activity. The presence

302   of predators did not influence any of these variables (Fig. 3, Table 1). However, predator

303   presence decreased the time spent in the pool for both enriched and standard fish (Fig. 3, Table

304   1).

305

306   Enriched fish had higher growth during the experiment, but growth was not influenced by the

307   presence of predators (Fig. 4, Table 1). Among tanks without predators, a higher number of

308   changes between stream and pool associated with lower growth (GLMM, $F_{1,\,201.2} = 11.82$, p <

309   0.001). Moreover, fish that spent more time in the pool had lower growth, but this effect was

310   found only in uninfected fish (GLMM, time in pool × infection interaction: $F_{1,\,303.1} = 11.66$, p

311   < 0.001).

312

313   The survival of fish was 97.8 % in the control tanks and 72.5 % in the predator tanks. Within

314   predator tanks, enrichment did not influence the survival of those fish that entered the pool

315   [48/78 (61.5 %) of standard fish and 37/64 (57.8 %) of enriched fish were preyed upon] (Table

316   1). Rearing treatment had also no effect on survival when including all fish in the analysis, i.e.

317   also those that did not enter the pool (GLMM: $F_{1,308} = 1.53$, p = 0.216).

318

319   There were no significant interactions between rearing, predator presence and parasitism in any

320   of the tests, suggesting that parasitism did not alter the effects of enrichment and predation

321   (Table 1). Parasitism increased the probability of entering the pool and the number of changes

322   between stream and pool but had no impact on the latency to the first entry of pool (Fig. 3,

323   Table 1). The infection also decreased growth (Table 1) and more so among fish with the

324   highest cataract coverage (Fig. S1, Table S2). Moreover, parasitism had no overall effect on

325   the survival of those fish that entered pool in the predator tanks (Table 1), but among the

326   infected fish, increasing coverage of parasitic cataracts decreased the probability of survival

327   (Table S2). Enriched and standard fish did not differ in parasite-induced cataract coverage

328   (GLMM: $F_{1, 303} = 0.01$, $p = 0.911$).

329

330   *Experiment 2*

331

332   In the over-winter experiment, fish growth differed between the time points so that the fish had

333   higher growth in the latter part of the experiment in March-July compared to the preceding

334   winter period in October-March (Fig. 4, Table 2). In both periods, growth was lower among

335   infected fish compared to uninfected, but did not differ between enriched and standard fish

336   (Fig. 4, Table 2). Higher coverage of parasitic cataracts also associated with lower growth in

337   both periods (Fig. S2, Table S2).

338

339   Average survival was high during October-March (308/314 survived), but significantly lower

340   during March-July (276/308 survived, Table 2). Rearing treatment did not influence survival,

341   but there was a marginally significant interaction between parasitism and time (Table 2).

342   Further analysis separately on the time periods indicated that infected fish had lower survival

343   than uninfected fish during March-July (GLMM: $F_{1, 296} = 7.17$, $p = 0.008$). Among the infected

344   fish, survival was negatively associated with cataract coverage, but only during March-July

345   (significant cataract × time interaction, Table S2).

346

347   **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

373 (habitat presumably with lower resources) between enriched and standard fish, it is possible

374 that enriched individuals were able to secure the best feeding grounds within the streams

375 regardless of their slightly smaller initial size. Second, enriched fish showed lower activity,

376 which could be related to securing territories (see above), but could also suggest lower overall

377 energy expenditure and/or metabolic rate (Millidine et al. 2006), resulting in positive effects

378 on growth. It is also possible that standard fish showed higher activity due to boldness and

379 explorative behaviors, which can be promoted by a traditional rearing environment (Sundström

380 et al. 2004). Enrichment can also decrease boldness and risk-taking behaviors in salmonids

381 (Roberts et al. 2011), although several studies have shown also the opposite of increased

382 boldness, aggression and exploratory behavior (Berejikian et al. 2001; Braithwaite and

383 Salvanes 2005; Brunet et al. 2022; Lee and Berejikian 2008). Third, enriched individuals may

384 also be more flexible in adjusting their behaviors (Braithwaite and Salvanes 2005; Campbell

385 et al. 2013; Salvanes et al. 2013; Strand et al. 2010), which can facilitate recovery from stress

386 after introduction. This can be related to earlier familiarization or habituation to river-like

387 conditions, thus reducing fear, anxiety and random movements in a novel environment (Lee et

388 al. 2019). Overall, all these factors, alone or in concert, could underlie the differences in activity

389 and growth between enriched and standard salmon.

