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Yaripour, Sareh

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# Does enriched rearing during early life affect sperm quality or skin colouration in the adult brown trout?

**Sareh Yaripour[1*], Jukka Kekäläinen[1], Pekka Hyvärinen[2], Sirpa Kaunisto[1], Jorma Piironen[3], Anssi Vainikka[1], Marja-Liisa Koljonen[4], Jarmo Koskiniemi[5], Raine Kortet[1]**

[1]University of Eastern Finland, Department of Environmental and Biological Sciences, P.O. Box 111, FI-80101, Joensuu, Finland

[2]Natural Resources Institute Finland (Luke), Aquatic population dynamics, Manamansalontie 90, FI-88300 Paltamo, Finland

[3]Natural Resources Institute Finland (Luke), Aquatic population dynamics, Yliopistonkatu 6, FI-80100 Joensuu, Finland

[4]Natural Resources Institute Finland (Luke), Production systems, Animal genetics

[5] University of Helsinki, Department of Agricultural Sciences, FI-00014 Helsinki, Finland

*Corresponding author, E-mail: sareh.yaripour@uef.fi ; phone: +358 414790915

**Abstract**

Enriched rearing has been demonstrated to shape the phenotype of hatchery-reared salmonids and improve their post-release survival in the wild, thus having an important applied value in conservation. However, it is unclear if rearing conditions or survival selection during the early life stages induce long-term fitness effects on adult phenotypes. Using a paired full-sib set-up, we investigated the influence of the environmental enrichment at the egg and fry stages on the milt quality and skin colouration of the adult brown trout (*Salmo trutta* L.). Overall, males had a higher number of skin spots than females. Notably, the total numbers of spots and black spots were significantly lower in fish raised in an enriched environment than in their full siblings reared in a conventional hatchery environment. However, neither sperm motility nor sperm swimming behaviour differed between full-sib males reared in different environments. Our results suggest that rearing method may shape the colouration of brown trout, either by ecological carry-over effects or by selective survival during the rearing process. This, in turn, indicates that ecological conditions at early life can have long-prevailing phenotypically plastic or microevolutionary effects on the adult traits of fish. These effects should be taken into consideration to better understand the ecological role of rearing methodology in salmonid conservation.

**Keywords** Enriched rearing, Plasticity, Milt quality, Colouration, Conservation.

## 1. Introduction

Globally, a significant proportion of fish stocks is threatened by direct or indirect human impacts, including habitat degradation, pollution, and overexploitation (Myers et al., 2004; Barnosky et al., 2011; Näslund et al., 2014). In order to mitigate the negative impact of these anthropogenic stresses on natural fish populations, billions of captive origin fish are released to nature every year. However, accumulating evidence indicates that many hatchery-support programmes have failed to meet their original targets of increasing wild fish stocks (Brown and Day, 2002; Fraser, 2008). One of the most important reasons for these failures has been the reduced fitness of hatchery-reared fish in the wild (Araki et al., 2008). In accordance with this view, hatchery-reared salmonids show altered growth rates (Vainikka et al. 2010), decreased survival (McNeil, 1991) and lower reproductive success (Svåsand et al., 2000; Brown et al., 2003; Brockmark and Johnsson, 2010) in the natural environment compared to their wild conspecifics.

One of the primary factors behind the reduced fitness of hatchery-reared fish seems to be that the traditional hatchery practices often select for phenotypes that are well adapted to hatchery conditions, but maladapted to natural conditions (e.g. Araki et al., 2008; Saikkonen et al., 2011). Reduced fitness of hatchery-reared fish in the wild has also been linked to the absence of opportunities for learning critical life skills, such as predator avoidance, or foraging of natural prey in complex natural habitats (Brown and Laland, 2001; Johnsson et al., 2001; Christie et al., 2014). Furthermore, captive environments may favour maladaptive behaviours like altered aggressiveness and boldness (Deverill et al., 1999) that may increase vulnerability of hatchery-reared fish to predation (Kekäläinen et al., 2008; Roberts et al., 2011; Alioravainen et al., 2018).

Virtually all species respond to environmental changes by adjusting their phenotypes to prevailing environmental conditions (phenotypic plasticity), which may constrain natural and human-induced evolutionary processes. However, the effects of selection and phenotypic plasticity are often

difficult to disentangle (Hidalgo et al., 2014). Many studies have demonstrated that the impact of early life conditions on individual phenotypes can last throughout an individual's life span (referred to as ecological carryover effects) and can even be transferred to future generations through maternal and epigenetic mechanisms (Miner et al., 2005; Brockmark and Johnsson, 2010; O'Conner et al., 2014). Harrison et al. (2011) defined carryover effects as "events and processes occurring in one season that result in individuals making the transition between seasons in different states (levels of condition) consequently affecting individual performance in a subsequent period". A wide spectrum of such consequences from the individual level to community structure may appear in response to environmental changes (Harrison et al., 2011). Carryover effects that arise during hatchery rearing might thus play an important role in affecting the fitness of the hatchery origin fish in the wild (Araki et al., 2009). On the other hand, Araki et al. (2009) showed that also genetic effects of hatchery rearing may persist longer than for one generation in the wild despite natural selection tends to remove the least fit genotypes.

