

Effects of the rearing environment on average behaviour and behavioural variation in steelhead

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In the context of conservation hatcheries that seek to bolster wild populations by releasing captive-reared fishes into the wild, steelhead *Oncorhynchus mykiss* were used to test the hypothesis that naturalistic rearing environments promote adaptive behaviour that might otherwise not develop in typical hatchery environments. When comparisons were made among fish reared in barren, structured or structurally variable environments (*i.e.* the location of the structure was repositioned every 2–3 days), structure in the rearing environment increased future exploratory behaviour, but only if the structure was stable. Under conditions of high perceived predation risk, the fish no longer exhibited increased exploratory behaviour, suggesting that it is expressed in an adaptive, context-dependant manner. Another concern with hatcheries is that relaxed selection over multiple generations in captivity can increase maladaptive behavioural variation. Compared to rearing in hatchery-typical barren environments, rearing in structured-stable environments decreased behavioural variation. This effect, which occurred during development and did not involve selection, demonstrates a different mechanism for change in behavioural variation in captivity. These experiments show that effects of structure and structural stability occur at the level of both average behaviour and behavioural variation, and suggest that these effects should be considered when fishes are reared in hatcheries for later release into the wild.

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INTRODUCTION

Species that are declining in nature may be buffered from extinction by fishes that are reared in captivity and eventually released into the wild (Kleiman, 1989; Flagg & Nash, 1999). The likelihood, however, that released individuals will survive and contribute to the wild population depends in large part on their ability to behave adaptively after release (Brown & Day, 2002). This ability may be hampered by rearing in unnatural captive environments, since

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environmental cues that are available early in life in nature, but possibly not in captivity, may be necessary to guide individuals towards learning or otherwise developing behaviour that will be adaptive in the future (Kieffer & Colgan, 1992; Snyder *et al.*, 1996; Huntingford, 2004). Thus, an understanding of how the environment influences future behaviour is crucial to the success of programmes that seek to bolster wild populations through releases of captive reared individuals.

While some studies have tested for effects of environmental stimuli on future behaviour (Brown *et al.*, 2003), fewer studies have explored the additional factor of variability in environmental stimuli. In two recent studies (Braithwaite & Salvanes, 2005; Salvanes & Braithwaite, 2005), cod *Gadus morhua* L. that were reared in aquaria where structure was periodically moved around in the aquarium throughout rearing (*i.e.* spatially variable, 'structured-unstable') behaved differently from fish raised in barren aquaria. Compared to fish reared in barren environments, fish reared in structured-unstable environments more quickly left a 'start-box' to explore a novel aquarium and more quickly resumed a normal opercular beat rate after a simulated predatory attack (Braithwaite & Salvanes, 2005). Start-box and opercular beat rate tests have been used in fishes to quantify boldness and stress, respectively (Brown & Braithwaite, 2004; Brown *et al.*, 2005a). When cod from barren and structured-unstable treatments were allowed to interact in aquaria, the latter fled less, were more likely to attack cod reared in barren aquaria than tank-mates reared in the same structured-unstable tank, were less active, and were more likely to use shelter (Salvanes & Braithwaite, 2005). These two studies suggested that exposing fishes to variable environments may help them to behave adaptively after release into the wild (Braithwaite & Salvanes, 2005; Salvanes & Braithwaite, 2005).

These stimulating studies generated a series of questions. First, can the effect of structured-unstable rearing environments be generalized to other species? Braithwaite & Salvanes (2005) put their results into the context of programmes that seek to bolster declining, wild populations of fishes with hatchery releases. Many of these programmes have been unsuccessful, partly because of maladaptive behaviour by hatchery fishes. The use of variable rearing environments, however, may benefit programmes for a range of species since, for example, the exploratory behaviour that was induced by such environments in cod should be adaptive in the wild (Braithwaite & Salvanes, 2005). But the generality of this approach depends on whether the effect of variable rearing environments on behaviour can be generalized to other species. Here, juvenile steelhead *Oncorhynchus mykiss* (Walbaum) were tested to determine if they exhibit a similar response in exploratory behaviour to rearing in enriched environments.