390

391 The presence of piscine predators in the pool section of the experimental tanks increased the

392 occupancy of the stream section, but did not influence the other activity measures, indicating

393 that the fish did not detect predator presence prior entering the pool. However, there was no

394 difference in susceptibility to predation between enriched and standard fish. In general,

395 predators can have strong regulatory effects particularly among young, inexperienced

396 individuals (Ferrari et al. 2015). With pike, this is emphasized in predation on newly stocked

397 fish (Hyvärinen and Vehanen 2004; Kekäläinen et al. 2008). Earlier studies using visual or

398   chemical predation cues, in some cases in connection with enriched rearing, have indicated that

399   predator-conditioned fish show more efficient anti-predator behaviors (e.g. Berejikian et al.

400   (2003); Berejikian et al. (1999); Roberts et al. (2011), see also Tetzlaff et al. (2019) for a review

401   on animal translocations). It is possible that the enrichment used here did not sufficiently

402   "train" the fish against live predators in an open environment such as the pool, although we

403   assumed it to enhance shelter seeking behaviors (use of stones) in general. The latter idea is

404   indirectly supported by the lower activity observed among enriched fish (see above). Unlike in

405   nature, our setup also did not allow predators to enter the streams, where the salmon had more

406   opportunities to hide. Thus, possible beneficial effects of this type of enriched rearing on

407   susceptibility to predation needs to be explored further.

408

409   Parasite infection in a central sensory organ did not remove the beneficial effects of enrichment,

410   despite the overall negative effects of the infection (Crowden and Broom 1980; Karvonen and

411   Seppälä 2008). In general, parasite infections can be acquired in nature after release and/or,

412   like simulated here, during rearing prior to release. The fluke *D. pseudospathaceum* is

413   commonly found in aquaculture (Field and Irwin 1994; Karvonen et al. 2006) as well as in

414   fresh water and coastal brackish water areas (Seppälä et al. 2011; Valtonen et al. 1997).

415   Cataracts caused by the infection impair the vision of fish, which was seen here as negative

416   effect on growth, likely because of a reduction in foraging efficiency [reviewed in Karvonen

417   (2012)]. Also, the positive effect of time spent in the primary feeding habitat, the stream, on

418   growth was not observed in infected fish, suggesting that these individuals benefited less from

419   the available resources. Interestingly, however, enriched fish had lower activity and higher

420   growth than standard fish regardless of the infection and similar coverage of cataracts. This

421   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

447   present data suggest that higher cataract coverage (and higher infection intensities that cause

448   them, see Karvonen et al. (2004)) can increase the susceptibility of natural fish hosts also to

449   live piscine predation. These effects are also likely to be long-lasting (see above) and can have

450   important implications for the survival of stocked fish depending on their infection history

451   during farming and susceptibility to further infections in the wild.

452

453   To conclude, our results suggest that enrichment of the rearing environment of aquaculture fish

454   can improve their performance after stocking, resulting, for example, in higher growth. These

455   effects were evident regardless of exposure of fish to common biological interactions such as

456   predation and parasitism. Overall, interactions between enrichment, parasitism and predation

457   are important not only from ecological and evolutionary perspectives but have also important

458   applied implications. Many natural salmonid populations are currently endangered.

459   Aquaculture fish stocked to support these populations typically face a range of predators and

460   parasites in the wild. Habituation through enriched rearing could potentially improve survival

461   and fitness of fish and, consequently, the success and economic viability of these actions.

462

466

467   **Competing Interest**

468   The authors declare that there are no competing interests.

469

473

474   **Data availability**

475   All data will be made available in JYX Digital Repository of the University of Jyvaskyla

476   upon acceptance.

477

478   **References**

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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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Figure legends

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

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

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Figure 3. Predicted mean probability of entering the pool section of a tank (a), latency to first entry to pool section (b), number of changes between stream and pool sections (c), and 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.