Recent studies suggest that enrichment of early rearing conditions may have positive carryover effects for the parasite resistance and post-release survival of hatchery-reared salmonids (e.g. Rodewald et al., 2011; Hyvärinen and Rodewald, 2013; Karvonen et al., 2016). Positive effects can occur also due to direct survival selection during rearing, as selective mortality during rearing is difficult to eliminate. Enriched rearing methods may include addition of physical structures (gravel and shelters) into the otherwise plain rearing tanks, irregular changes of water inflow, volume and direction, increase of variation in food particle size provided, and alterations in the feeding regimes (Karvonen et al., 2016). Importantly, environmental enrichment can shape the behaviour, survival, disease resistance, growth, and physiology of the salmonids in a way that is likely adaptive in nature (Roberts et al., 2011; Hyvärinen and Rodewald, 2013; Rosengren et al., 2017). Many of the effects are likely mediated by the lowered stress levels in enriched environments (Näslund et al., 2013). However, the influence of early

environmental enrichment on primary and secondary sexual traits have remained virtually unexplored despite their potential importance for the reproductive success of the stocked fish.

Skin pigmentation in fish has a crucial role in mate choice and camouflage (Parolini et al., 2018). Most pigment-based colours are produced by melanins (black, brown and grey colours) and carotenoids (red, orange and yellow colours). Melanin-based dark colours are synthetized by the animals and are assumed to be less sensitive to the environmental conditions than carotenoid-based colours (Badyaev and Hill, 2000). Melanin pigments have also been shown to be associated with numbers of behavioural and morphological traits as well as physiological functions (e.g. Roulin, 2016). Carotenoid-based bright colours instead cannot be synthetized by the fish but must be obtained along with diet and thus have been thought to signal the foraging success of the individuals. However, whether carotenoid-based ornaments could reflect the health and vigour of brown trout (*Salmo trutta*) has remained largely unclear (Parolini et al., 2018).

Melanin-based pigmentation typically functions as cryptic colouration (Wedekind et al., 2008). Accordingly, Maynard et al. (1995, 1996) have demonstrated that seminatural environments support the development of cryptic body colouration of salmon in a stream environment. Moreover, Donnelly and Whoriskey (1991) showed that cryptically coloured brook trout (*Salvelinus fontinalis*) encountered lower predation mortality compared to the fish that were not acclimated to the background colour. Furthermore, Chinook salmon (*Oncorhynchus tshawytscha*) that were reared in a seminatural environment, enriched with a cover and more natural stream structure, had better cryptic body colouration and 50% higher post-release survival than fish reared in conventional conditions (Maynard et al., 1995). Maynard et al. (1995) suggested that 25-50% of mortality during post-release migrations was explained by the individual differences in the development of camouflage and skin colouration. During the dispersal to new environments, the released brown trout face conditions that may differ in bottom substrate colourations and structures; therefore, development of spotting patterns influenced by

the environment can be an important factor in improving post-stocking survival. The main exception occurs during smoltification, when the migratory forms of salmonids prepare for pelagic environment and lose their carotenoid-based spots and dark lateral colouration.

Here, we investigated the impact of early environmental enrichment on the milt quality (primary sexual trait) and skin colouration (secondary sexual trait) of adult brown trout by partially controlling for direct survival selection by comparing differently reared full sibs. Our primary aim was to study if the rearing conditions during early life stages could induce ecological carryover effects on the phenotype of the fish as adults. Understanding possible responses of fish to early rearing conditions has potentially important implications for aquaculture and the production of high-quality fish for releases. In order to study this possibility, we produced full-sib families by artificial fertilisation and then reared the offspring of the same families both in replicated standard and enriched hatchery conditions. We predicted that fish would show differences in milt quality and skin colouration between the early rearing methods, because spermatogenesis and melanin production in the fish skin are physiologically linked to stress responsiveness (Campbell et al., 1992; Van der Salm et al., 2004; Kittilsen et al., 2009), potentially reflecting early environmental conditions. Finally, we predicted that the skin colouration and spotting pattern shows differences between sexes and thus might act as a secondary sexual ornament in trout (c.f. Wedekind et al., 2008).

## 2. Material and methods

### *Experimental fish and rearing treatments*

The brown trout is an economically important species, well-known for its evolution, adaptation to environmental challenges and high degree of intraspecific diversity (Kittilsen et al., 2009; Kocabas et al., 2016). It has also repeatedly been used as an ecological model organism for stock management and

conservation planning (Frank et al., 2011). Brown trout's life cycle typically includes juvenile stages in freshwater habitats, but adults can be both anadromous and potamodromous. Some individuals stay resident in their natal habitat for their whole life while others perform a feeding migration to a larger waterbody (Jonsson et al., 1989). The resident forms of brown trout have both dark and red spots for their whole life (thus the name brown trout), while the migratory (silvery) forms maintain mainly their dark spots during their feeding migration (e.g. Wedekind et al., 2008).

All animal experimentation was conducted in accordance with the Finnish National Animal Experiment Board's approval (ESAVI/2458/04.10.03/2011) and it meets the ABS/ASAB guidelines for the ethical treatment of animals and comply with the current Finnish legislation. The study was carried out in the Kainuu Fisheries Research Station (www.kfrs.fi) of Natural Resources Institute Finland (64° 23′ 20″ N 27° 30′ 23″ E) in 2012 – 2017. We first produced full sibling offspring (N = 32 families) by artificial fertilisation and reared the eggs and juvenile fish either in standard or enriched rearing conditions. Experimental fish originated from the Rautalampi water course hatchery-bred brood stock (wild fish originally captured from Äyskoski (63° 0′ 31.023″ N 26° 41′ 6.555″ E), Tyyrinvirta(62° 40′ 8.077″ N 26° 50′ 0.414″ E), Siikakoski (62° 37′ 0.140″ N 26° 20′ 29.925″ E) and Simunankoski (62° 22′ 49.874″ N 26° 10′ 30.904″ E). Fertilisations were performed on 11 October, 2012 from fifth and sixth-generation hatchery parents (16 males: 567 ± 28 mm, 2146 ± 285 g and 8 females: 576 ± 20 mm, 2262 ± 188 g) by crossing two females with four males in four independent fertilisation blocks (2 females × 4 males × 4 blocks = 32 families in total).

