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## Environmental enrichment reduces maladaptive risk-taking behavior in salmon reared for conservation

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## ABSTRACT

Hatcheries often produce bold fish that are maladapted to survive in the wild, as absence of predators and selection for fast growth tend to favour risk-taking behaviors. Not surprisingly, losses of hatchery fish through predation can be high immediately after release and this may account for the failure of many *ex-situ* fish conservation programmes. For supportive-breeding to be useful, it is essential that released fish are able to display natural behaviors. We compared the performance of juvenile Atlantic salmon reared in environmentally-enriched tanks receiving natural prey and subjected to simulated predator attacks with fish reared under standard hatchery conditions while keeping densities constant. No differences were detected between controls and environmentally enriched fish in survival, final size or nutritional status. Yet, changes in rearing conditions had rapid and marked effects on risk-taking behavior. Environmentally enriched fish were 2.1 times less willing, and took significantly longer to leave shelter, than controls within two weeks of enrichment. Thus our study indicates that it is possible through environmental enrichment to modify at least one component of fishes' behavior known to have clear adaptive implications, i.e. the propensity of hatchery-reared fish to take excessive risks. *Ex-situ* conservation could therefore benefit from rearing fish in naturalized, structurally complex environments with natural prey to promote the development of more natural behaviors.

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### 1. Introduction

Escalating human impacts have resulted in a severe reduction in biodiversity (Allan et al., 2005; Lévêque et al., 2008), leading to an increased reliance on reintroduction and captive-breeding programmes for restoring endangered and over exploited species (Seddon et al., 2007; IUCN, 2008). Captive rearing, however, can result in domestication (the adaptation of organisms to the captive environment) due to artificial selection, lack of behavioral feedbacks and abnormally high survival rates (Price, 1999; McDougall et al., 2006), rendering artificially-reared animals poorly suited to survive in the wild (Olla et al., 1994; Brown and Day, 2002).

Artificial rearing can have profound effects on behavior when animals are reared at unnaturally high densities or are isolated from natural stimuli (Farmed Animals Welfare Council, 1996; Garner, 2005). The simple housing cages, uniform rearing conditions, and predictable and localised food sources typical of captive rearing create a void in the animal's experiences that would otherwise be filled in the wild (Huntingford, 2004). The development of maladapted behaviors has long been recognized as one of the most pervasive shortcomings of artificial rearing (Dawkins, 2003;

Huntingford and Adams, 2005; Garner, 2005), and probably explains the failure of many animal reintroductions (Griffin et al., 2000; Seddon et al., 2007). Compared to wild individuals, captive-reared animals often forage less efficiently (Ellis and Nash, 1998; Sol et al., 2002), take longer to detect and avoid predators (Alvarez and Nicieza, 2003), and are less successful at locating and claiming quality territories (Deverill et al., 1999; Mathews et al., 2005). For example, artificially-reared bank voles (*Clethrionomys glareolus*) appear unable to use key food resources, while poor post-release survival of golden lion tamarins (*Leontopithecus rosalia rosalia*) has been attributed to poor locomotor and foraging skills developed in captivity (Stoinski and Beck, 2004). Recent evidence also indicates that artificially-reared fish can have smaller brains and lower cognitive abilities than their wild counterparts (Marchetti and Nevitt, 2003; Kihlslinger and Nevitt, 2006).

Perhaps the most striking effect of domestication across taxa (Price, 1999) is the reduced ability shown by captive-bred animals to react to environmental stimuli and to escape from predators (Koolhaas et al., 1999; Fischer and Lindenmayer, 2000; Armstrong and Seddon, 2007). While some changes induced by domestication appear to be the direct consequence of artificial selection and unnaturally high survival rates, others are probably the result of phenotypic plasticity and muted natural selection. Captive-reared animals can develop maladaptive behaviors compared to wild

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counterparts due to genetic differences arising from differential selection pressures (e.g. Fernö and Järvi, 1998; Petersson and Järvi, 2006), developmental and environmental effects (e.g. absence of copying and learning; Brown, 2003; Vilhunen et al., 2005), as well as from genotype by environment interactions (e.g. Bleakley et al., 2007; Sundström et al., 2007). Individuals with maladapted behaviors are not normally preyed on in captivity, and will therefore tend to persist under artificial conditions. But can the negative effects of domestication be avoided, or at least be reduced?