Second, are behavioural effects due to instability *per se*, or to structure itself? Braithwaite & Salvanes (2005) used two spatial treatments, barren and structured-unstable, so it is not possible to determine whether the effects on exploratory behaviour were due to structure itself or to variation in the spatial position of the structure. To differentiate between these would require a third treatment using structure that remains in the same location through time; this is what was done in this study with juvenile steelhead. Braithwaite & Salvanes (2005) did conduct a controlled experiment on variability, but with regards to feeding

regimens; these feeding effects may not necessarily be generalized to the structural treatments.

Third, does current predation risk affect the relationship between past rearing conditions and current exploratory behaviour? Braithwaite & Salvanes (2005) found that cod reared in structured-unstable environments more quickly left start-boxes to explore novel habitat than fish from barren environments. They raised the possibility that this could be costly in nature if it leads to higher mortality rates (*e.g.* through predation), and suggested repeating the study under conditions where high predation risk was perceived.

Perceived predation risk can be manipulated in juvenile steelhead, which exhibit anti-predator behaviour in response to chemical cues released from injured conspecifics ('alarm cue'; Scheurer *et al.*, 2007). Juvenile salmonids, like many animals, are known to exhibit trade-offs between foraging and predator avoidance behaviours (Lima & Dill, 1990). Juvenile steelhead may also make 'decisions' regarding the trade-offs associated with exploratory behaviour, *i.e.* they may reduce or delay exploratory behaviour when high predation risk is perceived. Here, experiments tested how those decisions are affected by past experience with structure and the stability of that structure in the rearing environment.

Finally, might rearing conditions and current predation risk affect not only the average behavioural response of a population, but also individual variation within a population? Because of the traditional focus on behavioural means, few studies have paid attention to the potential for environmental stimuli to affect behavioural variation among individuals. Recently, an increasing number of studies have documented among-individual behavioural variation (Wilson *et al.*, 1994; Bell & Stamps, 2004; Sih *et al.*, 2004; Brown *et al.*, 2005b; Overli *et al.*, 2005). Behavioural variation among individuals may be important from both evolutionary and conservation perspectives (Wilson, 1998; Huntingford, 2004; McDougall *et al.*, 2006). Evolutionarily, behavioural variation can be non-adaptive noise around an adaptive peak, or alternatively could represent adaptive responses to heterogeneous selective pressures (*e.g.* negative frequency dependence and heterogeneous environments). From a conservation perspective, some attention has been given to altered behavioural variation in captive *v.* wild populations (McPhee, 2004; MCPhee & Silverman, 2004; Hakansson & Jensen, 2005; McDougall *et al.*, 2006), but most of this has been within the context of relaxed or otherwise altered selection over multiple generations, as opposed to within-lifetime environmental effects that do not require changes in gene frequencies through time. Thus, the data were further explored with additional analyses that tested for effects of different rearing treatments on future behavioural variation.

All four questions were addressed by rearing juvenile steelhead in aquaria under three treatment conditions: barren environments, structured-stable environments (*i.e.* with structure that is not moved) and structured-unstable environments (*i.e.* the spatial position of structure was changed through time). Behaviour was assayed in novel aquaria, both in the presence and absence of an alarm cue that is perceived by juvenile steelhead as indicating the presence of predators in the immediate environment. Juvenile steelhead are easily reared in aquaria, and their behaviour can be readily assayed, making them amenable

to studies of early environmental effects on behaviour. These fish are also currently reared in hatcheries and released into the wild to supplement natural populations, so knowledge about environment-dependant behavioural development may allow hatcheries to produce juveniles that behave like wild fish (Mobrand *et al.*, 2005; Salvanes & Braithwaite, 2006).