The rearing treatments began immediately after fertilisation (Fig. 1), when we divided 50 newly fertilised eggs from each of the 32 families into two rearing treatments (25 eggs in standard and 25 eggs in enriched rearing per family) resulting 1600 eggs in total: 800 eggs in standard and 800 eggs in enriched incubation. In the enriched rearing treatment, the eggs were incubated with grey-brownish gravel (Ø 30-50 mm), whereas in standard rearing treatment eggs were incubated without gravel in

grey trays. Incubation tray (0.16 $m^2$, height 20 mm, 3.5 mm mesh size) was similar in both rearing treatments. For egg incubation, we used 4 flow-through chutes (367 x 50 x 20 cm), three incubation trays in each chute: one tray for standard rearing treatment and two for enriched rearing. Each standard tray was divided into 8 blocks with round plastic frames (⦰ 10cm) giving 32 incubation units (25 full sib eggs per units). In enriched rearing 100 half sib eggs (from 1 female sired by 4 different males) were kept on one tray. The eggs from the standard rearing treatment were transferred into separate incubation tubes (⦰ 11 cm, one for each family) floating in two circular tanks (3.2 m2) on March 11 2013.

On 22 May 2013, the hatched fry were moved either in four 0.4 $m^2$ plain green (standard) tanks or in four identically coloured enriched tanks with 30-50 mm gravel (50% of the bottom surface). From 23 May 2013 onwards the fish were offered commercial feeds (Biomar INICIO Plus). On 6 August 2013, four grey-brownish stones (Ø 80-100 mm) were added in each of the four enriched tanks. Otherwise, the rearing conditions, such as feeding regime, water level, and water current were identical between the rearing treatments during the whole experiment. Water for each tank was taken from the nearby Lake Kivesjärvi, situated upstream of the facility. The water volume in all the tanks during the first two weeks was 80 L and was then raised to 160 L. Water flow between 23 May 2013 and 31 October 2013 varied between 12-17 litres per minute. Water temperature corresponded to natural fluctuations in the lake (2.6-19.0ºC).

Fish were maintained in the aforementioned rearing tanks until 31 October 2013, when we haphazardly selected 40 fish from each of the eight tanks (in total of 160 fish from standard tanks and 160 fish from enriched tanks) and tagged them under benzocaine anaesthesia (40 mg $L^{-1}$) with 12 mm HDX PIT tags (Texas Instruments Inc.) in the body cavity. A small fin clip sample (ca. 2 $mm^2$) was taken for the parental analysis (see below). The realized mean mortality was 24.1 % (± 5.18% SD, n = 274): in standard rearing treatment mortality was 22.99 % (± 6.46% SD, n = 148) and in enriched

194 rearing treatment 25.13 % (± 4.37% SD, n = 126) by 6 November 2013. During the first four months (1
195 November 2013 – 10 March 2014), all the pit-tagged fish were kept outdoors in eight similar semi-
196 natural streams (40 fish per stream) with constant directional flow and gravel bottom (Vainikka et al.,
197 2012). Standard- and enriched-reared fish were kept in four randomized separate tanks per treatment. In
198 10 March 2014, the fish were pooled and moved indoors into one 3.2 $m^2$ standard rearing tank, and, in
199 2 July 2014, they were further moved outdoors in one 50 $m^2$ standard concrete rearing tank in which all
200 the fish were kept for the rest of the study period (until 20 October 2017).
201
202 *Parental analysis and sample selection for sperm, skin spot, and colouration analyses*
203
204 In total of 826 individually pit-tagged fish were genotyped using a DNA-microsatellite panel of 16 loci
205 as in Koljonen et al. (2014). The family structure was solved with the COLONY-software package v.
206 2.0.6.2 (https://www.zsl.org/science/software/colony) (Wang, 2004; Wang and Santure, 2009; Jones
207 and Wang, 2010). Family structure was assessed using random mating model (Wang, 2016). The
208 analysis was run twice, using a medium run length. The results of the two runs were identical. Due to
209 the set-up, the numbers of potential sires and dams were sixteen and eight, respectively. For both sexes
210 polygamy was assumed as the mating system. No prior criteria was used for sibship size.
211 In October 2017, the within-family (i.e. standard vs. enriched reared) pairs of fish, identified by the
212 pit tags, were sampled for sperm motility, skin spot and colouration analyses (Table A.1). We controlled
213 for the genetic variation among families by randomly selecting standard vs. enriched-reared pairs of
214 individuals equally within the families. In total of 25 within-family pairs of females (25 fish from both
215 enriched and standard rearing) were selected from 16 families (one to three pairs per family). Similarly,
216 a total of 30 within-family pairs of males were selected from 21 families (one to four pairs per family).
217

*Fish measurements and gamete collection*

On 20 and 21 October 2017, the selected fish (50 females and 60 males) were anaesthetised with MS-222 (100 $mgL^{-1}$), stripped for their gametes (males) and then measured for their total length and body mass. Digital photographs were taken from the lateral side of all the fish with a digital single-lens reflex (DSLR) camera (Nikon D500) under constant lighting and exposure settings for later skin colour and ornamentation analyses. To prevent milt sample contamination (see below), genital pore area of each mature male was cautiously dried, and milt was stripped on individual petri dishes.