Recent advances in reintroduction biology have advocated the use of pre-release training as a means of counteracting some of the negative effects of domestication, and many *ex-situ* conservation programmes for mammals now make use of predator conditioning or some form of environmental enrichment prior to release (Maran et al., 2009). For example, predator conditioning now precedes the reintroduction of black-tailed prairie dogs (*Cynomys ludovicianus*) and tamar wallabies (*Macropus eugenii*), as this has been shown to enhance post-release survival (Blumstein et al., 2002; Shier and Owings, 2006). Yet, the use of environmental enrichment and predator conditioning in *ex-situ* conservation of other taxa is still very limited (e.g. Alberts, 2007). For example, amongst fishes, environmental enrichment has been shown to improve foraging efficiency (Brown, 2003) and enhance exploratory behavior (Berejikian et al., 2001; Braithwaite and Salvanes, 2005; Lee and Berejikian, 2008), which could also enhance post-release survival, but the application of enrichment in the conservation of highly fecund fish is still relatively rare, probably due to the difficulty of simultaneously conditioning a large number of individuals. Salmonids are probably the best studied group amongst fishes, and comparative results indicate that hatchery-reared salmon and trout typically survive 2–5 times worse in the wild than wild conspecifics (Aprahamian et al., 2003; Araki et al., 2008), largely due to increased predation (Jepsen et al., 1998; Henderson and Letcher, 2003; Kekäläinen et al., 2008) on individuals selected for fast growth (Fleming et al., 2002). Thus, while there appears to be considerable scope for enhancing post-release survival of salmonids and other fish via pre-release conditioning, it is not clear how enrichment could be scaled up to benefit the vast number of juveniles typically reared under real conservation scenarios.

To address this question, we reared juvenile Atlantic salmon (*Salmo salar*) in environmentally-enriched tanks provided with natural prey and subjected to simulated predator attacks (collectively referred to as 'enriched fish'), and compared their performance to fish reared under standard hatchery conditions while keeping densities constant. We assessed common measures of fish performance, such as growth, body condition, and fin damage, as well as boldness, measured as the time taken to leave shelter (Wilson and Stevens, 2005). Boldness is a context specific behavioral trait that is known to be influenced by different motivations, having both demographic and intrinsic properties (Wilson et al., 1993; Coleman and Wilson, 1998). We assayed the motivation to leave shelter because it tends to correlate well with other tests of boldness (Sneddon, 2003; Wilson and Stevens, 2005), and because it is known to differ substantially between wild and artificially-reared fish (Huntingford and Adams, 2005; Sundström et al., 2004) and other taxa (e.g. foxes Bremner-Harrison et al., 2004; prairie chicken, Cusato and Morrow, 2003), and can thus be expected to reflect behavioral deficits in captivity. We predicted that environmentally enriched fish would be more cautious and less variable in boldness than controls (Lee and Berejikian, 2008, but see Braithwaite and Salvanes, 2005 for the opposite effect), reflecting a greater similarity to wild fish and thereby demonstrating an improvement in anti-predatory behavior. We also expected the addition of conspecific chemical alarm cues (known to affect anti-predatory behavior, Chivers and Smith, 1998) to increase latency to leave shelter, particularly amongst fish reared under the

threat of predation. Ultimately, we wanted to test whether changes in artificial-rearing protocols could promote more naturalistic behaviors under real, large scale hatchery conditions, something that would be of value for *ex-situ* fish conservation programmes.

## 2. Methods

### 2.1. Environmental enrichment and predator conditioning

Atlantic salmon broodstock from the River Taff in South Wales (50 maiden males, 25 maiden females, plus 25 reconditioned female kelts) were stripped during the winter of 2005–2006 and their progeny reared for c. 12 months under standard hatchery conditions (5 m × 1 m GRP circular tanks, flow 120 l min<sup>-1</sup>, fed *ad libitum* on Skretting pellets via automatic feeders) at the Environment Agency Cynrig Fish Culture Unit (Powys, Wales). From 18th to 24th June 2007, 12,088 first generation hatchery juveniles (1+ parr, mean body mass 29.3 g) were haphazardly selected from six tanks containing a mixture of 50 families and randomly allocated to four identical 5 m × 1 m circular GRP tanks (3022 fish per tank; loading density = 6.1 kg m<sup>-3</sup>; average flow rate = 120 l min<sup>-1</sup>), and batch-tagged in the snout using coded wire tags (NMT, Shaw Island, USA) as part of the Environment Agency restoration program in the River Taff. Two tanks were randomly chosen to be enriched and two tanks were kept under standard hatchery rearing conditions (as above) to serve as controls.

On 19th July (10 days before the first behavioral assay) we enriched the two experimental tanks by anchoring ten branches of Lawson cypress (*Chamaecyparis lawsoniana*) in the bottom of the tanks (to provide submerged cover) and by hanging brown and green camouflage netting on top (to provide c. 30% overhead cover). We also added 10 g of frozen bloodworms (Chironomidae) at random through one of four equally spaced plastic pipes ten times a day to provide natural prey stimuli. The purpose of this was to encourage fish to forage throughout the water column as opposed to the surface, a common maladaptive feeding strategy observed within hatchery fish (Tatara et al., 2008). The addition of natural prey amounted to less than 5% of total food rations and, coupled with the simulated predator attacks (see below), was designed to provide natural stimuli, encourage foraging skills and discourage surface orientation, not to increase food intake. Primary feeding in all tanks was achieved via commercial feed (Skretting) dispensed through Arvotec automatic feeders at a rate of c. 2.4% of body weight day<sup>-1</sup>. This ensured feeding to or near satiation according to manufacturer's guidelines. Although, ostensibly, enriched fish received c. 5% more food than controls, control and enriched fish did not differ in survival, condition factor or final weight at the end of the study (see Section 3).