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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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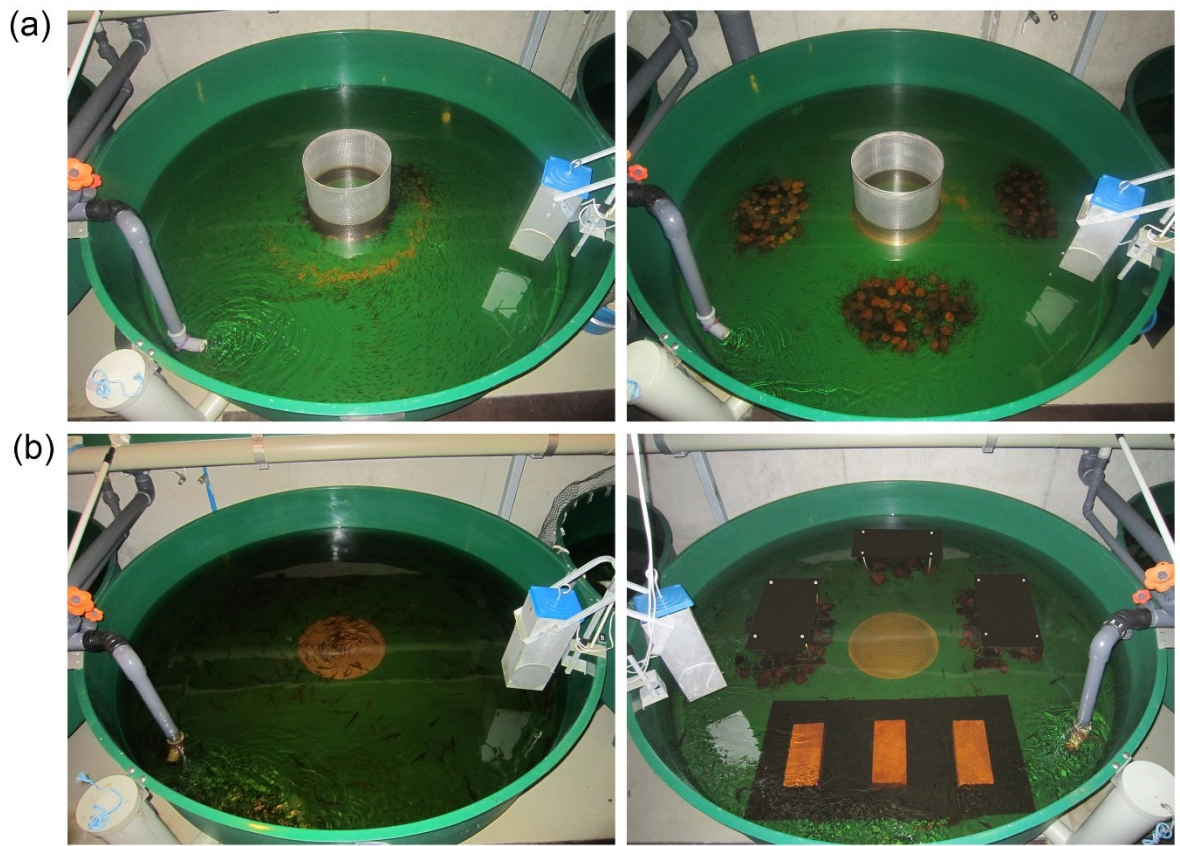
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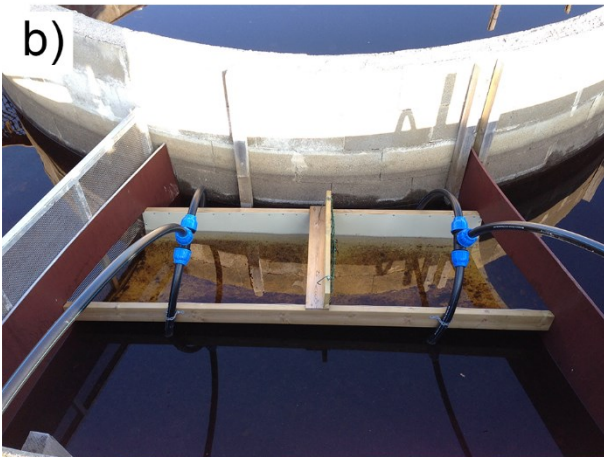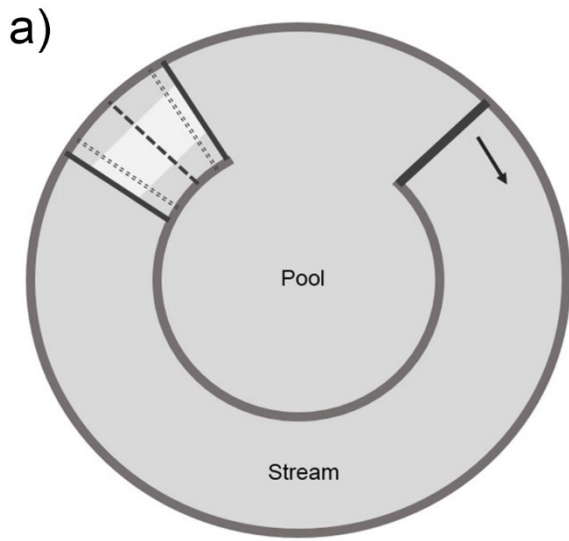836