*Sperm motility analyses*

Sperm motility parameters were measured after stripping using computer-assisted sperm analysis (Integrated Semen Analysis System, Proiser, Spain) with B/W CCD camera (capture rate 60 frames $s^{-1}$) and negative phase contrast microscope (100 × magnification). In the analyses, 0.1 μL of milt was first added into two-chamber (chamber height, 20 μm; volume, 6 μL) microscope slides (Leja, Nieuw-Vennep, The Netherlands) and then the sperm cells were activated with 3 μL of 4°C natural water or with the pooled water:ovarian fluid mixture (1:1) of 10 females. Sperm motility parameters (curvilinear velocity, VCL; percentage of rapid sperm cells, % Rapid cells; and linearity of sperm swimming tracks, LIN) were recorded for 10 s and 40 s after the sperm activation (two replicate measurements/male/activation type).

*Skin spot and abdominal colouration analyses*

The number of red and black spots were determined by calculating the numbers of spots from two specified body areas (Fig. 2a). The number of total skin spots were determined by calculating the sum

of red and black spots. Abdominal colouration was measured with Image J program (version 1.51j8) from two separate body areas (Fig. 2b). Abdominal colouration was later determined using HSB colour coordinates (Hue, Saturation, and Brightness). Hue presents colour wavelength in a range from 0 ° to 360 °. Saturation defines the intensity of the colour, ranging from 0% to 100%, whereas brightness refers to the lightness (or darkness) of the colour and ranges from 0 (black) to 100 (white).

*Statistical analyses*

The effect of sex and rearing treatment on fish body mass, total length, skin spot numbers (black, red and total skin spots) and abdominal colouration was tested using linear mixed effect models (LMM). In these models, sex and rearing treatment acted as fixed factors and family × rearing tank -interaction as a random factor (to account for the common-environment effects within families). The effect of rearing treatment and sperm activation method on sperm motility was tested in otherwise identical model, but instead of sex, we added sperm activation method (water vs. ovarian fluid) as a second fixed factor. Assumptions of all the models were graphically verified using Q-Q plots and residual plots. Statistical analyses were performed using *lmerTest* package in R (version 3.5.1, R Foundation for Statistical Computing, Vienna, Austria).

## 3. Results

*Body mass and total length*

The mean size of standard reared brown trout (25 females and 30 males) was 1777.86 ± 447.29 (SD) g and 516.47 ± 40.57 mm, whereas the size of the enriched reared fish (25 females and 30 males) was 1749.11 ± 314.47 g and 514 ± 30.81 mm. Interaction effect between rearing treatment and sex was not

statistically significant (LMM, length: $df$ = 85.03, $t$ = -0.01, $P$ = 0.991; body mass: $df$ = 98.04, $t$ = 0.03, $P$ = 0.979), indicating that the effect of rearing treatment on body size was similar in both sexes. Neither body mass nor length differed between the rearing treatments (Table. 1). However, males were heavier than females in both standard and enriched groups, but total length did not differ between sexes.

*Sperm motility*

Interaction effect between rearing treatment and sperm activation method was statistically insignificant for all the measured sperm traits (LMM, VCL: $df$ = 71.96, $t$ = -0.23, $P$ = 0.82; LIN: $df$ = 84.70, $t$ = -0.02, $P$ = 0.985; % Rapid cells: $df$ = 73.89, $t$ = -0.15, $P$ = 0.880). There was no difference in the measured sperm traits (VCL, LIN, % rapid cells) between enriched and standard groups, but sperm had higher motility (VCL) in ovarian fluid than in pure water (Table. 2).

*Skin spot numbers and abdominal colouration*

In all skin spot models, the interaction effect between rearing treatment and sex was statistically insignificant (LMM, total skin spots: $df$ = 86.243, $t$ = 0.16, $P$ = 0.875; black spots: $df$ = 85.64, $t$ = 0.28, $P$ = 0.783; red spots: $df$ = 88.05, $t$ = 0.30, $P$ = 0.769), indicating that males had more spots than females in both rearing treatments. Standard-reared fish had higher number of total skin spots than their enriched-reared counterparts, and they tended to have more black spots (LMM, $df$ = 43.294, $t$ = -1.982, $P$ = 0.065, Table. 3, Fig. 3A), but there was no difference in the number of red spots between the rearing treatments (LMM, $df$ = 43.89, $t$ = -0.86, $P$ = 0.397). Males had higher number of black spots and more spots in total than females (Table 3, Fig. 3B), but the number of red spots did not differ

between sexes (LMM, *df* = 89.*25*, *t* = 0.59, *P* = 0.560). In abdominal coloration models, there was no interaction between rearing treatment and sex (LMM, hue: *df* = 92.954, *t* = 1.88, *P* = 0.065; saturation: *df* = 92.954, *t* = 0.71, *P* = 0.482). Mean hue and saturation of the abdominal colouration did not differ between the rearing treatments (Table. 4, Fig. 4A). Mean hue did not differ between sexes, but males had more saturated abdominal colouration than females in both rearing treatments (Fig. 4B).