Enriched and control tanks were cleaned daily in the same way, which consisted in flushing away settled sediment and removing any mortalities. Natural cypress branches used in the enriched tanks were replaced by new ones on two occasions (8th August and 1st September 2007). For this, the tanks were half drained, the branches were replaced by new ones and the tanks quickly refilled. This procedure was replicated within the control tanks by draining them for the same duration as the enriched tanks, and by moving a hand net which imitated the removal of branches thereby ensuring similar levels of disturbance.

To condition fish to the presence of predators, we used a full size plastic model of a heron (*Ardea cinerea*), a common predator of juvenile salmonids. The head of the heron was submerged into the surface of the tank several times in rapid succession, immediately after which 100 ml of a solution of conspecific alarm cues (see below) was injected into the tank via a syringe. This was repeated three times a day in each enriched tank to simulate three series of

predator attacks. The chemical alarm cues were made according to Ferrari et al. (2008). Briefly, two fish of approximately 140 mm in fork length were sacrificed each day from each of the enriched tanks with a rapid blow to the head (in accordance with HO schedule 1). A cut was then made with a scalpel behind the gill cleft and along the ventral surface and c. 2 cm<sup>2</sup> of skin was removed, taking care not to remove any muscle or fat as these may trigger different behavioral responses. The skin was then macerated to release alarm cues from specialized skin cells via mechanical damage. It was then suspended in 300 ml of tank water at ambient temperature and stirred for 30 min. This was repeated daily for the duration of the study.

Environmental enrichment and predator conditioning were done concurrently, beginning on 18th July and finishing on 18th September 2007, hence for a total of just over 2 months. This is referred to simply as 'enrichment'. Test-water temperatures during the study period ranged from 10 to 14 C.

## 2.2. Behavioral analysis

We measured latency to come out of a hide as a measure of boldness, as this has been shown to correlate well with risk-taking behaviors (Sneddon, 2003; Wilson and Stevens, 2005). The experimental set ups consisted of two identical hatchery troughs (280 cm L × 39 cm W × 16 cm D) fitted with start boxes (16 cm L × 39 cm W × 16 cm D) at the outflow to serve as hides (Braithwaite and Salvanes, 2005). The start boxes afforded 50% overhead cover and were fitted with gates that could be operated remotely via a pulley. The experiments were conducted under natural light conditions, and the experimental arenas were shielded from observers to prevent disturbance. Flow rates in each trough were maintained at c. 19 L min<sup>-1</sup> via submerged perforated spray bars at the inlets, resulting in a surface velocity of 10–13 cm s<sup>-1</sup> and even flows to each test arena. During the behavioral assays, two fish were haphazardly dip-netted from the four rearing tanks, and randomly allocated (using a pre-established allocation based on a random number generator) to one of the two test arenas. Following a 15 min acclimatisation period, the doors in each start box were slowly lifted and the time taken for fish to leave the shelter completely (entire body) was recorded with a stop watch. If the fish had not come out within 15 min they were allocated the maximum time score of 900 s. A 15 min observation period had previously been shown to be adequate for studies of anti-predatory behavior in another salmonid (Vilhunen and Hirvonen, 2003).

Forty-six fish from each tank were assayed for boldness under two experimental conditions (blank water and water scented with conspecific alarm cues) on three time periods spaced approximately 3 weeks apart: on 27 July–7 August (9–20 days after enrichment begun), on 13–25 August (26–38 days since enrichment) and on 6–18 September (50–62 days since enrichment). In total we assayed 1104 fish in a fully balanced design. The use of two parallel experimental troughs meant that we could assay between 26 and 36 fish per day (30 fish on an average day). Each fish was tested only once and the order of treatments was randomly selected on each occasion. We chose to test fish singly to maintain statistical independence and because juvenile Atlantic salmon are territorial and so risk-taking behavior may be expected to be primarily an individual, rather than a group decision.

To test for response to conspecific alarm cues, alarm cues were made in a similar way as for predator conditioning described above. One litre of alarm cue solution (made from a fish originating from the same tank as the test fish) was dosed from an IV drip bag positioned at the upstream end of the test arenas, out of sight of the fish. Twenty seconds before the door was released the scent was added at a rate of 44 ml min<sup>-1</sup>. Following each behavioral assay, the two test arenas were drained, cleaned, and carefully

washed with 90% ethanol to prevent carry-over effects from previous scents (Vilhunen and Hirvonen, 2003).

## 2.3. Nutritional status and body condition

Following each of the three sampling periods, a sample of 30–46 fish per tank were humanely killed by an overdose of anaesthetic, measured (fork length, mm), weighed (wet weight, g), sexed, and their condition factor, mesenteric fat score (Rikardsen and Sandring, 2006) and stomach fullness score (Johnsson et al., 1999) visually determined on a scale of 0 (nil) to 3 (full). A health score was calculated by assessing the degree of opercular (left, right) and fin (caudal, dorsal) erosion on a scale from 0 (nil) to 3 (heavily eroded) using a procedure similar to that described in Hoyle et al. (2007). Individual erosion scores were summed up to obtain an aggregated health score (ranges 0–12). The left and right pectoral fins were measured with callipers to the nearest 0.1 mm.