MATERIALS AND METHODS

STUDY SUBJECTS

The juvenile steelhead used in this study were offspring of five pairs of artificially spawned Skookumchuck River fish, which in turn were offspring from an integrated broodstock in which 30% of the parental stock each year is made up of wild fish. The offspring were obtained as eyed embryos from the Bingham Creek Hatchery (Washington Department of Fish and Wildlife) in March 2006, and reared at NOAA Fisheries, the Manchester Research Station, Manchester, WA, U.S.A., in a 1.2 m outdoor holding tank.

EXPERIMENTAL REARING

On 14 June 2006, juvenile steelhead were netted from the holding tank and stocked into 12 208 l aquaria (24 fish per aquarium, totalling 288 fish). The aquaria were located inside a 9.0 by 1.5 m flume that was surrounded by a black curtain, and lit for 12 h day⁻¹. The aquaria were divided into three treatments: barren, structured-stable and structured-unstable. To avoid bias in aquarium position, treatments were alternated within the flume. Twenty-four fish per treatment were randomly selected across replicate tanks and measured [mean \pm s.e. standard length (L_S): barren 47.46 \pm 0.54 mm; structured-stable 47.92 \pm 0.57 mm and structured-unstable 49.46 \pm 0.47 mm]. Barren aquaria contained no structure. Each structured aquarium contained five rocks (c. 70–100 mm in diameter) and two 580 by 60 mm plastic planks that anchored a total of four 280 mm plastic plants. Each aquarium was supplied with 3 l min⁻¹ of well water, a portion of which was re-circulated through a pump (Little Giant, PMO-650) and carbon filter. Fish were fed *ad libitum* once per day, five times per week. Structured-unstable aquaria were identical to structured-stable aquaria except the spatial position of rocks and plants in structured-unstable aquaria was randomly moved every 2–3 days throughout the rearing period. Differences in disturbance were minimized among treatments by conducting the spatial repositioning during tank-cleanings on days in which tanks from all treatments were cleaned. The additional time spent shifting rocks and plastic planks for the structured-unstable treatments was only c. 10 s, which was probably negligible compared to the c. 5 min taken to clean each aquarium. Thus, some aquaria were barren, others contained stable structure and others contained structure that varied in position through time. Experiment 1 was conducted after the fish had been reared under these conditions for 4 weeks (mean \pm s.e. L_S : barren 54.90 \pm 0.78 mm; structured-stable 53.67 \pm 0.86 mm; structured-unstable 53.63 \pm 0.91 mm); experiment 2 was conducted after 8 weeks of rearing (mean \pm s.e. L_S : barren 63.86 \pm 3.55 mm; structured-stable, 54.00 \pm 2.01 mm). Mortality was low (c. 5%) and was randomly scattered among the treatments.

EXPERIMENT 1: EXPLORATORY BEHAVIOUR WITH AND WITHOUT ALARM CUE

Approximately 1 month following the stocking of the rearing tanks, the experiment followed Braithwaite & Salvanes (2005) in using 'start-box' releases to assay the tendency of fish to explore a novel area. Start-boxes were 140 mm lengths of 76 mm diameter

PVC pipe that were sealed at one end with plastic disks. The other end was closed-off by a door that could be lifted remotely by pulling on a string attached to the door. Each fish was netted from its 208 l rearing aquarium and placed into its own start-box, which in turn was placed into a 38 l aquarium. Prolonged chasing with a net can influence stress and boldness scores (Brown *et al.*, 2007). When netting the fish, the amount of chasing with the net was minimal, and roughly similar for the three treatments. Water entered each 38 l aquarium through a PVC pipe, and exited through another PVC pipe at the other end of the aquarium. After a 10 min acclimation period, the start-box door was remotely opened and the number of seconds until the fish left the start-box was recorded.