## 4. Discussion

Brown trout that were reared in environmentally enriched conditions as juveniles had lower number of skin spots as adults than their standard-reared full siblings. This demonstrates that environmental conditions, including background colour, during early life-history can have long-lasting effects on adult phenotype. We also found that males had more skin spots and more saturated abdominal colouration than females in both rearing treatments. This provides support to the idea that, along with skin colouration, spot patterns may play a role in sexual selection in the brown trout (Wedekind et al., 2008). However, rearing method during the early life-history did not affect body size or milt quality of the adult fish. As our paired design within full-sib groups harmonised the genetic composition of fish between the rearing backgrounds, it is plausible that ecological carryover effects at least partially explained our results, while not completely excluding survival selection.

Animal pigmentation patterns generally have a strong heritability (Hoekstra, 2006; Colihueque, 2010), and melanin-based colours especially have been found to be genetically regulated with a heritability estimate of 0.83 in brown trout (Wedekind et al., 2008). However, contradicting results have been observed for heritability of carotenoid-based colour traits in the brown trout (Blanc et al., 1994; Wedekind et al., 2008). In the present study, using a paired design, we found that early rearing environment affected the number of melanin-based black spots, which indicates that the heritability of melanin-based colour patterns might be lower than has been assumed, or that strong within-family

317 survival selection operated on this trait during early rearing. The mortality rate was potentially large
318 enough to result in observable group differences if the mortality was selective with regard to the
319 colouration or any physiologically correlated trait. The contradiction between the current study and that
320 of Wedekind et al. (2008) can also result from different experimental designs. Our study population has
321 been bred for six generations in the hatchery while Wedekind et al. (2008) captured fish from their
322 natural spawning ground and raised them in a semi-natural environment. On the contrary, our
323 experiment was based on two different environments, in which the offspring were reared separately.
324 These two environments could have directly affected the formation of the background-matching cryptic
325 colouration (Donnelly and Whoriskey, 1991; Maynard et al., 1995). Fishes are known to show
326 adaptation to background as means as changes in skin colouration (Leclercq et al., 2010). Nevertheless,
327 early rearing environment seems to induce population-level effects that last at least several years.
328 Animal colouration is likely based on a complex genetic architecture (Greenwood et al., 2011)
329 and various colour patterns are known to have many critical functions both in intra- and interspecific
330 signalling. For example, colour ornaments can act as signals both in mate choice and intra-sexual
331 competition (dominance behaviour) and may also convey signals between predators and their prey, act
332 as species recognition signals, and offer camouflage (Protas and Patel, 2008). Melanin-based colour
333 patterns in salmonids have been thought to play particularly important role in camouflage (Westley et
334 al., 2013). Furthermore, in the brown trout, skin melanin concentration has been shown to be positively
335 associated with aggressiveness, and darker coloured males may have higher energetic costs of
336 reproduction than paler males (Jacquin et al., 2017). Melanin-based colours seem to act also as an
337 indicator for high stress tolerance as darker coloured males sire offspring with high tolerance to
338 stressful conditions (Jacob et al., 2010). Captive rearing conditions may favour more spotted salmonid
339 phenotypes, and indeed salmon raised in a farm environment have been shown to have a higher number
340 of spots than salmon raised in a river environment (regardless of their genetic origin) (Jørgensen et al.,

2018). This information, together with developments of enriched rearing methodology that may lower fish stress levels (Näslund & Johnsson 2014; Karvonen et al. 2016), could offer valuable implications for fish welfare in aquaculture. Interestingly, unintended selection in captive environments seems also to favour aggressive and bold phenotypes that have a good competitive ability in hatchery conditions but may have reduced fitness in the nature (Sundström et al., 2004; Saikkonen et al., 2011).

Together with these earlier findings, our results suggest that enrichment of early rearing environment might produce less aggressive and more 'natural' brown trout phenotypes (as signalled by their skin spot patterns). Such phenotypes may have lower fitness in standard rearing environments, but higher performance in the wild (Brockmark et al., 2007; Näslund et al., 2013). In the present study, fish from both rearing treatments were combined into one plain concrete pool for long-term rearing. After three years of maintenance in these conditions, no within-pair differences were detected in the size of the fish. Thus, any potential differences in competitive ability between the differentially treated fish might not have manifested in the low-density conditions used in our study, compared to typical fish densities in commercial hatcheries.

Besides demonstrating the effect of early rearing environment on the fish phenotype as a whole, we also found that males had a significantly higher number of spots than females. In general, earlier work has produced mixed evidence for sex differences in spotting patterns in salmonids (Agapova et al., 2002; Lin et al., 2008). Contrary to our finding, Kocabas et al. (2011) observed no sex difference in the spotting pattern of wild-captured sub-species of brown trout (*Salmo trutta macrostigma*). In our study population, males had more spots than females in both rearing conditions, indicating that spots act as secondary sexual signals and that the differences in early rearing environments may not affect the development of these traits.

The rearing conditions were not found to affect sperm motility (male primary sexual traits). Interestingly, sperm motility has repeatedly been found to be linked to male dominance in salmonids

(e.g. Rudolfsen et al. 2006). Given that the milt quality is largely dependent on nutrition (Rurangwa et al., 2004; Cabrita et al., 2014) and both fish groups had identical diet during the whole study period, this finding may not be surprising. Astuarino et al. (2001) reported that enriched diet pellet which included essential polyunsaturated fatty acids (PUFAs), caused a longer spermiation period, higher milt volume, and higher survival of embryo in male Sea bass, but did not have any effect on milt volume or embryo survival in the rainbow trout.