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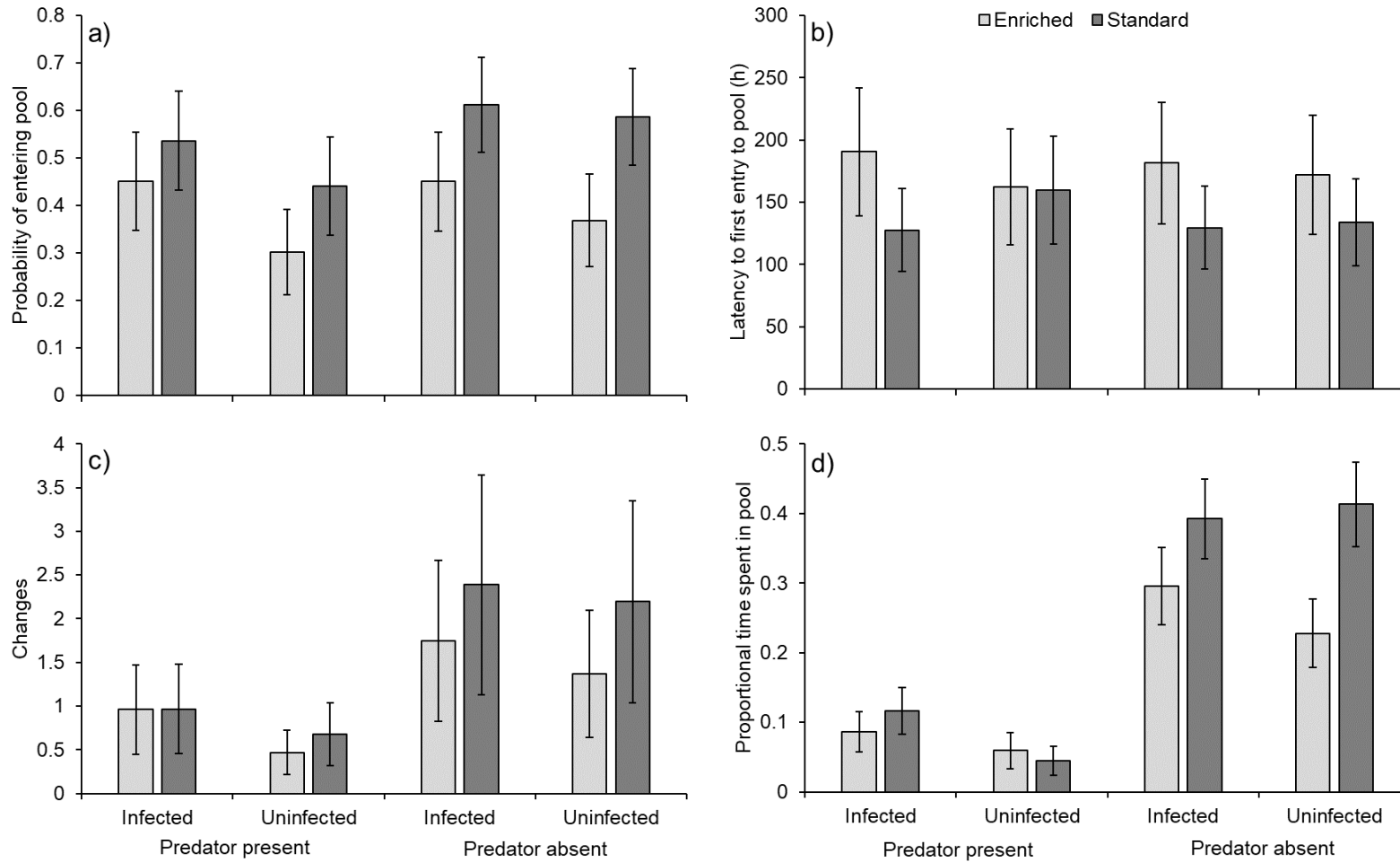842