Another set of replicates run concurrently with the predator-free start-box experiment used the methods described above, but with one difference. One minute before remotely opening the start-box door, 40 ml of alarm cue was added into the aquarium. This was done *via* an opening in the flow-through system that allowed the alarm cue to be remotely added to the water normally passing into the tank. Thus, 'alarm cue' aquaria received influxes of water and alarm cue, whereas 'non-alarm-cue' aquaria received influxes of just water. Alarm cue consisted of juvenile steelhead muscle and skin that had been mixed with water, liquified and run through filter floss following the methods of Berejikian *et al.* (2003). Like many other fishes, juvenile salmonids have an innate response in which they apparently interpret alarm cue to mean that a conspecific has been injured (perhaps by a predator), and show a fright response that basically consists of a reduction in activity (Brown, 2003; Scheurer *et al.*, 2007). Forty millilitres of alarm cue consisted of *c.* 15 mm² of liquified and filtered muscle and skin. This concentration (40 ml per 38 l aquarium) is effective in generating a fright response in juvenile steelhead (C. Tatara, pers. comm.), and is a substantially greater concentration than that used by Scheurer *et al.* (2007).

DATA ANALYSIS

The Kruskal–Wallis test was used to test for differences in time to leave the start-box; this test was followed with non-parametric *post hoc* tests (Siegel & Castellan, 1988). Data were also analysed with Bonferroni corrected, pair-wise Mann–Whitney *U*-tests. Possible differences in variance among non-alarm cue treatments were tested with O'Brien, Brown-Forsythe, Levene and Bartlett tests. The Pearson correlation was used to test whether time to leave the start-box correlated with L_S .

EXPERIMENT 2: FEEDING BEHAVIOUR ASSAY

Results from experiment 1 showed differences in behavioural variation between the barren and structured-stable treatments. Next, a different assay tested whether these behavioural variation differences were repeatable for another measure: feeding behaviour. At 1600 hours on day 1, single fish were placed into their own 38 l aquarium ($n = 7$ for the barren rearing treatment and $n = 7$ for the structured-stable treatment). The fish were fed thawed, previously frozen *Daphnia* sp. at 1630 hours on day 1 and at 0830 hours on day 2. At 1200 hours on day 2, fish were again fed thawed *Daphnia* sp., and the number of bites taken in the 300 s following the release of *Daphnia* sp. into the aquarium were counted and recorded. In nature, juvenile steelhead eat, among other prey, larval aquatic insects that drift passively downstream. The *Daphnia* sp., which drifted 'downstream' in the flow-through aquaria, mimicked these larval aquatic insects. The *Daphnia* sp. differed from the larger and denser pellet food on which the steelhead were raised.

Larger sample sizes and additional alarm cue and predator odour treatments were originally intended, but equipment problems forced an early end to the experiment. Nevertheless, this experiment still allowed behavioural variation differences between the two treatments that showed the greatest variation differences in experiment 1 to be tested.

DATA ANALYSIS

The Mann–Whitney U -test was used to test for a difference in the number of bites taken on the *Daphnia* sp., and the O'Brien, Brown–Forsythe, Levene and Bartlett tests were used to test for differences in variation. The Pearson correlation was used to test for a relationship between the number of bites taken and L_S .

RESULTS

EXPLORATORY BEHAVIOUR WITH AND WITHOUT ALARM CUE

Fish almost always appeared active within each start-box and hovered over the bottom of the start-box. There were significant differences in time to leave the start-box among the treatments that did not involve alarm cue (Fig. 1; Kruskal–Wallis test statistic = 8.15, $n = 18$, $P < 0.05$). *Post hoc* tests (Siegel & Castellan, 1988) on these data not involving alarm cue showed that fish from structured-stable rearing environments left their start-boxes significantly earlier than did fish from structured-unstable environments (*post hoc*, $n = 18$, $P < 0.05$; $z = 13.42$; critical value of $z = 12.55$). Structured-stable fish also tended to leave their start-boxes earlier than fish from barren environments, but the z -score (12.42) was slightly less than the critical value of 12.55. No difference was detected between barren and structured-unstable treatments (*post hoc*, $n = 18$, $P > 0.05$; $z = 1.00$; critical value of $z = 12.55$). Data were also analysed by conducting pair-wise Mann–Whitney U -tests, and using the Bonferroni correction for multiple comparisons ($P_{crit} = 0.0167$). When this was done, there was a significant difference between fish from structured-stable and structured-unstable rearing environments (Mann–Whitney U -test = 241.5, $n = 18$, $P < 0.0167$), a marginally insignificant difference between fish from structured-stable