Some previous studies suggest that fish spot patterns may not be dependent on the environment (Kause et al., 2004). For example, Maynard et al. (1996) did not find difference in the number of dorsal spots between conventional hatchery and semi-natural rearing treatments in Atlantic salmon. However, there are studies indicating that early environmental factors can affect the development of spotting patterns in adult fishes (Blanc et al., 1982; Leclercq et al., 2010; Lehtonen and Meyer, 2011). These studies are well in line with our novel results showing that brown trout spots actually can be shaped by the hatchery environment. Different brown trout strains are known to differ in their colouration (Skaala and Jørstad, 1988; Aparicio et al., 2005), and in certain cases environmental factors, especially salinity and stress, can potentially affect the spotting pattern (Kocabas and Başçina, 2013). Koljonen et al. (2014) showed that the Finnish sea trout that mainly originate from large-scale stockings were generally more spotted than the wild Estonian sea trout populations.

To conclude, our study showed that the rearing method during early life-history can affect the distribution of adult skin colouration traits, either via ecological carryover effects or differential survival of siblings during egg and fry stages. Overall, our study suggests that increased number of black spots in brown trout might be an indicator of unintended acclimatization to standard hatchery rearing which is likely to be associated with changes in the physiology and behaviour of the fish.

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## Data availability

The original data of the study is available upon request from the corresponding author.

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## Tables

**Table 1.** General linear mixed effect model statistics for fish body mass and length.

| **Effects** | **Body mass** | | | **Length** | | |
|---|---|---|---|---|---|---|
| **Random** | $\chi^2$ | d.f. | *P*-value | $\chi^2$ | d.f. | *P*-value |
| Tank:Family | 0.01 | 1 | 0.914 | 0.91 | 1 | 0.341 |
| **Fixed** | *t* | d.f. | *P*-value | *t* | d.f. | *P*-value |
| Treatment | -0.38 | 25.83 | 0.709 | -0.26 | 24.48 | 0.800 |
| Sex | -2.66 | 98.90 | 0.009 | - 1.19 | 85.94 | 0.238 |

Statistically insignificant treatment × sex interactions were removed from the final model.

**Table 2.** General linear mixed effect model statistics for sperm motility parameters.

| **Effects** | **VCL** | | | **LIN** | | | **% Rapid cells** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Random** | $\chi^2$ | d.f. | *P*-value | $\chi^2$ | d.f. | *P*-value | $\chi^2$ | d.f. | *P*-value |
| Tank:Family | 7.64 | 1 | 0.006 | 1.67 | 1 | 0.196 | 3.90 | 1 | 0.048 |
| **Fixed** | *t* | d.f. | *P*-value | *t* | d.f. | *P*-value | *t* | d.f. | *P*-value |
| Treatment | -0.81 | 33.82 | 0.425 | -0.34 | 35.85 | 0.736 | -0.01 | 33.57 | 0.990 |
| Activation | 13.13 | 72.98 | < 0.001 | 9.91 | 74.26 | < 0.001 | 12.18 | 74.81 | < 0.001 |

Statistically insignificant treatment × activation method interactions were removed from the final model.

**Table 3.** General linear mixed model statistics for fish skin spot numbers.

| **Effects** | **Total skin spots** | | | **Black spots** | | | **Red spots** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Random** | $\chi^2$ | d.f. | *P*-value | $\chi^2$ | d.f. | *P*-value | $\chi^2$ | d.f. | *P*-value |
| Tank:Family | 6.91 | 1 | 0.008 | 8.70 | 1 | 0.003 | 7.52 | 1 | 0.006 |
| **Fixed** | *t* | d.f. | *P*-value | *t* | d.f. | *P*-value | *t* | d.f. | *P*-value |
| Treatment | -2.17 | 41.90 | 0.036 | 1.89 | 43.29 | 0.065 | -0.86 | 43.89 | 0.397 |
| Sex | -5.14 | 87.55 | < 0.001 | - 5.34 | 86.99 | < 0.001 | 0.59 | 89.25 | 0.560 |

Statistically insignificant treatment × sex interactions were removed from the final model.

**Table 4.** General linear mixed model statistics for fish abdominal colouration.

| **Effects** | **Mean Hue** | | | **Mean Saturation** | | |
|---|---|---|---|---|---|---|
| **Random** | $\chi^2$ | d.f. | *P*-value | $\chi^2$ | d.f. | *P*-value |
| Tank:Family | 2.76 | 1 | 0.097 | 3.77 | 1 | 0.052 |
| **Fixed** | *t* | d.f. | *P*-value | *t* | d.f. | *P*-value |
| Treatment | 0.95 | 41.03 | 0.347 | -0.81 | 44.14 | 0.420 |
| Sex | -1.34 | 93.29 | 0.182 | -11.36 | 93.98 | < 0.001 |

Statistically insignificant treatment × sex interactions were removed from the final model.

## Figure legends

**Figure 1.** A schematic diagram of experimental procedures.

**Figure 2.** Areas for skin spot calculation (a) and abdominal colour measurements (b). Number of skin spots (black spots, red spots and total spots) and abdominal colour were determined for two skin areas (1 and 2).