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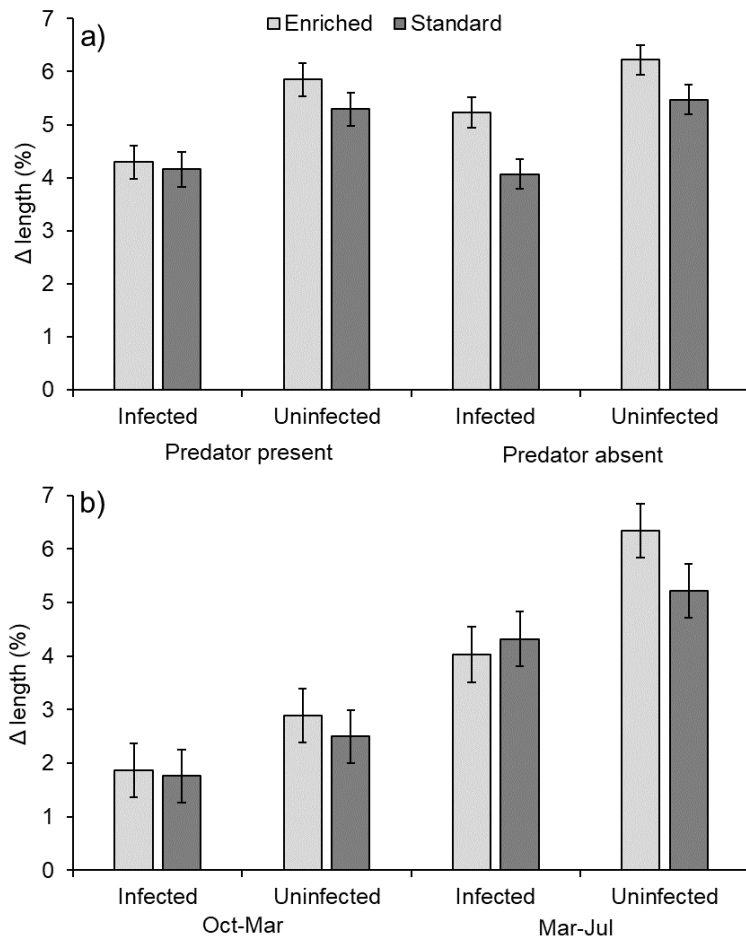
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Fig. 4