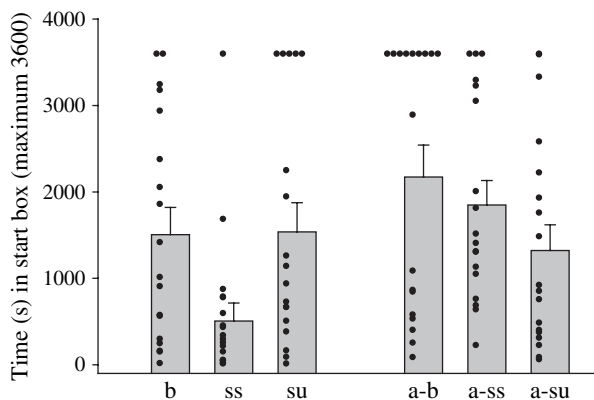


FIG. 1. Exploratory behaviour of steelhead. Fish from barren (b), structured-stable (ss) and structured-unstable (su) rearing environments were assayed for exploratory behaviour in a novel aquarium under conditions of normal and high (alarm cue, a-b, a-ss and a-su) perceived predation risk. A shorter period of time in the start-box corresponds to higher exploratory behaviour. Values are means + S.E. and individual data points.

and barren rearing environments (Mann–Whitney U -test = 237.5, $n = 18$, $P > 0.0167$), and no difference between barren and structured-unstable treatments (Mann–Whitney U -test = 155.0, $n = 18$, $P = 0.82$).

Among-individual variation in time to leave the start-box seemed to be greater in fish raised in barren environments than fish raised in structured-stable environments (Fig. 1), but tests for unequal variances among the three non-alarm cue treatments were inconsistent (O'Brien: $F = 2.23$, $n = 18$, $P > 0.05$; Brown–Forsythe: $F = 3.97$, $n = 18$, $P < 0.05$; Levene: $F = 6.87$, $n = 18$, $P < 0.01$ and Bartlett: $F = 2.05$, $n = 18$, $P > 0.05$). The s.d. for each treatment were barren 1306, structured-stable 860 and structured-unstable 1400, and coefficients of variation (CV) for each treatment were barren 0.83, structured-stable 1.45 and structured-unstable 0.88.

When alarm cue was added to aquaria before start-box doors were opened, fish from each of the three rearing treatments did not differ in time to leave the start-box. There were no significant differences between fish from barren rearing environments tested with and tested without alarm cue (Mann–Whitney $U = 211.0$, $n = 18$, $P > 0.05$; Fig. 1), or between fish from structured-unstable rearing environments tested with and tested without alarm cue (Mann–Whitney $U = 142.5$, $n = 18$, $P > 0.05$; Fig. 1). Fish from structured-stable rearing environments tested with alarm cue spent significantly longer in the start-boxes before leaving than those tested without alarm cue (Mann–Whitney U -test = 281.0, $n = 18$, $P < 0.001$; Fig. 1).

When no alarm cue was added to aquaria, data appeared in one cluster at moderately low values and in a second cluster at the maximum value for the structured-unstable treatment, but not for the barren treatment (Fig. 1). In contrast, when alarm cue was added to aquaria, data appeared in two clusters for the barren treatment but not for the structured-stable or structured-unstable treatments (Fig. 1).