## 2.4. Pilot study and power analysis

Because the study was conducted under realistic, high density hatchery conditions the number of replicate tanks available was limited to two per treatment. Therefore a preliminary pilot study was conducted to determine statistical power and to check for potential tank effects. A sample of 46 fish, haphazardly dip-netted from each tank ( $n = 184$ ), was assayed for boldness and morphometric variation on 6th–18th July 2007, before enrichment began. There were no statistical differences in boldness ( $F_{3,180} = 2.091$ ,  $P = 0.103$ ), body size ( $F_{3,180} = 2.623$ ,  $P = 0.052$ ), health score (KW test = 0.439,  $df = 3$ ,  $P = 0.932$ ), condition factor ( $F_{3,180} = 1.279$ ,  $P = 0.283$ ), mesenteric fat (KW test = 1.160,  $df = 3$ ,  $P = 0.763$ ) or stomach fullness (KW test = 1.577,  $df = 3$ ,  $P = 0.665$ ) among tanks at the beginning of the experiment, indicating that our random assignment of fish to tanks had been effective. There were also no differences in boldness between control tanks and tanks designated for enrichment ( $F_{1,179} = 1.449$ ,  $P = 0.230$ ), but fish destined to be reared under enriched conditions were 3.8 mm larger on average (mean = 132.1 mm) than control fish (mean = 128.3 mm) at the beginning of the study ( $t_{182} = -2.366$ ,  $P = 0.019$ ), had slightly higher condition factor (enriched = 1.29; controls = 1.32;  $t_{182} = 1.984$ ,  $P = 0.049$ ) but did not significantly differ in weight (enriched = 30.39 g; controls = 28.28 g;  $t_{182} = 1.984$ ,  $P = 0.068$ ). From the pilot study, we estimated that we would be able to detect a 60 s. difference in mean latency between treatments with at least 80% power.

## 2.5. Statistical analysis

Fish were tested singly in each test arena, and were tested only once. Therefore we are confident that the conditions of statistical independency were met.

To model variation in latency, we employed a mixed-effects factorial ANOVA with latency as the dependent variable, treatment (control vs. enriched) and test odour (control vs. scented with alarm cues) as fixed factors, tank (nested within treatment) as random factor, and body length (Log<sub>10</sub>-transformed), water temperature (C) and duration of enrichment (days) as continuous covariates. Latency to leave shelter was square-root transformed to improve normality and homogeneity of variances and only significant terms and interactions were retained in the final, reduced model.

As in most studies of boldness (e.g. Brown et al., 2005), we assigned a maximum ceiling value (900 s in our case) to those fish that did not come out of the hide for ANOVA. However, because latency data from such fish may introduce errors due to right-censoring, we also employed a Cox proportional hazards function

(Budaev, 1997) to properly model the effects of environmental enrichment and alarm cues on truncated boldness data. Also, in order to make the most efficient use of data provided by fish which did not leave the shelter (and which therefore provided only right-censored data on latency) we classified fish as 'bold' or 'shy' depending on whether they emerged or remained in the shelter within the allocated 15-min time period, respectively; we then employed binary logistic regression to model the effects of treatment, test odour, and duration of enrichment on willingness to leave shelter using SPSS 16.0.

Variation between treatments in health scores (fin and opercula erosion) and nutritional status (mesenteric fat and stomach fullness) was tested by non-parametric Kruskal–Wallis analysis of variance. General linear models (GLMs) were used to evaluate how environmental enrichment affected survival, growth, condition factor, and size-adjusted fin lengths. Body size (fork length, mm; weight, g), pectoral fin lengths (mm) and condition factor were log<sub>10</sub>-transformed, while survival (%) was arcsine transformed to improve normality and homogeneity of variances.

We used the coefficient of variation (CV) to examine the extent of individual variation in performance traits, and employed Levene's test to compare homogeneity of variances between controls and environmentally enriched fish at the end of the study.

## 2.6. Ethical note

The research described here was discussed at Swansea University Research Ethics Committee and conducted in consultation with the UK Home Office Inspector. A pilot test was used to refine sample sizes, which were kept to a minimum for the desired statistical power. No physical contact occurred during conditioning with a simulated model predator, and fish were able to hide in refuges when alarm cues were released into the rearing tanks, as well as during the boldness tests. Survival during the study was very high (average = 98.3%) and not different from survival figures typically found at the Cynrig hatchery.