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
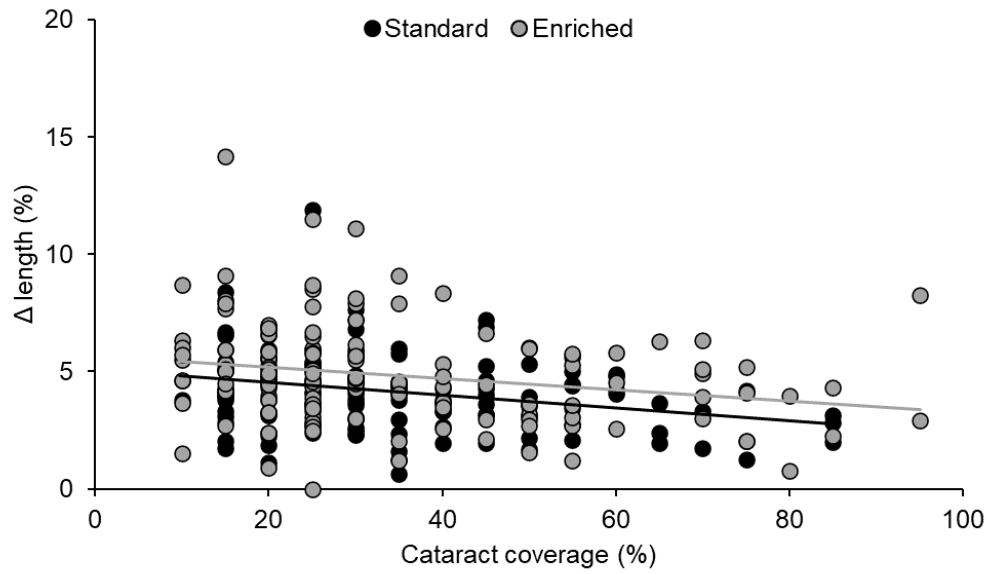
Figure S1. Relationship between eye cataract coverage caused by *Diplostomum pseudospathaceum*, and the percentage change in length in Atlantic salmon with standard and enriched rearing background in Experiment 1. The fitted lines are linear regressions to illustrate the direction of the relationships.
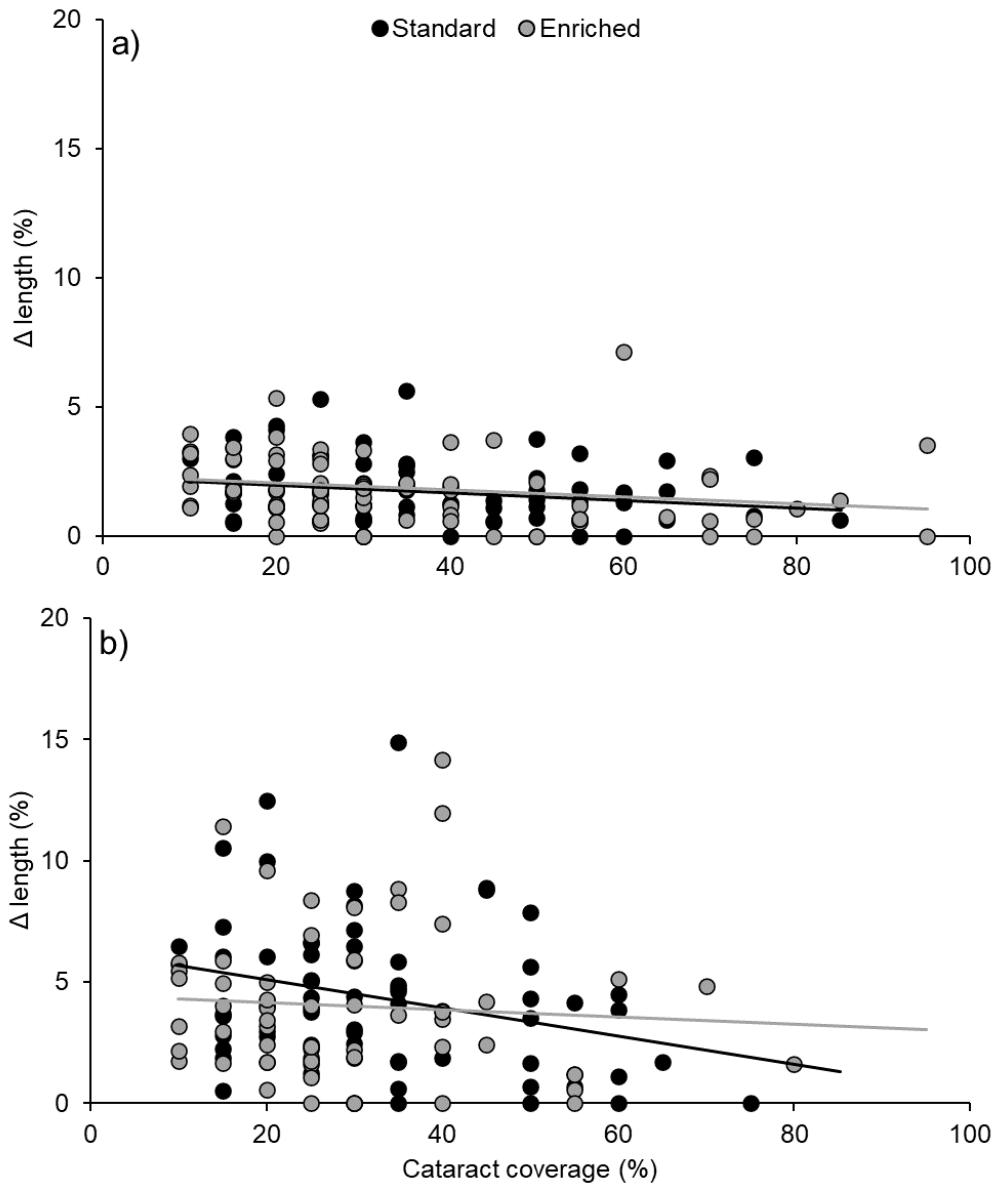
Figure S2. Relationships between eye cataract coverage caused by *Diplostomum pseudospathaceum*, and the percentage change in length in Atlantic salmon with standard and enriched rearing background in Experiment 2 in October 2017-March 2018 (panel a) and March-July 2018 (panel b). The fitted lines are linear regressions to illustrate the direction of the relationships.

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