Time to leave the start-box was not predicted by L_S , whether data were analysed from the six treatments separately (alarm cue barren, alarm cue structured-stable, alarm cue structured-unstable, barren, structured-stable and structured-unstable (Pearson correlation, all $n = 18$, $P > 0.05$) or together (Pearson correlation, $n = 108$, $P > 0.05$).

FEEDING BEHAVIOUR ASSAY

Fish raised in barren and structured-stable environments did not differ in the number of bites taken on the *Daphnia* sp., though sample sizes were low (Fig. 2; Mann–Whitney U -test = 26.00, $n = 7$, $P > 0.05$). Variation in the number of bites taken by fish from barren environments was greater than that for fish from structured-stable environments (Fig. 2; O'Brien: $F = 5.91$, $n = 7$, $P < 0.04$; Brown–Forsythe: $F = 15.08$, $n = 7$, $P < 0.003$; Levene: $F = 12.09$, $n = 7$, $P < 0.005$; Bartlett: $F = 7.56$, $n = 7$, $P < 0.006$). Standard deviations for each treatment: barren – 40.0; structured-stable – 10.84. Coefficients of variation for each treatment: barren – 0.69; structured-stable – 0.19.

The number of bites taken did not correlate with L_S , whether data were analysed from the two treatments together (Pearson correlation, $n = 14$, $P > 0.05$) or separately (barren or structured-stable; Pearson correlation, both $n = 7$, $P > 0.05$).

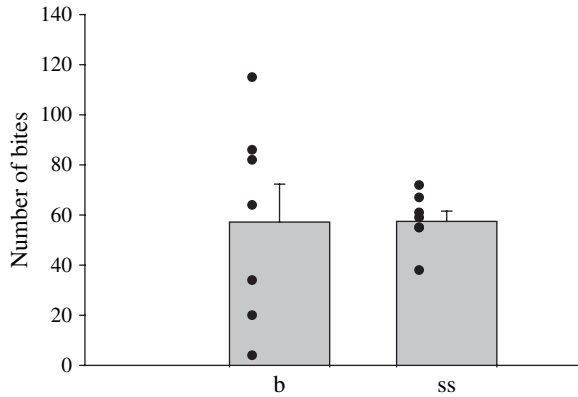


FIG. 2. Feeding behaviour of steelhead. Fish from barren and structured-stable rearing environments were assayed for feeding behaviour. Values are means + S.E. and individual data points.

DISCUSSION

EFFECTS OF REARING ENVIRONMENTS AND PREDATION RISK ON EXPLORATORY BEHAVIOUR

The hypothesis that structured-unstable environments enhance exploratory behaviour in juvenile steelhead was not supported. Fish reared in structured-stable environments, however, tended to leave start-boxes sooner than those reared in structured-unstable and barren environments, suggesting that effects of structure on steelhead exploratory behaviour actually required stability; movement of the spatial position of structure through time reduced exploratory behaviour. Thus, while it was not possible to test whether effects of structured-unstable environments [as found with Braithwaite & Salvanes' (2005) cod] are due to structure itself or to temporal variation in the spatial position of structure, this experiment does demonstrate that exploratory behaviour can be affected by the spatial stability of structure.

Why did not structured-unstable steelhead show the same results as the structured-unstable cod from Braithwaite & Salvanes (2005)? The importance of environmental variability for proper behavioural development may differ between the two species. Newly settled juvenile cod roam through various microhabitats as they forage; thus their environment changes on a regular basis and exposure to this variation may be necessary for proper behavioural development (A. G. V. Salvanes, pers. comm.). Juvenile steelhead can also experience changing environments in the rivers in which they rear in nature (Quinn, 2005). When water levels fluctuate, juvenile steelhead may be forced to move from location to location. Even if an individual does not move, fluctuating water levels can rapidly and frequently change a sedentary juvenile's surrounding environment. There is no 'typical' early life experience for juvenile steelhead, however, and environmental variability is not necessarily experienced by all individuals. A dominant juvenile under conditions of stable water levels may be able to defend a particular area for several weeks; this juvenile would experience a relatively stable environment (Keeley, 2001). Perhaps the existence

of this stability within the range of natural juvenile steelhead experiences makes instability during hatchery rearing less important to the behavioural development of juvenile steelhead.