## 3. Results

### 3.1. Survival

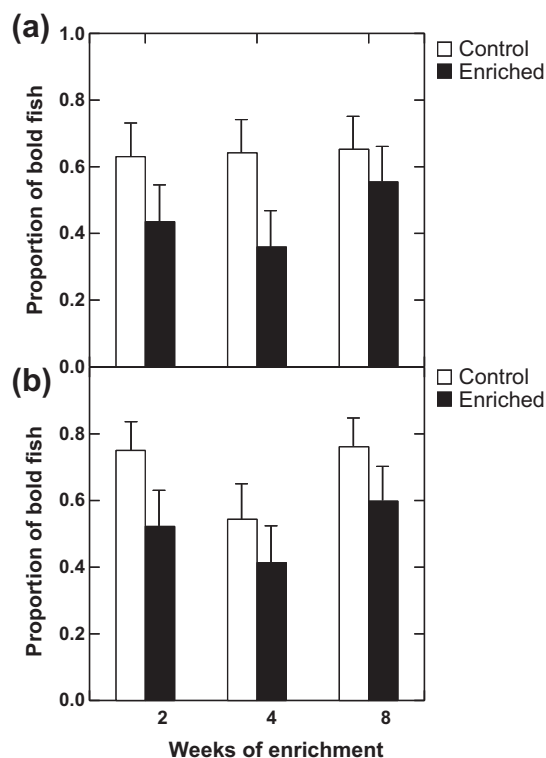
Survival in the tanks during the course of the study was very high (mean = 98.3%, SE = 0.24), and not significantly different ( $F_{1,2} = 10.151$ ,  $P = 0.086$ ) between controls (mean = 97.9%) and experimentally enriched tanks (mean = 98.7%).

### 3.2. Growth

Fish reared under environmentally enriched conditions were statistically longer than controls at the end of the experiment (enriched – 141.7 mm, controls – 139.8 mm  $F_{1,364} = 4.127$ ,  $P = 0.043$ ), though the difference was very small (2 mm, or c. 1.4% size difference), and this largely reflected the initial size differences at the beginning of the study. Thus, estimated specific growth rates (in length) were 7.27% for enriched fish and 8.96% for control fish. There was no difference in final mean weight (enriched – 36.7 g, controls – 35.6 g,  $F_{1,363} = 1.889$ ,  $P = 0.170$ ) or in condition factor (enriched – 1.28, controls – 1.29,  $F_{1,363} = 1.294$ ,  $P = 0.256$ ) between treatments.

### 3.3. Willingness to leave shelter

Results of logistic regression indicated that willingness to emerge from the shelter (Fig. 1) was significantly affected by rearing conditions (Wald statistic = 37.281,  $df = 1$ ,  $P < 0.001$ ), but not by



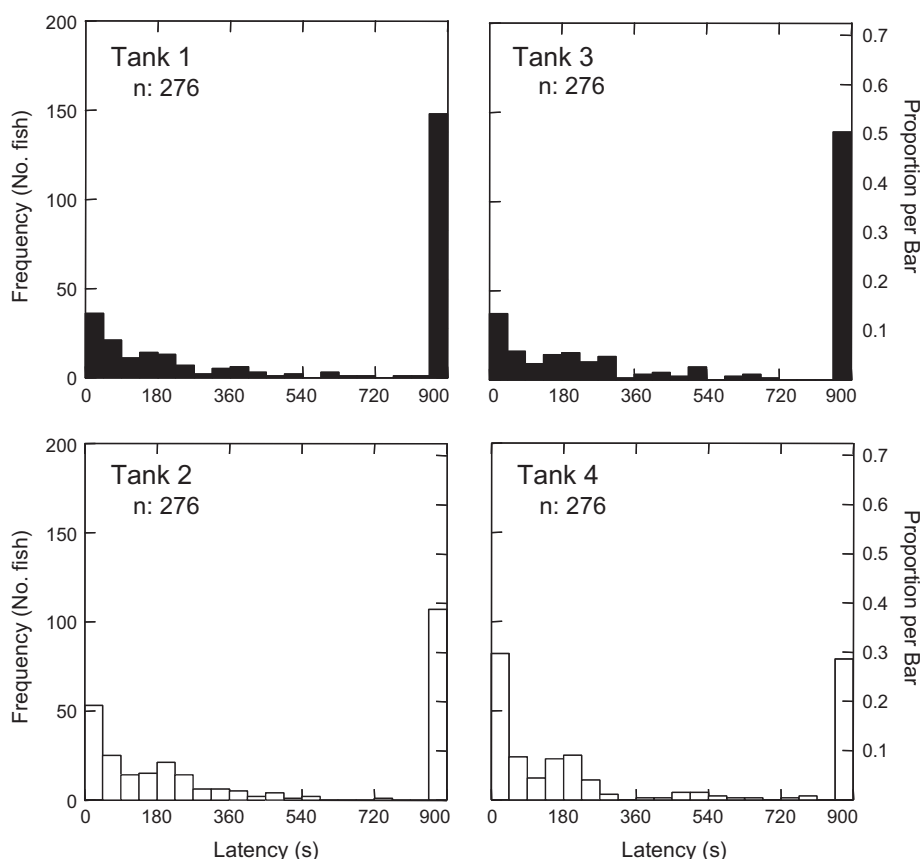
**Fig. 1.** Proportion of juvenile Atlantic salmon coming out of a hide after 15 min ('bold' fish, binomial 95% CI's) in relation to duration of rearing (weeks of enrichment) and rearing environment (□ control hatchery tanks, ■ environmentally-enriched tanks) when tested under (a) blank water and (b) water scented with conspecific alarm cues. Environmentally enriched fish are less willing to emerge from the shelter than controls ( $P < 0.001$ ).

test odour (Wald statistic = 3.220,  $df = 1$ ,  $P = 0.073$ ) or days of enrichment (Wald statistic = 3.418,  $df = 1$ ,  $P = 0.064$ ). Analysis of odds-ratios indicated that environmentally enriched fish were 2.1 times more likely to remain in the shelter than controls (95% CI = 1.675–2.727).