**Figure 3.** Skin spot numbers in different rearing treatments (a) and sexes (b). LMM, *: $P < 0.05$; ***: $P < 0.001$ (see also Table 3)

**Figure 4.** Hue (a) and saturation (b) values of abdomen skin area in different rearing treatments. LMM, ***: $P < 0.001$ (see also Table 4).

**Figure 1.**

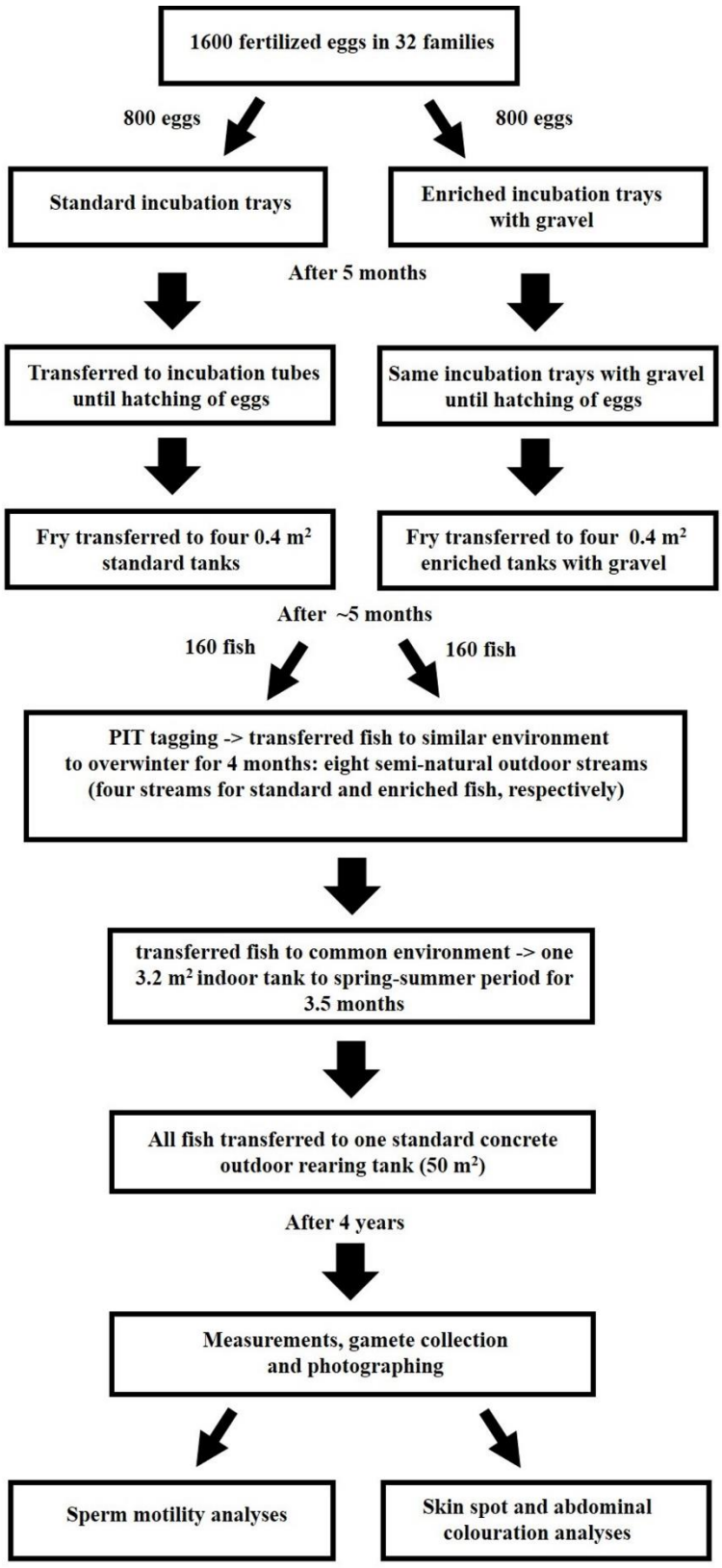

1600 fertilized eggs in 32 families
800 eggs
800 eggs
Standard incubation trays
Enriched incubation trays with gravel
After 5 months
Transferred to incubation tubes until hatching of eggs
Same incubation trays with gravel until hatching of eggs
Fry transferred to four 0.4 m² standard tanks
Fry transferred to four 0.4 m² enriched tanks with gravel
After ~5 months
160 fish
160 fish
PIT tagging -> transferred fish to similar environment to overwinter for 4 months: eight semi-natural outdoor streams (four streams for standard and enriched fish, respectively)
transferred fish to common environment -> one 3.2 m² indoor tank to spring-summer period for 3.5 months
All fish transferred to one standard concrete outdoor rearing tank (50 m²)
After 4 years
Measurements, gamete collection and photographing
Sperm motility analyses
Skin spot and abdominal colouration analyses