Another possibility is that the effect requires a longer rearing duration or less frequent habitat disruptions; Braithwaite & Salvanes (2005) reared the cod for 3.5–5 months and disrupted the habitat once per week, whereas the juvenile steelhead were reared for 1 month and the habitat disrupted two to three times per week. This level of disturbance was within the range experienced by juvenile steelhead in nature, but perhaps the optimal level of disturbance is at a lower level (Odling-Smee & Braithwaite, 2003). Experiments that include a variety of rearing duration and disturbance level treatments are necessary to determine whether the discrepancy between the steelhead and cod results is due to methodological or species differences.

Braithwaite & Salvanes (2005) reasonably proposed that exploratory behaviour should allow fishes to find resources in nature, but also raised the question of whether such behaviour could lead to increased mortality when predation risk is high. If steelhead that are exposed to structured-stable environments exhibit increased exploratory behaviour, do they pay a cost when predators are present? The efficacy of alarm cue in steelhead enabled similar behavioural trials to be conducted with a higher level of perceived predation risk. When steelhead were exposed to alarm cue before the lifting of the start-box door, the among-rearing-treatment differences that were apparent without alarm cue no longer existed. On average, alarm cue did not appear to increase time to leave the start-box for barren-reared and structured-unstable-reared fish. In contrast, structured-stable fish took significantly longer to exit the start-boxes when alarm cue was present, compared to when it was not. This suggests that the exploratory behaviour that was promoted by rearing in structured-stable environments might not necessarily result in higher mortality, since on average, fish from that rearing environment compensated by reducing their exploratory behaviour in response to high perceived predation risk, whereas fish from the other treatments did not. Thus, rearing steelhead in structured-stable environments may promote exploratory behaviour that is adaptively flexible, and thus increase the chances that fish will survive and succeed after release into nature. Additional effects of enriched environments are given by Berejikian & Tezak (2005), Berejikian *et al.* (2000) and Tatara *et al.* (in press).

THE BEHAVIOURAL VARIATION PERSPECTIVE

While most studies focus on behavioural means, attention to behavioural variation is increasing (Bourke *et al.*, 1997; Wilson, 1998; Salvanes *et al.*, 2007). Studies that test for consequences of change in behavioural variation are still scarce, but such changes probably have important consequences (McPhee & Silverman, 2004; Watters & Meehan, 2007). For example, variation in food preferences and habitat preferences can be important for habitat partitioning (Bourke *et al.*, 1997); reduced variation may lead to increased competition for a smaller range of food or habitat types. Variation in exploratory behaviour could be relevant since exploratory behaviour may affect the likelihood that individuals will discover new habitats or sources of food.

Behavioural variation can also cause different members of a single population to succeed differentially in alternative environments. Not having all of the population's 'eggs in one basket' may allow populations to persist in changing environments or to exploit new ones, even if the variation evolved for a different reason (Kirschner & Gerhart, 1998). Another way that variation can affect populations is through frequency dependence (Maynard Smith, 1982). That is, the fitnesses of different behavioural phenotypes can depend on the frequencies of other phenotypes (Sinervo & Lively, 1996), so altering the frequency distribution of different phenotypes from that in nature can affect fitness.

Variation is not always adaptive. In some situations, variation may increase past the range seen in nature. If increased behavioural variation represents an increase in maladaptive behaviour (*e.g.* when changes in variation are driven by relaxed selection in captivity; McPhee, 2004), then increased variation may lead to lower rates of survival in nature.