### 3.4. Latency to leave shelter

Observed variation in latency to leave shelter was consistent with a behavioral continuum between two extreme strategies, with the majority of fish leaving shelter within the first 60 s or remaining in hiding for the full duration of the assay (Fig. 2), seemingly depending on treatment and tank identity. Results of mixed nested ANOVA (Table 1) indicated that fish reared under enriched conditions took significantly longer to leave the shelter than controls (Fig. 3a). In contrast, fish tested with water scented with conspecific alarm cues took significantly less time to leave the shelter than controls tested with blank water (Fig. 3b). Latency also differed with tank identity, duration of rearing, and water temperature, but not with body size. Fish took significantly longer to leave shelter at warmer temperatures, although the range in test-temperature during the study was only 4 °C (from 10.0 to 14.0 °C), and the effect was very small ( $r = +0.099$ ,  $P = 0.001$ ).

None of the two-way interactions were significant, indicating that control and environmentally enriched fish responded in the same way to the presence of alarm cues and to duration of rearing. Response to alarm cues was also unaffected by rearing time. Differences in latency between control and enriched tanks were apparent within the first two weeks of enrichment (Fig. 4), suggesting that behavioral conditioning took place very rapidly.



**Fig. 2.** Variation in latency to come out of a hide (s) of juvenile salmon reared under control (□) and enriched (■) conditions. Individuals which did not come out of the hide after the 15 min test period were given the maximum 900 s. score.

**Table 1**  
Mixed effects ANOVA on latency to come out of the hide (square-root transformation). Treatment (control vs. enriched) and test odour (blank vs. water scented with conspecific alarm cues) were used as fixed factors and tank identity was treated as a random factor nested within treatment. Temperature, duration of rearing, and fish size were used as covariates. Fish length ( $P = 0.634$ ), and the three two way-interactions treatment  $\times$  test odour ( $P = 0.606$ ), treatment  $\times$  duration of rearing ( $P = 0.380$ ) and test odour  $\times$  duration of rearing ( $P = 0.547$ ) were non-significant and were excluded from the final, reduced model.

Source	SS	df	MS	F-ratio	P
Treatment	5017.961	1	5017.961	49.263	0.000
Test odour	564.790	1	564.790	5.545	0.019
Duration of rearing	1061.973	1	1061.973	10.426	0.001
Water temperature	958.745	1	958.745	9.412	0.002
Tank effect (treatment)	993.529	2	496.765	4.877	0.008
Error	111740.178	1097			

Analysis of latency data by Cox proportional hazards regression confirmed the results of ANOVA, and indicated that fish reared under environmentally enriched conditions took longer to emerge from the shelter than controls (Fig. 5a;  $P < 0.001$ ), and that fish tested with water scented with alarm cues left the shelter sooner than controls tested with blank water (Fig. 5b,  $P = 0.029$ ).

3.5. Health scores and nutritional status

At the end of the study there was no difference in health scores (Mann–Whitney = 6686.5,  $df = 1$ ,  $P = 0.365$ ), amount of mesenteric fat (Mann–Whitney = 8028.5,  $df = 1$ ,  $P = 0.332$ ) or stomach fullness (Mann–Whitney = 7173,  $df = 1$ ,  $P = 0.322$ ) between control fish and fish reared under environmentally enriched conditions. Likewise,

there was no difference in the size-adjusted length of the pectoral fins between controls and enriched fish (left pectoral,  $F_{1,234} = 0.076$ ,  $P = 0.782$ ; right pectoral  $F_{1,234} = 0.452$ ,  $P = 0.502$ ).

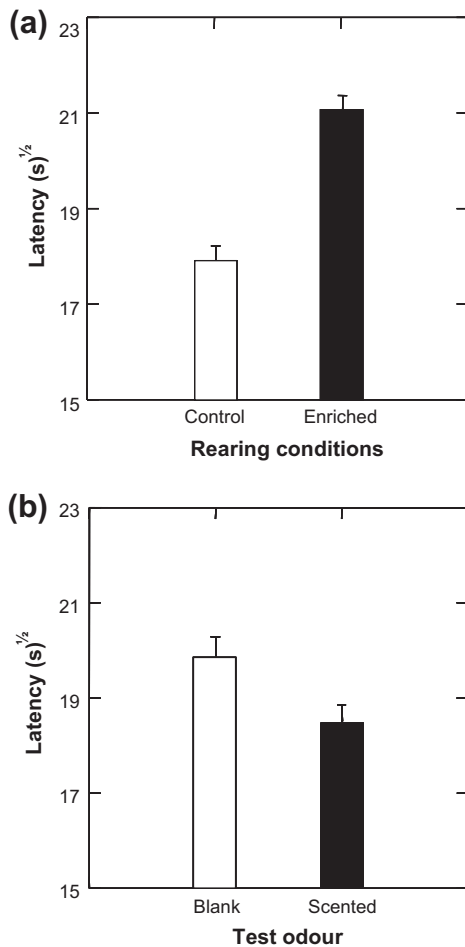
3.6. Extent of individual variation

Environmental enrichment had no significant effects on individual variation in fork length (Levene's test  $P = 0.0971$ ), weight (Levene's test  $P = 0.960$ ), or condition factor (Levene's test  $P = 0.052$ ).