**Figure 2.**

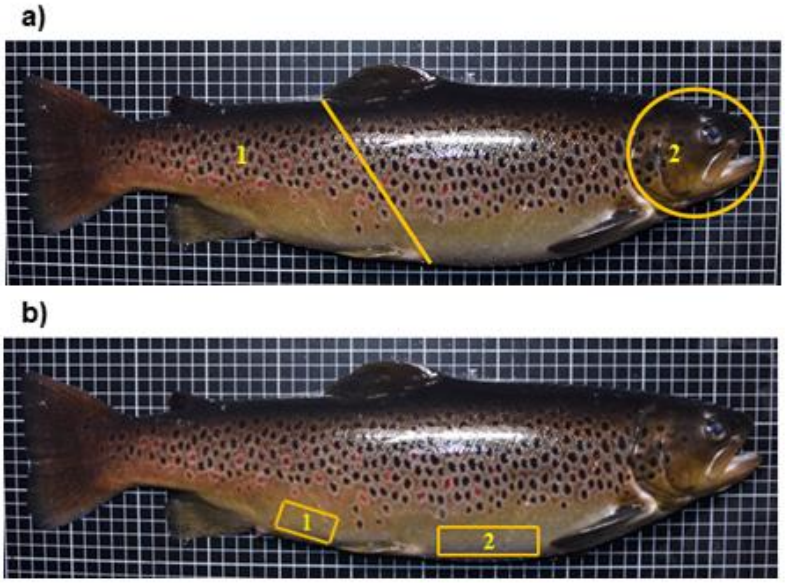
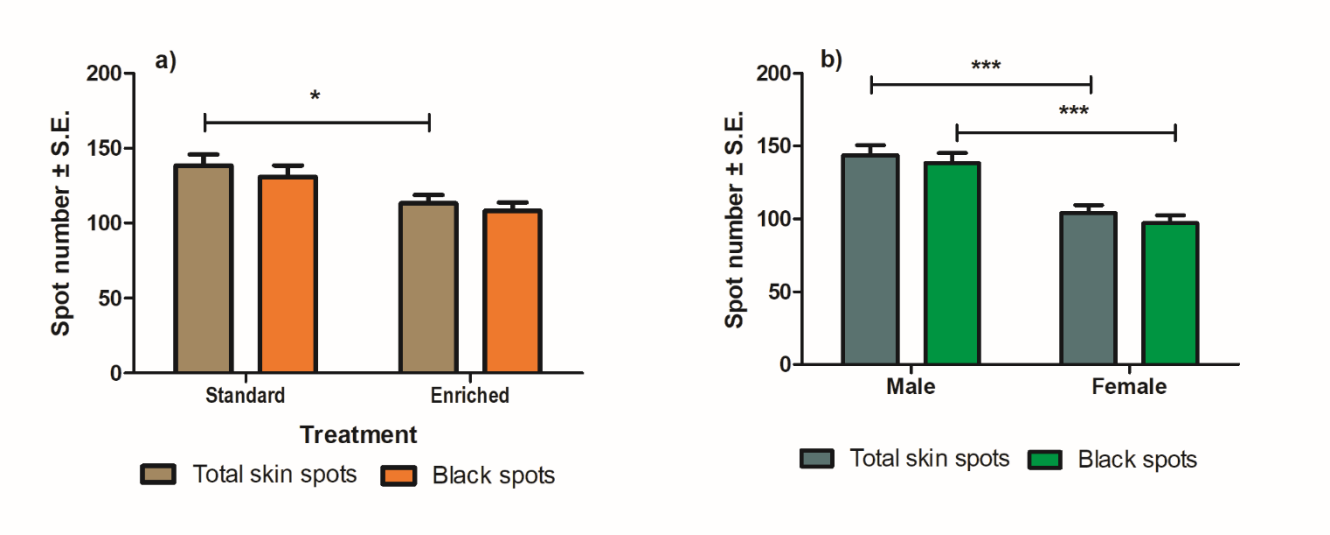
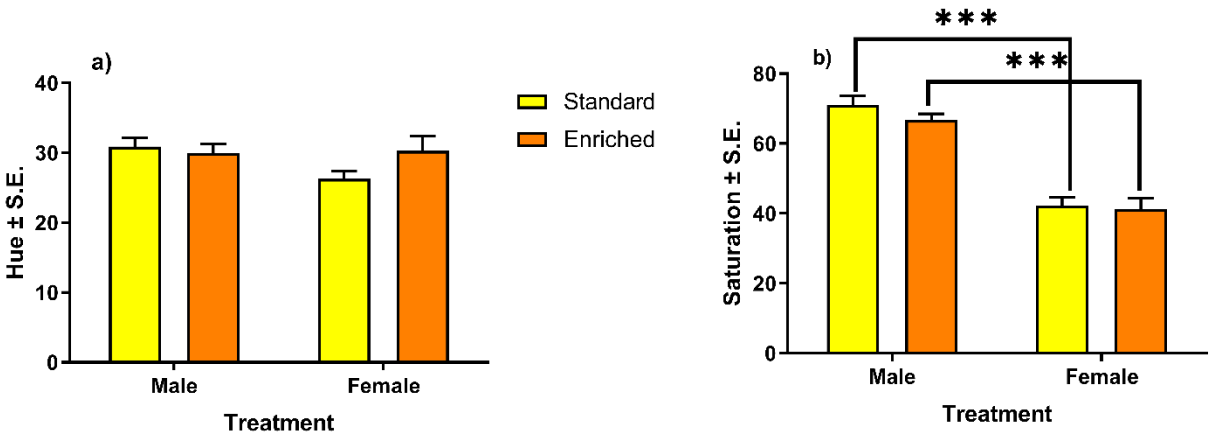


**Figure 3.**


a)
200
150
100
50
0
Spot number ± S.E.
*
Standard
Enriched
Treatment
Total skin spots
Black spots
b)
***
***
Male
Female
Total skin spots
Black spots

715 **Figure 4.**

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