In experiment 1, barren and structured-stable fish differed in not only their means, but also in the degree of dispersion around the means; data appeared more dispersed around the mean for the barren data than for the structured-stable data. In feeding data from experiment 2, variation was again greater in the barren-reared fish; this was unaccompanied by any mean difference. While some studies have shown how relaxed selection over multiple generations can increase behavioural variation (McPhee, 2004), data from both experiments 1 and 2 of this study illustrate another way that the typically barren captive environment can change behavioural variation: by altering the behaviour of individuals through development (Zimmermann *et al.*, 2001).

While the mechanisms through which rearing environments affected behavioural variation are unknown, it is well known that alternative tactics (*i.e.* increased behavioural variation) may be adopted in response to intense competition (Maynard Smith, 1982; Brockmann, 2001). The intensity and nature of competition experienced by steelhead probably differed between structured and barren aquaria. For example, structural complexity in natural streams can visually isolate juvenile salmonids and reduce 'intruder pressure' (Mesick, 1988), meaning that each fish receives fewer attacks from a smaller number of competitors. Consistent with this finding, steelhead cultured in structured rearing tanks and a natural stream exhibited significantly lesser nipping-induced fin damage than those reared in barren tanks (Berejikian & Tezak, 2005). Reduced aggression in structured habitats has been demonstrated in other fishes as well, *e.g.* zebra fish *Danio rerio* (Hamilton) (Basquill & Grant, 1998). Structure also promotes the ability to obtain and defend territories (*i.e.* dominance; Berejikian *et al.*, 2000, 2001). Thus, fishes reared in structured-stable environments probably experience greater stability in their location, and in the presence and position of competitors and food. In barren environments, perhaps fishes employ a broader range of behaviours to respond to the increased intensity and different nature of competition they endure and induce.

Alternatively, increased behavioural variation in barren tanks may not represent alternative, adaptive behavioural tactics, but rather may be maladaptive noise resulting from historically weak selection. In nature, selection may select against maladaptive behaviours, thus reducing behavioural variation around

the most adaptive response (stabilizing selection, assuming a single optimum). If a particular environment is rarely encountered in nature (*e.g.* the barren environment), however, then there will rarely be an opportunity for natural selection to select against maladaptive responses to that particular environment (Fry, 1996). Thus, the large amount of behavioural variation exhibited by fishes reared in barren aquaria may reflect an historical lack of opportunity for natural selection to shape a single response to completely barren environments, since completely barren environments are not encountered in nature. Rearing fishes in such a novel environment in captivity may allow for the expression of this variation, or 'noise'. Increased variation due to release from selection in a particular environment in nature has been demonstrated in a plant (T. Griffith & S. Sultan, unpubl. data).

The perspective of behavioural variation also provides a different view of behavioural responses to perceived predation risk. In response to alarm cue, the group reared in barren environments appeared to move from a continuous to a discontinuous distribution, whereas the group reared in structured-unstable environments appeared to move in the opposite direction. There still remains much to learn about why the directions of these shifts depended on the rearing environment, and what the shifts themselves mean. Field data that quantify the extent, distribution, and adaptive value of behavioural variation in nature will probably provide valuable insight into the interpretation of rearing treatment effects such as those in this study.

This study was unable to support the hypothesis that structured-unstable rearing environments increase exploratory behaviour in juvenile steelhead. This could be due to methodological differences between this study and the study by Braithwaite & Salvanes (2005), or to species differences between steelhead and cod. Nevertheless, with the 1 month rearing period and structural instability occurring two to three times per week, exploratory behaviour was affected by environmental instability, though in the opposite direction of the effect found by Braithwaite & Salvanes (2005) in cod: stable structure in steelhead rearing environments resulted in greater exploratory behaviour than did unstable structure. Further, this behavioural effect may not lead to substantially greater predation risk since fish reared with stable structure expressed their enhanced exploratory behaviour in a context-specific manner, only when perceived predation risk was low. Thus for steelhead, rearing with stable structure appears to increase exploratory behaviour and may improve post-release success. Finally, data from the exploratory and feeding trials suggest that greater attention should be paid to behavioural variation, since rearing environments can affect variation around means as well as the means themselves.

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