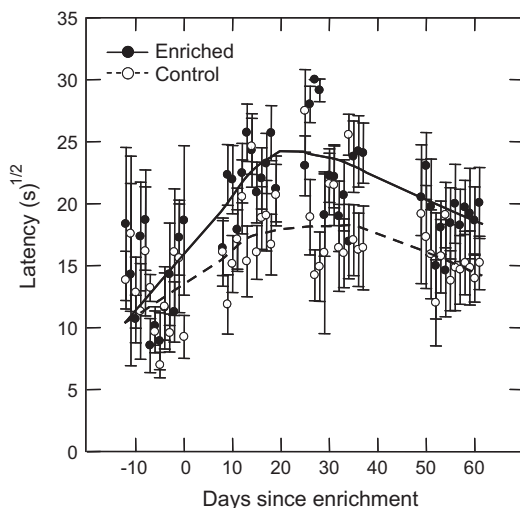
4. Discussion

The results of this study reveal that fish reared in enriched hatchery tanks with natural prey and under the threat of predation take fewer risks than fish reared under standard hatchery conditions. Such difference was observed throughout the study period, and was not affected by fish densities, which were similar for each treatment throughout the experiment. Fish reared under environmentally enriched conditions were not only less willing to leave shelter, but those that emerged from shelter did so considerably later than control fish. This implies that a clearly identifiable behavioral transformation had occurred, with enriched fish adopting a less risky strategy, comparable to that of their wild counterparts (Johnsson et al., 2001; Alvarez and Nicieza, 2003).

Relaxed natural selection in captivity tends to increase individual variation in maladaptive behaviors such as extreme boldness (Cusato and Morrow, 2003; McPhee, 2003; Lee and Berejikian, 2008; but see Braithwaite and Salvanes, 2005), and our study shows that this can also be reversed by appropriate environmental stimuli. Purging of extreme risk-taking behaviors should have

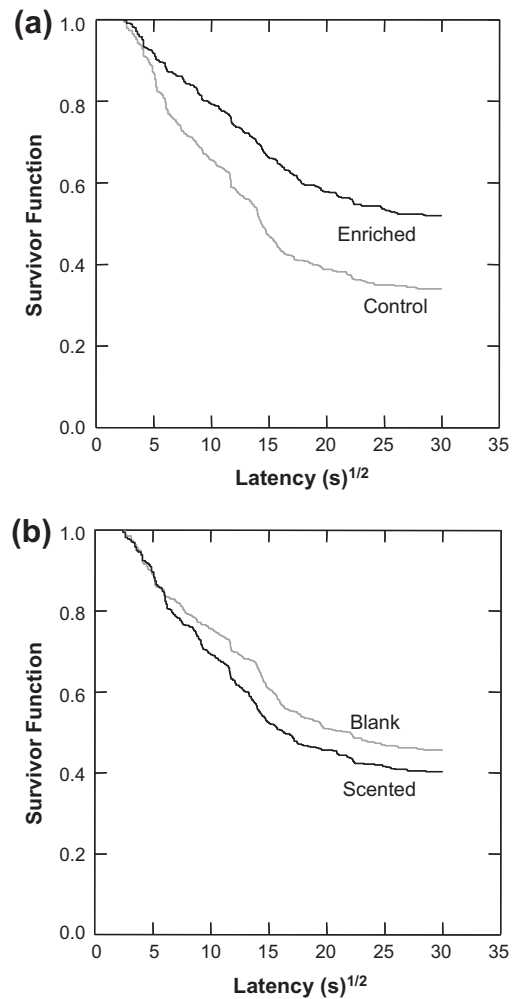


**Fig. 3.** Least squares marginal means ( $\pm$ S.E.) of latency (seconds, square-root transformation) of juvenile Atlantic salmon to come out of a hide in relation to (a) rearing environment ( $P < 0.001$ ), (b) test odour ( $P = 0.019$ ).



**Fig. 4.** Effect of duration of enrichment on mean latency to come out of a hide (seconds, square-root transformation;  $\pm$ S.E.) of juvenile salmon reared under control (○) and enriched (●) conditions. Trend lines show Lowess smoother of raw data. Tanks were enriched at time zero.

adaptive value in the wild by improving predator avoidance (Lima and Dill, 1990), and therefore help reducing one of the main sources of post-release mortality (Jepsen et al., 1998; Brown and



**Fig. 5.** Cox proportional hazards estimation of latency to come out of a hide (square root, s) in relation to (a) rearing environment ( $P < 0.001$ ) and (b) test odour ( $P = 0.029$ ). Survival plots show fraction of fish not yet emerging from a hide.

Day, 2002). As the test fish used in this study had no prior experience of predators, they must have been responding to changes in environmental conditions through learning. Hence our results indicate that it is possible to modify at least one component of fishes' behavior known to have clear adaptive implications, i.e. the propensity to leave shelter, even in individuals which have been kept in captivity for over a year. This opens the possibility of using environmental enrichment to improve post-release survival in supportive-breeding and reintroduction projects where the aim to establish self-sustaining populations, although it is not clear for how long enrichment would need to be prolonged for. For example, in salmonids Berejikian et al. (1999) found improved anti-predatory behavior after only one conditioning event, whereas double conditioning led to a weaker response. In contrast, Vilhunen (2006) reported improved predator avoidance following repeated conditioning. In our study two weeks of enrichment were enough to elicit a significant decrease in risk-taking behavior, but no further improvement was apparent with repeated conditioning.

Fish reacted to the presence of chemical alarm cues in the test-water by leaving shelter sooner, rather than later. Such behavior would appear counterintuitive as predation is thought to be a common cause of post-release mortality, and a more cautious approach under the threat of predation might have been expected (e.g. Lee and Berejikian, 2008). However, selection may be expected to favour a correct assessment of escalating predation threats

(Wisenden, 2000; Brown, 2003), resulting in either avoidance or inspection behavior (e.g. Brown and Godin, 1999; Ferrari et al., 2005). Fish can use diet-related alarm cues to initiate inspection without sight of a predator (Brown and Godin, 1999), and as visual cues were absent in our test arenas, this may have resulted in increase willingness to leave shelter under scented conditions. What seems clear is that fish tested in the presence of alarm cues responded by leaving shelter to explore a novel environment sooner than controls, and that this behavior was unaffected by environmental enrichment.

Poor survival of hatchery fish has been a pervasive problem in supportive-breeding and reintroduction programmes in general, but our findings show that rearing conditions can be easily modified to reduce maladaptive risk-taking behaviors developed in captivity. This is in line with studies that show that it is also possible to condition artificially-reared fish to recognise predators, further enhancing anti-predatory behaviors (Berejikian et al., 2003; Vilhunen, 2006), just as it had previously been shown for birds (e.g. Maloney and McLean, 1995; McLean et al., 1999) and mammals (e.g. McLean et al., 1996; Jule et al., 2008).

The rearing protocols adopted in this study were deliberately tailored to be easily incorporated into standard hatchery procedures, included the supplemental addition of natural prey items, and had no detrimental effects on fish health or stocking densities. The addition of small quantities of natural prey items (frozen invertebrates readily available in the aquarium trade) was an integral part of the enrichment protocol, and was designed to encourage fish to forage throughout the water column as opposed to just the surface, as this is a maladaptive feeding strategy commonly shown by hatchery fish (Tatara et al., 2008). Our experimental design, however, does not allow us to dissociate the effects of adding natural prey items from other concurrent manipulations carried out under enrichment, namely exposure to a predator model and increase in tank complexity. We know, however, that enriched fish that were initially 3.8 mm longer than controls, continued to be 2 mm longer at the end of the study, suggesting that both groups must have grown at similar rates and, perhaps more importantly, that body size had no significant effect on latency to leave shelter.

Given that over 70 million fish are reared in hatcheries in the UK alone (Lymbery, 2002), the scope for environmental enrichment is considerable. However, there are still large gaps in our understanding of the merits of environmental enrichment and naturalistic rearing systems. For example, it is not clear what is the best number and frequency of conditioning events, or the duration, intensity and timing of enrichment (Tatara et al., 2008); our results indicate that behavioral conditioning is rapid and can take place in as few as two weeks of enrichment. For most species, it also remains to be determined to what extent environmental enrichment results in increased post-release survival (Tatara et al., 2009), or what are the relative benefits of habitat manipulation over predator training (Berejikian et al., 1999), or simply over lowering rearing densities (Riley et al., 2009; Brockmark and Johnsson, 2010; Brockmark et al., 2010). Yet, given that over 1.7 billion hatchery fish are released into the wild every year in the US alone (Halverson, 2009) – and similar numbers are probably released in European waters – even modest incremental increases in post-release survival brought about by changes in rearing conditions would translate into significant gains for aquaculture-based fisheries and conservation projects alike.

Most projects involving environmental enrichment and pre-release conditioning have been carried out on birds and mammals (Griffin et al., 2000, 2001; Seddon et al., 2007), but as our results and those of others increasingly suggest (Riley et al., 2009; Tatara et al., 2009; Brockmark and Johnsson, 2010), fish can probably benefit too. This can pave the way for improving post-release survival in other traditionally neglected taxa such as amphibians, reptiles

and even invertebrates. More generally, our study provides empirical evidence for the benefits of enrichment in reverting human-altered phenotypes under relaxed selection scenarios (Lahti et al., 2009), and highlights the need to consider behavioral traits in reintroduction biology, a discipline in desperate need for a paradigm shift (Blumstein and Fernández-Juricic, 2004; Armstrong and Seddon, 2007).

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